UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

KAMILA FERREIRA CHAVES

DESENVOLVIMENTO DE ORGANOGÉIS PARA APLICAÇÃO EM *SPREADS* DE CHOCOLATE

DEVELOPMENT OF ORGANOGELS FOR CHOCOLATE SPREADS APPLICATION

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

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

Orientadora: Profª. Dra. Ana Paula Badan Ribeiro

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RESUMO

Nos últimos anos, alguns avanços na estruturação das bases lipídicas permitiram uma influência direta na modificação de propriedades de óleos e gorduras para aplicação em produtos alimentícios. Os mecanismos estruturantes das bases lipídicas podem ser classificados como convencionais ou não convencionais. Mecanismos não convencionais trazem a tecnologia de organogéis como uma tendência, que consiste no uso de agentes de auto-montagem para reter o óleo líquido, resultando em uma rede de gel estruturada. Questões controversas sobre o papel dos ácidos graxos *trans* e saturados nos alimentos levaram a mudanças progressivas na legislação de vários países para incluir mais informações para os consumidores. Neste contexto, os organogéis têm sido indicados como uma alternativa viável para a obtenção de gorduras semi-sólidas com reduzido teor de ácidos graxos saturados (AGS) e propriedades compatíveis para aplicação em alimentos. O objetivo desta tese de doutorado foi apresentar os estudos que abordam os organogéis lipídicos como uma alternativa para a aplicação de alimentos, caracterizar os *spreads* de chocolate comerciais e suas respectivas fases lipídicas, visando estratégias de reformulação dessa categoria de produto, além da caracterização físico-química de estruturantes de grau alimentício com potencial uso no desenvolvimento de organogéis, avaliar o efeito de estruturantes de grau alimentício isoladamente, em misturas binárias, ternárias ou mais, em diferentes concentrações, sobre a formação de organogéis de óleo de girassol alto oleico e produzir *spreads* de chocolate com redução do teor de AGS. Os *spreads* de chocolate comerciais analisados neste estudo mostraram estabilidade, uma vez que não houve exsudação de óleo líquido durante o período de estabilização, no entanto, eles poderiam apresentar menores níveis de AGS para atender a demanda de consumidores que buscam alimentos mais saudáveis. Todos os estruturantes apresentaram propriedades semelhantes, como alta concentração de ácidos graxos saturados, alto teor de sólidos na temperatura analisada, baixo tempo de indução de cristalização, alta resistência térmica, bem como parâmetros uniformes quanto à morfologia e dimensões cristalinas. Os organogéis analisados são bases lipídicas com potencial para serem usados como substitutos de gordura em processos industriais, para atender uma demanda de consumidores que buscam por alimentos mais saudáveis. Os *spreads* de chocolate com organogel mostraram alta estabilidade, indicando que o uso de organogéis como substituto de bases lipídicas convencionais em *spreads* de chocolate foi eficiente, uma vez que apresentaram comportamento similar ao padrão produzido com óleo de palma e redução de AGS variando entre 69,79 a 76,04%. Dessa forma, é possível produzir um produto de qualidade utilizando baixa concentração de estruturante e reduzindo o teor de AGS.

Palavras-chaves: organogel, agentes estruturantes, ácidos graxos saturados, *spreads* de chocolate.

ABSTRACT

In recent years, some advances in the structuring of lipid bases have allowed a direct influence on the modification of properties of oils and fats for application in food products. The structuring mechanisms of the lipid bases can be classified as conventional or unconventional. Unconventional mechanisms bring organogel technology as a trend, which is the use of self-assembling agents to retain liquid oil, resulting in a structured gel network. Controversial questions about the role of trans and saturated fatty acids in food have led to progressive changes in legislation in several countries to include more information for consumers. In this context, organogels have been indicated as a viable alternative to obtain low saturated fatty acid (SFA) semisolid fats and compatible properties for food application. The aim of this doctoral dissertation was to present the studies that approach lipid organogels as an alternative for food application, to characterize the commercial chocolate spreads and their respective lipid phases, aiming at strategies of reformulation of this product category, besides the physical-characterization. Chemistry of food grade structurants with potential use in organogel development, to evaluate the effect of food grade structurants alone, in binary, ternary or more mixtures, at different concentrations, on the formation of high oleic sunflower oil organogels and produce chocolate spreads with reduced SFA content. The commercial chocolate spreads analyzed in this study showed stability as there was no liquid oil exudation during the stabilization period; however, they could have lower levels of SFA to meet the demand of consumers seeking healthier foods. All structurants had similar properties, such as high concentration of saturated fatty acids, high solids content at the analyzed temperature, short crystallization induction time, high thermal resistance, as well as uniform morphological parameters and crystalline dimensions. The analyzed organogels are lipid bases with potential to be used as fat substitutes in industrial processes, to meet a demand of consumers who are looking for healthier foods. The organogel chocolate spreads showed high stability, indicating that the use of organogels as a substitute for conventional lipid bases in chocolate spreads was efficient, since they presented similar behavior to the palm oil pattern and reduction of SFA ranging from 69.79 to 76.04%. Thus, it is possible to produce a quality product using low concentration of structurant and reducing the SFA content.

Key-words: organogel, structuring agents, saturated fatty acids, chocolate spreads.

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1. Tecnologia de lipídios

Lipídios são nutrientes essenciais da dieta humana, apresentando papel vital mediante o fornecimento de ácidos graxos essenciais e energia. Quimicamente, óleos e gorduras naturais consistem de misturas multi-componentes de triacilgliceróis (TAGs), que são ésteres de glicerol e ácidos graxos. Cada ácido graxo pode ocupar diferentes posições na molécula (*sn-1*, *sn-2* ou *sn-3*), possibilitando uma grande diversidade de combinações (O'Brien, 2008). Os ácidos graxos de ocorrência natural em fontes vegetais apresentam número par de átomos de carbono em uma cadeia não ramificada com número de carbonos entre 12 e 24 (Scrimgeour, 2005).

Os TAGs correspondem à classe lipídica que fornece energia a organismos vivos, podendo ser armazenados no tecido adiposo, ou hidrolisados liberando glicerol e ácidos graxos que são transportados ao fígado, rins e músculos, onde são catabolizados como fontes de energia para o organismo (Curi, 2002). Em adição às qualidades nutricionais, os óleos e gorduras provêm consistência e características de fusão específicas aos produtos que os contêm, atuam como meio de transferência de calor durante o processo de fritura e como carreadores de vitaminas lipossolúveis e aroma. Além disso, os lipídios afetam a estrutura, estabilidade, sabor, aroma, qualidade de estocagem, características sensoriais e visuais dos alimentos (O'Brien, 2008).

Um aspecto importante das propriedades físicas de óleos e gorduras está relacionado às mudanças de fase sólido-líquido e líquido-sólido; fusão e cristalização, respectivamente. Os diversos fenômenos térmicos relacionados aos óleos e gorduras são verificados pelo monitoramento de mudanças de entalpia e de transição de fases das diversas misturas de TAGs. As características polimórficas dos TAGs tornam complexo o estudo das propriedades térmicas e estruturais em sistemas lipídicos. O comportamento térmico reflete, portanto, as propriedades gerais de funcionalidade e aplicabilidade de lipídios, e mostra-se dependente dos perfis de TAGs nos óleos e gorduras comestíveis (Tan, Che Man, 2002).

A estruturação de fases lipídicas determina, portanto, importantes propriedades dos alimentos: (i) a consistência e plasticidade de produtos ricos em gordura, durante as etapas de produção e estocagem; (ii) propriedades sensoriais, como sensação de fusão na boca; (iii) estabilidade física, com respeito à formação ou sedimentação de

cristais, exsudação de óleo e coalescência de partículas e emulsões; (iv) aparência visual, a exemplo do brilho em chocolates e coberturas (Foubert et al., 2007).

No escopo da estruturação não convencional, sistemas lipídicos compostos por TAGs insaturados, a exemplo de óleos vegetais líquidos ou semi-sólidos, podem ser estruturados como géis, que podem formar redes contínuas de pequenas moléculas que se reúnem em cristais líquidos, micelas ou redes fibrilares formadas a partir de agregados de micelas, desenvolvendo estruturas de bicamadas inversas na forma de bastonetes (Pernetti et al., 2007). Este tipo particular de estruturação caracteriza sistemas reconhecidos como organogéis, que compreendem um grupo de compostos que possuem características de gel, onde a fase imobilizada é um composto orgânico, o que o difere de outros géis formados basicamente por compostos hidrossolúveis (Rogers, Wright, Marangoni, 2011).

1.2. Tecnologia de organogéis

Os organogéis podem apresentar diferentes estruturas, formadas pelos mais diversos compostos orgânicos, sendo que as duas mais importantes referem-se à dispersão de sólidos em uma fase líquida (pequenas partículas inertes, sólidos cristalizados, gotas) ou ainda mecanismos específicos, como auto-sustentação (comumente observados nos agentes estruturantes de baixo peso molecular). Ambos formam redes tridimensionais que são capazes de imobilizar uma fase líquida. O tamanho e forma dessa estrutura e suas interações estão diretamente relacionados com as características físicas e propriedades dos estruturantes (Pernetti et al., 2007).

A estruturação de fases orgânicas baseia-se na dispersão de uma fase lipídica externa com agentes estruturantes, através de mecanismos moleculares particulares. Diversos componentes podem atuar como estruturantes para as redes tridimensionais que são requeridas para a consistência de produtos gordurosos específicos. Alguns elementos estruturantes que podem ser utilizados são macromoléculas, como polímeros e proteínas, compostos de baixo peso molecular de natureza lipídica. Os principais compostos capazes de formar organogéis possuem cadeias carbônicas bastante longas, como os ácidos graxos de cadeia longa, álcoois graxos, ésteres de cera e alcanos (Daniel, Rajasekharan, 2003). Esses agentes estruturantes permitem que duas fases distintas se combinem em um estado quase homogêneo. Os componentes específicos, utilizados isoladamente ou em conjunto, bem como suas interações, determinam a estrutura do produto final, e consequentemente, suas propriedades de consistência e plasticidade (Cerdeira et al., 2006).

De acordo com o mecanismo relacionado à estruturação de TAGs, os agentes estruturantes podem ser classificados em dois grupos, referentes às partículas cristalinas e aos sistemas de self-assembly (ou auto-associação). As partículas cristalinas estão associadas ao fenômeno clássico de nucleação, crescimento e estabilização da rede cristalina; enquanto nos sistemas de self-assembly a estruturação é promovida por um mecanismo de auto-organização molecular de seus componentes na fase orgânica. Interações covalentes, de Van der Waals, eletrostáticas e pontes de hidrogênio são exemplos de forças de interação intermolecular dos agentes estruturantes responsáveis pela formação da rede de gel tridimensional. Componentes com estruturas moleculares e químicas semelhantes frequentemente apresentam interações positivas quanto aos fenômenos de partículas dispersas e auto-associação (Dassanayake, Kodali, Ueno, 2011; Siraj et al., 2015; Godoi, Barrera Arellano, Ribeiro, 2017).

Óleos vegetais são exemplos de materiais orgânicos que podem ser estruturados com uma concentração relativamente baixa de agente estruturante, geralmente inferior a 10%. Em termos físico-químicos, organogéis são definidos como materiais viscoelásticos compostos por estruturantes e uma fase líquida apolar. São sistemas semi-sólidos, onde uma fase oleosa é imobilizada por uma rede tridimensional auto-sustentada do estruturante (Dassanayake et al., 2009). Tais estruturas têm sido desenvolvidas para diferentes aplicações, tais como farmacêuticas, cosméticas e indústrias de alimentos. Portanto, uma das maiores habilidades dos organogéis refere-se às características e propriedades reológicas de um sólido, embora sua composição majoritária (~98%) seja representada por um líquido orgânico (Pernetti et al., 2007).

1.3. Formulação de organogéis

Os métodos de obtenção dos organogéis são variados, bem como suas propriedades físico-químicas. Segundo Co and Marangoni (2012), as possibilidades de formação destes compostos ocorrem através de partículas cristalinas, fibras cristalinas, fitas poliméricas, dentre outros. Esses métodos variam de acordo com o estruturante utilizado e as condições de processo aos quais as matérias primas são expostas (Chaves et al., 2019).

O óleo de girassol alto oleico é considerado uma matéria-prima premium, geralmente utilizado em aplicações alimentícias que requerem o emprego de óleo líquido com estabilidade oxidativa excepcional. Possui sabor e aroma neutros,

característica associada ao seu alto potencial de aplicação em alimentos, cosméticos e fármacos (Gunstone, 2005). O óleo de soja (OS), rico em ácidos graxos poliinsaturados (aproximadamente 54% de ácido linoléico e 7% de ácido linolênico), é barato e facilmente disponível. É usado como uma fase orgânica na adição de agentes estruturantes para a formulação de organogels (O'BRIEN, 2009).

Uma opção de baixo custo e alto potencial para estruturação de fases lipídicas são os óleos vegetais totalmente hidrogenados, denominados *hardfats*. Estes componentes são considerados sistemas-modelo em termos de composição em ácidos graxos e TAG, que representam fatores importantes na determinação do efeito estruturante e modificador dos processos de cristalização em fases lipídicas contínuas ou emulsionadas. O *hardfat* de óleo de palma é caracterizado pelo hábito polimórfico β', propriedade de cristalinidade que o direciona para aplicações em alimentos de base lipídica (Oliveira et al., 2015; Omonov, Bouzidi, & Narine, 2010; Ribeiro, Basso, & Kieckbusch, 2013).

Outros componentes descritos recentemente pela literatura científica com potencial para estruturar matrizes lipídicas complexas referem-se aos emulsificantes (Rogers, 2009; Siraj et al., 2015). Os monoacilgliceróis são moléculas lipídicas que possuem apenas um ácido graxo esterificado a molécula de glicerol, que pode variar quanto ao tamanho de cadeia e grau de insaturação (Chen & Terentjev, 2010). A estruturação de óleos vegetais por monoacilgliceróis ocorre através do mecanismo self-assembly, pela formação de micelas ou fases lamelares inversas durante o resfriamento do sistema formado (Lopez-Martínez et al., 2015; Valoppi et al., 2016; Wang et al., 2016).

O monoestearato de sorbitana é um surfactante emulsificante não-iônico hidrofóbico, utilizado para modificar as propriedades de cristalização em sistemas lipídicos (Marangoni & Narine, 2002). Demonstra capacidade para formação de dispersões viscosas em solventes orgânicos e óleos comestíveis, através do mecanismo de self-assembly (Co & Marangoni, 2012; Smith et al., 2011). Estudos recentes sobre o efeito estruturante do monoestearato de sorbitana sugerem seu uso como potencial elemento de estruturação em óleos vegetais, além de uma interação positiva com os triacilgliceróis (Cerqueira et al., 2017; Oliveira et al., 2015; Sonwai, Podchong, & Rousseau, 2017).

1.4. Aplicação de organogéis em alimentos

Embora a aplicação de organogéis em alimentos represente um campo de grande interesse para utilização destes materiais, estudos relativos à obtenção e caracterização de alimentos de base lipídica contendo organogéis mostram-se recentes na literatura científica. A aplicação de organogéis vem sendo estudada há alguns anos e incluem a estabilização de emulsões de água em óleo e também meio de liberação controlada de produtos farmacêuticos e nutracêuticos. Aplicações na indústria de alimentos incluem o uso potencial dos estruturantes para minimizar a migração de óleo líquido nos alimentos, como por exemplo, recheio de chocolates, margarinas, produtos de panificação como biscoitos e *cookies*, massas folhadas, *spreads* e para estruturar óleos comestíveis, reduzindo a necessidade de utilizar ácidos graxos *trans* e saturados. Emulsões de organogel são mais apropriadas para alimentos emulsionados, tais como margarina, iogurte, queijos em barra e processados, maionese e molhos (Chaves, Barrera Arellano, Ribeiro, 2018).

Spreads de chocolate padrão e *spreads* preparados por substituição total e parcial de óleo de palma (27%) por organogéis de goma laca foram avaliados quanto à viscosidade, com parâmetros similares aos *spreads* comerciais. Os *spreads* padrão e formulado com organogel não mostraram exsudação quando armazenado a 30ºC por mais de 4 semanas (Patel et al., 2014).

Os produtos de chocolate são alimentos de base lipídica cujas propriedades físicas são dependentes de sua estrutura cristalina. Consequentemente, os óleos e gorduras utilizados na formulação tem efeito significativo sobre a qualidade do produto (MAYFIELD et al., 2015).

2. OBJETIVOS

2.1. Objetivo Geral

Desenvolvimento de *spreads* de chocolate com teores reduzidos de ácidos graxos saturados (*low-sat*), produzidos mediante a substituição parcial de óleo de palma por organogéis de óleo de girassol alto oleico, estruturados por diferentes agentes estruturantes (cera de candelilla monoestearato de sorbitana, monoacilgliceróis e *hardfat* de óleo de palma).

2.2. Objetivos Específicos

- ✓ Apresentação da visão geral a respeito da cristalização convencional e não convencional das fases lipídicas e também dos organogéis lipídicos como uma alternativa para a aplicação de alimentos;
- ✓ Caracterização de *spreads* comerciais e de suas respectivas fases lipídicas, visando estratégias para reformulação desta categoria de produtos, utilizando a tecnologia de organogéis;
- ✓ Caracterização físico-química de estruturantes de grau alimentício com potencial uso no desenvolvimento de organogéis;
- ✓ Avaliação do efeito de estruturantes de grau alimentício isoladamente, em misturas binárias, ternárias ou mais, em diferentes concentrações, sobre a formação de organogéis de óleo de girassol alto oleico;
- ✓ Avaliação e caracterização de organogéis de óleo de girassol alto oleico, utilizando sistemas de misturas representados por diagrama ternário.
- ✓ Reformulação de *spreads* de chocolate utilizando a tecnologia de organogéis para redução do teor de ácidos graxos saturados.

3. APRESENTAÇÃO DO TRABALHO

Para atender os objetivos propostos, o trabalho foi desenvolvido em 9 capítulos:

CAPÍTULO 1 – CONVENTIONAL AND UNCONVENTIONAL CRYSTALLIZATION MECHANISMS

O capítulo 1 apresenta uma revisão de literatura geral a respeito da cristalização convencional e não convencional das fases lipídicas, a fim de ampliar o conhecimento sobre as propriedades cristalográficas, microestruturais e aspectos cinéticos associados à estruturação de óleos e gorduras comestíveis, dando embasamento científico para compreensão e ao mesmo tempo justifica os capítulos abordados subsequentes.

CAPÍTULO 2 – POTENTIAL APPLICATION OF LIPID ORGANOGELS FOR FOOD INDUSTRY

O capítulo 2 apresenta uma revisão de literatura que aborda os organogéis lipídicos como uma alternativa para a aplicação de alimentos, tendo em vista que os organogéis têm sido indicados como uma alternativa viável para a obtenção de gorduras semi-sólidas com reduzido teor de ácidos graxos saturados e propriedades compatíveis para aplicação em alimentos. Além do mais, a proposta desse capítulo foi dar destaque à importância deste trabalho na área de desenvolvimento de óleos e gorduras.

CAPÍTULO 3 – CHOCOLATE SPREADS: CHARACTERIZATION FOR REFORMULATION

O capítulo 3 apresenta um estudo de caracterização de *spreads* comerciais e de suas respectivas fases lipídicas, visando estratégias para reformulação desta categoria de produtos, utilizando a tecnologia de organogéis.

CAPÍTULO 4 – FOOD GRADE STRUCTURING AGENTS FOR OLEOGELS: A PHYSICOCHEMICAL STUDY

O capítulo 4 apresenta uma extensa caracterização físico-química de estruturantes de grau alimentício com potencial uso no desenvolvimento de organogéis. Os estruturantes mostraram propriedades semelhantes, como alta concentração de ácidos graxos saturados, alto teor de sólidos nas temperaturas analisadas, baixo

tempo de indução na cristalização, alta resistência térmica, hábito polimórfico preferencial β', bem como parâmetros uniformes quanto à morfologia e dimensões cristalinas.

CAPÍTULO 5 – STRUCTURING POTENTIAL AND MECHANISMS OF ORGANOGELS FORMED BY DIFFERENT STRUCTURANTS AND HIGH-OLEIC SUNFLOWER OIL

O capítulo 5 apresenta uma avaliação do efeito de estruturantes (cera de candelilla, óleo vegetal de palma totalmente hidrogenado, lecitina de soja padrão, misturas de monoacilgliceróis Grindsted Crystallizer 100 e monoestearato de sorbitana) de grau alimentício isoladamente, em misturas binárias, ternárias ou mais, em diferentes concentrações, sobre a formação de organogéis de óleo de girassol alto oleico. Organogéis formulados nas concentrações de 4, 5 e 6% de C, H, L, M e S como estruturantes isolados foram analisados quanto dureza e estabilidade. Organogéis a 6% foram elaborados segundo um diagrama de possíveis combinações de até cinco estruturantes e analisados quando ao conteúdo de sólidos, cinética de cristalização, microestrutura, dureza e estabilidade.

CAPÍTULO 6 – FOOD GRADE HYBRID ORGANOGELATOR SYSTEMS FOR STRUCTURING OF HIGH OLEIC SUNFLOWER OIL

O capítulo 6 apresenta um estudo de avaliação e caracterização de organogéis de óleo de girassol alto oleico, estruturados com cera de candelilla, monoestearato de sorbitana e monoglicerídio, utilizando sistemas de misturas representados por diagrama ternário.

CAPÍTULO 7 – FORMULATION OF LIPID BASES USING A TERNARY SYSTEM

O capítulo 7 apresenta um estudo de avaliação e caracterização de organogéis de óleo de girassol alto oleico, estruturados com cera de candelilla, monoestearato de sorbitana e *hardfat* de palma, no que diz respeito às propriedades físico-químicas e ao comportamento de cristalização dos sistemas lipídicos com menores teores de ácidos graxos saturados e maior quantidade de ácido oleico.

CAPÍTULO 8 – OBTAINING ZERO TRANS/LOW SAT LIPID BASES WITH NON-CONVENTIONAL STRUCTURING

O capítulo 8 apresenta um estudo de avaliação e caracterização de organogéis de óleo de girassol alto oleico, estruturados com monoestearato de sorbitana, *hardfat* de palma e monoglicerídio para a obtenção de bases lipídicas zero *trans* e *low sat* para aplicação em alimentos.

CAPÍTULO 9 – CARACTERIZAÇÃO DE FASE LIPÍDICA E REFORMULAÇÃO DE *SPREADS* DE CHOCOLATE PARA REDUZIR ÁCIDOS GRAXOS SATURADOS

O capítulo 9 apresenta um estudo de reformulação de *spreads* de chocolate utilizando as bases lipídicas desenvolvidas nos capítulos 6, 7 e 8, visando estratégias para reformulação desta categoria de produtos, utilizando a tecnologia de organogéis.

CAPÍTULO 1

CONVENTIONAL AND UNCONVENTIONAL CRYSTALLIZATION MECHANISMS

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Conventional and unconventional crystallization mechanisms

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Abstract

Understanding the crystallization behavior of fats and oils is essential to ensure certain desirable characteristics in a given industrial application. In recent years, some advances in the structuring of lipid phases have enabled a direct influence in the food properties. The structuring mechanisms of lipid bases can be classified as either conventional or unconventional. Conventional crystallization mechanisms consist of nucleation, growth, and maturation of the crystals, thus resulting in a crystalline lattice. Co-crystallization or seeding agents and emerging technologies such as ultrasound can be used to aid in crystallization and improve the physical properties of fats and oils. Unconventional mechanisms bring organogel technology as a trend, which consists in the use of self-assembly agents to entrap the liquid oil, resulting in a structured gel network. In this chapter, the formation process of crystalline networks and gel networks will be presented in stages, highlighting the main differences related to the mechanisms of formation and stabilization of both types of networks.

Keywords: Conventional crystallization, Unconventional crystallization, Crystallization mechanisms, Crystalline networks, Gel networks, Seeding agents, Emerging technologies.

1. Introduction

Edible oils and fats are essential nutrients in the diet as they provide essential fatty acids and energy. In addition, they have important functions in the production of processed foods. These ingredients are directly related to texture, stability, aeration and nutritional features of the products [1, 2]. Some fats used in food are known by the term shortening, also known as plastic fats. Selection of the best shortening is based on formulations (product to which it is intended), functions and process conditions. The production of shortening is related to the types of fats and oils used and the way in which they crystallize and form solid networks. This is because solid crystalline fat networks are responsible for providing the expected physical macroscopic functions of the various types of shortening systems, due to the way they melt and to the hardness, consistency and complacency of the network [3].

Oils and fats are one of three food macronutrients, represented by triacylglycerol (TAG) molecules, formed by an ester bond between glycerol and three fatty acids. TAG crystals are known to show polymorphism, which is the ability of a substance to have more than one structure or crystalline form. As the physical properties of fat crystals, such as melting point and rheology, are important for food texture, polymorphic control of fat crystals is crucial in the food industry, and it is thus necessary to understand the crystallization mechanisms of TAGs.

Crystallization consists of an arrangement of the system as a result of a driven force, characterized by the total or partial restriction of movement caused by physical or chemical bonds between TAG molecules. Due to the complexity of this system, TAGs can be compacted in different ways. This crystallization has implications in the industrial processing of foods, since these depend on fat crystals in products like margarines, chocolate, spreads, baked goods etc. [4, 5].

2. Lipids

Lipids are defined as compounds soluble in organic solvents and insoluble in water. These compounds are represented by oils and fats. Chemically, vegetable oils and fats are mainly composed of TAGs and may contain small amounts of diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, waxes and unsaponifiable constituents [6].

Glycerol has three positions in its molecule (*sn*-1, *sn*-2 and *sn*-3) in which the fatty acids are esterified to form the TAG. The features of these fatty acids influence the physical properties of oils and fats: (i) in relation to unsaturation and saturation: saturated fatty acids have a higher melting point compared to unsaturated ones, since unsaturation impairs the packing of molecules; (ii) in relation to the geometric isomerism: it refers to the possible arrangements of the hydrogen atoms around the double bond, presenting the *cis* or *trans* form. *cis* bonds reduce the melting point due to folding of the molecule, impairing packing. Conversely, fatty acids in a *trans* configuration have a melting point close to saturated fatty acids, due to torsion in the molecule, making it linear; (iii) in relation to chain size: the longer the chain, the higher the melting point. Physically, oils are liquid at room temperature, while fats are solid, semi-solid and plastic at room temperature. Therefore, the composition in TAGs determines the physical and functional properties of oils and fats, affecting the structure, stability, taste, and the sensorial and visual characteristics of foods [5].

3. Crystallization mechanisms

3.1 Conventional crystallization

Crystallization, melting and recrystallization of lipids are first order transitions of interest in food systems that affect the shelf life of food and food ingredients. The crystallization properties of food components may be highly complex due to soluble-solvent interactions, mixture of miscible components and polymorphic transitions. The production of solid fats, spreads and chocolates are excellent examples of the use of first order transition behaviors of food systems to manipulate properties in processing, structure formation and application performance [7]. Understanding the development of structures and the control of the crystallization of food components has evolved over the years, but there is still much to learn.

In lipids, the conventional crystallization process is fundamentally important for to understand what happens while processing foods such as: chocolates, spreads, bakery products, margarines, ice cream, etc. How can these crystals have such a great influence on the quality of a product? We can propose ice cream as an example. When consuming an ice cream, we want it to be creamy and without crystals leaving a sandy sensation in the mouth. Another classic example is chocolate, as a cocoa butter of good quality and properly crystallized provides a shiny and smooth chocolate that melts homogeneously [8].

Macroscopic properties of lipids in foods are particularly influenced by the microstructure of fats. Therefore, it is important to consider the effects of the microstructure for subsequent analysis of macroscopic properties. The formation and growth rate of the crystals and the polymorphic transformations are important to determine the function and application of oils and fats [4].. Therefore, understanding crystallization is fundamental to obtain products with adequate function, texture and sensory qualities and that are physically stable.

The following sections will address the mechanisms that occur in vegetable fats during their crystallization. We will also understand that some techniques can induce or delay this crystallization in different ways, in order to achieve the industrially desired result.

3.1.1. Definitions and mechanisms

Before discussing the conventional crystallization mechanism of vegetable fats, we need to define some concepts that are fundamental for a good understanding of the subject. We define conventional crystallization as an arrangement of the system characterized by total or partial restriction of movement, caused by the physical or chemical bonds between the molecules of TAGs. These bonds will lead to what we call crystals, which are molecules arranged in fixed patterns. Its high degree of molecular complexity allows the same set of TAGs to be compacted into several different and relatively stable structures [9, 10].

We can say that the crystallization behavior involves four phases: nucleation (formation of a crystalline phase in the liquid phase through the organization of TAGs in a crystalline network, that is, molecules in the liquid state bind to create a stable nucleus), growth (caused by the inclusion of other TAGs in the existing crystalline network), recrystallization (structural and chemical change of the crystals during storage) and polymorphism (which controls the order of packing of TAG molecules in the crystals). Before forming the first crystals, the system, in its liquid state, needs to reach the supersaturated zone, in which there is a driving force for crystallization [11, 12]. From this point, crystallization starts.

Nucleation: It starts with the appearance of a driving force to overcome the energy barrier for crystallization. As a result, molecules in the liquid state somehow bind to create a stable nucleus. Nucleation can be classified as (a) primary nucleation (homogeneous or heterogeneous) (Figure 1) and (b) secondary nucleation. Homogeneous primary nucleation occurs with the binding of isolated molecular species, which form dimers and trimers, and subsequently continue the accumulation process until a potential nucleus can be formed. That is, it is a molecular arrangement in a crystalline network, without external aid.

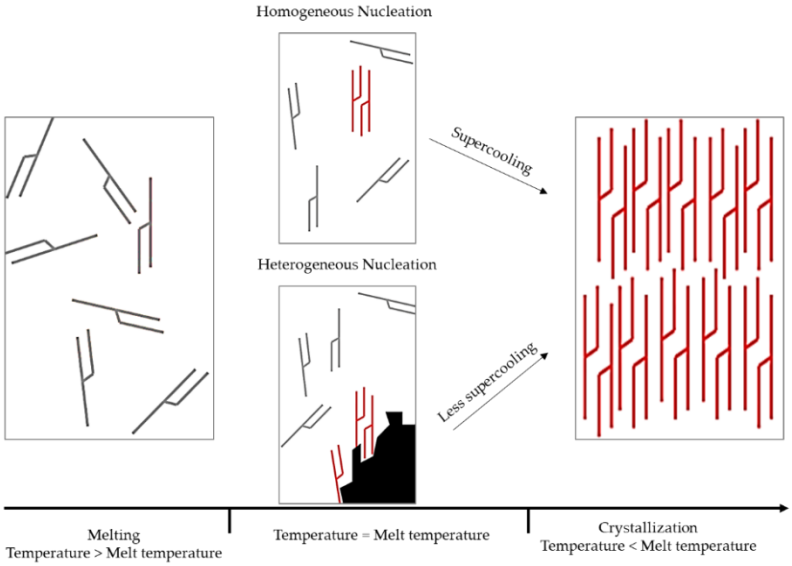

Figure 1. Nucleation mechanisms: crystal embryos formation in homogeneous nucleation and seeding or co-crystallization in heterogeneous nucleation (Adapted from [13]).

Heterogeneous primary nucleation occurs when the local arrangement of molecules to form the nucleus arises from interactions between the solid particle and the supersaturated fluid, by supplying external energy. Secondary nucleation is the formation of a new nucleus in the presence of existing crystals, which may occur if crystalline microscopic elements are separated from an already formed crystalline surface, resulting in fracture of crystals in small stable nuclei [5, 8]. Once a crystal core is formed, it starts growing due to the incorporation of other molecules of the adjacent liquid layer, which is continuously filled by the supersaturated liquid surrounding the crystal [12].

Growth: According to the theory, crystalline growth occurs through two features: 1) surface of the growth process, and 2) transport process in the face of the growing crystal [14]. The mechanism is based on how the face of the crystal incorporates growth units of the adjacent solution. According to Hartman and Perdock [15], for a three-dimensional crystalline face, the capture of growth units can be classified according to how many binding interactions form between the adsorbed growth unit and the surface of the crystal. If three orientations of surface bonds are involved, the incorporation is then of the Kinked face type; if two orientations of surface bonds are involved, the incorporation is of the Stepped face type; and if only one surface bond is involved, the incorporation is of the flat face type (Figure 2).

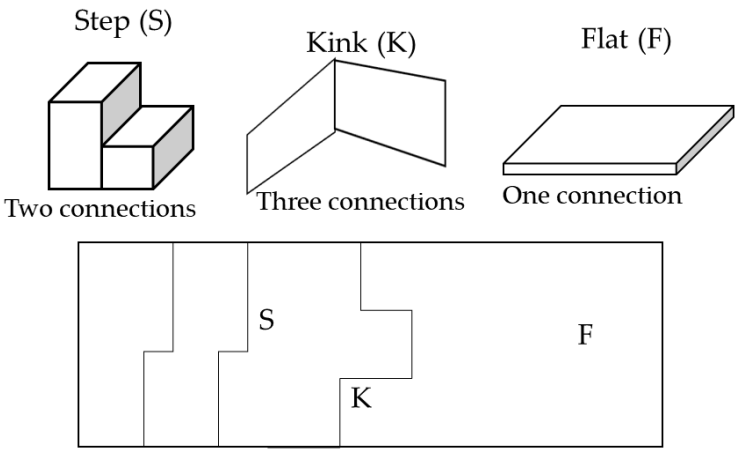

Figure 2. Classification of surface contacts according to growth theory (Adapted from [15]).

Once a crystal nucleus is formed, it starts growing due to the incorporation of other molecules of the adjacent liquid layer that is continuously filled by the supersaturated liquid that surrounds the crystal [12]. When the formed nuclei reach favorable dimensions, these elements become crystallites and their growth depends on external (supersaturation, solvents, temperature, impurities) and internal (structure, bonds, defects) factors. Therefore, the crystal growth rate can vary by several orders of magnitude. Growth occurs through the binding of molecules to a crystalline surface. While the molecules are attached to the surface of a crystal, some molecules are also deactivated. There is a continuous movement of molecules at the surface of the crystal and the result of these processes determines the growth rate, which is directly proportional to the subcooling and varies inversely to the viscosity system [5, 12]. Unless there is a kinetic constraint, growth continues until the system reaches equilibrium (driving force for crystallization of zero and maximum volume of the phase of the crystals [8].

Recrystallization: The final stage of crystallization in food occurs during storage, when crystals undergo a recrystallization step [8]. This phenomenon can be defined as a change in the size, shape, orientation and perfection of the crystals [16]. Basically, in this stage, small crystals, which are more soluble, disappear and larger crystals grow. The concern with this step is related to the changes in the products during their storage, such as fat bloom in chocolates and coatings and oil exudation in fat-rich products [12].

Polymorphism: When discussing lipid crystallization, we must relate this phenomenon to one of the most important physical properties, which is polymorphism. Polymorphism can be defined in terms of the ability to present different unit cell structures due to several molecular packages [5]. In lipids, there are three specific types of sub-cells (the smaller periodic structure that exists in the current cellular unit, which is defined as the transverse mode of packing of the aliphatic chains in the TAGs), the polymorphs: α, β' and β [4]. The α form is metastable, with hexagonal chain packing. The β' form has intermediate stability and orthorhombic perpendicular packing. Crystals in the β' form show greater function, since they are smoother, provide good aeration and creaminess properties. The β form has greater stability and triclinic parallel packing. The melting temperature increases with increasing stability (α → β' → β) due to differences in molecular packing density [5, 17]. The crystal structures also differ in chain length, which illustrates the repetitive sequence of acyl chains in a cell

unit along the axis of a long chain. The chain length structure ranges from double (DCL) to triple (TCL), fourth (QCL) or hexa (HCL). In addition, the conformation of the glycerol group also influences the phase behavior of fats. Two typical glycerol conformations, fork type and chair type, have been observed in TAG crystals. The fork-like conformation is favored by the presence of two identical fatty acids at positions 1,3 of the glycerol, forming a symmetrical TAG. On the other hand, the chair-type conformation is favored in asymmetric TAGs. Neighboring molecules of TAG crystals are stacked in layers, with the parallel hydrocarbon chains packed together and arranged more or less perpendicular to the parallel planes of the glycerol groups and to the methyl terminal groups planes. The distances between these molecules characterize the shorts spacings and the longs spacings, providing the polymorphic properties and melting point of each type of fat. The choice between the fork- or chair-like conformation is dictated by the need to accommodate fatty acids of different chain lengths and the distortions introduced by the double bonds [18, 19] (Figure 3).

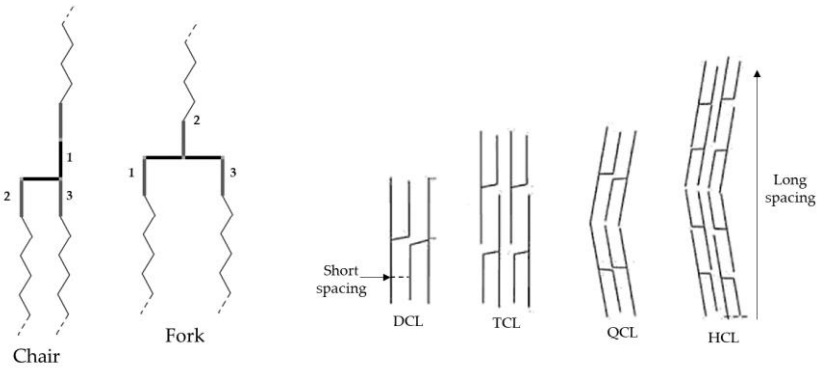

Figure 3. Conformation of glycerol groups in triacylglycerol crystals and chain length structures of triacylglycerol crystals (Adapted from [18]).

3.1.2. Crystallization Modifiers

The crystallization process can be modified using techniques that change the kinetics of the crystals, such as, for example, the use of additives and ultrasound.

Additives: Lipophilic additives are used as they influence the volume properties (consistency and texture), promoting or inhibiting crystallization. These compounds provide a modeling effect, i.e., seeding nuclei. For example, a higher melting point additive with chemical and structural similarities to the lipid is used as a seeding nucleus for heterogeneous crystallization [20]. An example of additives that may promote or inhibit crystallization are minor lipids, such as diacylglycerols, monoacylglycerols, free fatty acids, phospholipids and sterols. These lipids can interact with TAGs in the growth stage, causing a structural competitive effect or permanent incorporation into the crystalline structure, affecting the crystallization rate, polymorphic forms and microstructure of the crystals, positively or negatively [21].

Another technique that is being increasingly used is seeding. It consists of the addition of solid material with crystallization nucleating agent properties. The incorporation of crystallization seeds into liquid fats can promote two effects associated with the control of crystallization by the availability of numerous additional nuclei and/or surfaces for crystal growth. In addition, it may promote specific polymorphic forms [21].

The addition of emulsifiers changes the supersaturation characteristics of the solution and consequently its solubility, and thus the incorporation of growth units on the surface will also change. At low supersaturations, growth occurs by the incorporation

of the Flat face type, and, in general, the free energy (ΔG) decreases according to the sequence Kinked < Stepped < Flat [21]. Thus, the mechanisms of co-crystallization and seeding occur, leading to the template effect of the emulsifiers (Figure 4).

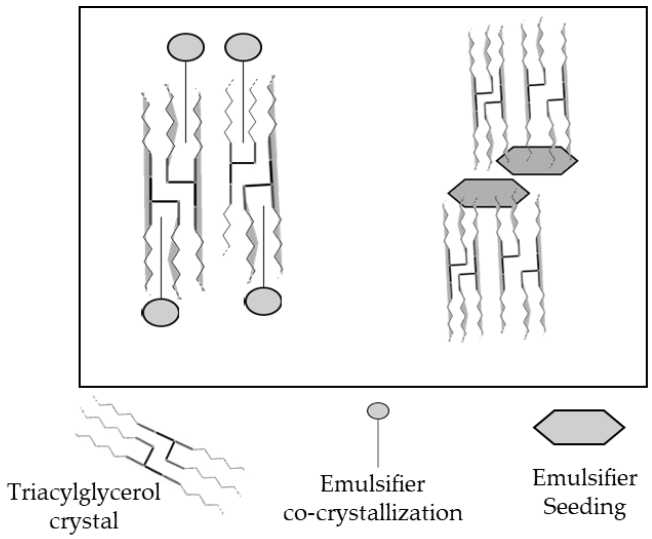

Figure 4. Template effect of emulsifiers: co-crystallization and seeding.

Ultrasound: This technique has been noted as an excellent alternative to promote crystallization, since the ultrasonic energy is sufficient to promote nucleation and growth by increasing the transfer of heat and mass. Through manipulation of nucleation and growth, ultrasounds can potentially provide improved control for the assembly of crystal structures in foods to control texture or improve separation [8]. It is believed that these ultrasonic effects are superior to conventional agitation to control and stimulate the reaction rate, supersaturation level, nucleation and crystal growth [22].

Care should be taken in relation to the intensity of ultrasound used, since the crystalline structure is highly dependent on the intensity of the ultrasound. For example, a palm oil where the ultrasound was applied at an intensity of 30 dB had little effect on kinetics but prevented the formation of large spherulitic crystals. When this intensity was increased to 35 dB, it produced smaller, more uniform crystals that tend to clump and fall to the bottom of the cell. A further increase in intensity to 40 dB, below the cavitational threshold, produced a uniform product of very small crystals, suggesting that there are numerous nucleation sites that produced crystals simultaneously [23].

The effects caused by the ultrasound include: (i) faster primary nucleation, which is quite uniform through the sonicated volume; (ii) relatively easy nucleation in materials that are generally otherwise difficult to nucleate; (iii) start of the secondary nucleation; and (iv) production of smaller, purer crystals of more uniform size. In addition, ultrasound can reduce clumping of crystals. The shock wave, which is caused by cavitation, decreases the contact between the crystals, preventing clumping, by controlling the population of local nuclei. The induction time is defined as the time elapsed between onset of supersaturation and appearance of crystals and decreases as supersaturation increases. This induction time is drastically reduced by the presence of sonication. This effect is more significant at low absolute supersaturations [24].

3.2 Unconventional crystallization

Studies with organogels led to the development of many applications in the food area, such as chocolate filling, margarines, baked products, such as biscuits and cookies,

pastries, spreads, sausages, ice cream, yogurt, unprocessed and processed cheese, mayonnaise and sauces, among other products [25–28]. Considering this, it is crucial to understand how unconventional crystallization occurs.

Many organogels are more desirable to consumers since they have a better nutritional profile than conventional fats. Therefore, organogel technology seems to be a potential solution for the reduction of saturated fatty acids and possibly the elimination of trans fatty acids from processed foods [26, 29, 30].

This technology is feasible in comparison to the technologies currently used, since it does not cause any chemical changes in the structure of fatty acids and TAGs and maintains the nutritional features of the oil; in particular, it maintains unsaturated fatty acids contents and natural regiospecific distribution without increasing the AGS content [31].

3.2.1. Definitions and mechanisms

Organogels are a class of materials that can hold large volumes of organic liquids in self-assembled networks and have many practical applications in the pharmaceutical, chemical and food industries regarding lipid technology [32]. Organogels are formed by structurants of low molecular weight and some organic solvents that are supported, through a three-dimensional network, forming the gel. Since organogels are biodegradable, they can be used in drugs as protein carriers and vaccines, for example [33–35].

The formation of these structures through supramolecular assembly is of great interest, since they can form semisolid phases that are produced at low cost [32]. Several researchers have focused their studies on the properties of structurants to form organogels [33–35].

In unconventional structuring, lipid systems composed of unsaturated TAGs, such as liquid or semi-solid vegetable oils, can be structured as gels, forming continuous networks of small molecules that assemble in liquid crystals, micelles or fibrillar networks formed from aggregates of micelles, developing inverse bilayer structures in the form of rods [39].

Organogels can be structured forming a fibrous 3D network, where the solvent is trapped in the structuring matrix, avoiding the flow of solvent. The network is stabilized by weak interactions between the chains, such as hydrogen bonds, van der Waals forces and π staking [40–43]. Although it is known that organogels are formed through weak intermolecular interactions between the structurant molecules, which generates three-dimensional networks [44], there is still lack of fundamental understanding of the type of interactions that are required [32].

The physically driven process of structuring organogels depends on many factors, such as structural adjustment of structurant molecules, solvent effects, concentration, temperature, use of ultrasounds and shear. When these factors change, the structuring properties are affected due to the precise balance between the interaction of the structurant molecules, the interaction between the solvent and the structurant, and the applied external stimulus [45, 46].

The structurants are insoluble in almost all solvents at room temperature and the solvent-structurant and structurant-structurant interactions are the main force for the formation of organogels. However, they dissolve after heating and jellify after cooling; thus, the gelation process is an equilibrium between dissolution and precipitate in the solvents [47].

Organogel networks may range from the assembly of surfactants into solution by physical interactions (e.g., micellization, lyotropism and crystallization) [48], also known as molecular organogels, to the formation of flexible polymer networks (e.g., swelling) , known as polymer organogels [49]. The molecular organogels known today are at the interface between complex fluids [50] and solids, and, regardless of the nature of the structure, are composed of thermoreversible semisolid materials [32].

3.2.2. Structuring Modifiers

The group of molecular organogels can be subdivided according to the mode of self-organization of the structurant in liquid crystals [51], platelet crystals, elliptic networks [52, 53] and inverted micelles [54, 55].

Several solvent parameters have now been used to correlate with gelation ability [56], such as protic, dipolar aprotic and apolar aprotic (low polarity), which were too broad to quantify solvent effects [57]. More specifically, solvent parameters are divided into three categories: physical properties, solvatochromic and thermodynamic properties, including dielectric constant, Reichardt ET-30 parameter [58], Kamlet-Taft parameters [59], Hildebrand solubility parameter [60] and Hansen's solubility parameters [61, 62].

The structures of the organogel molecules significantly influence the resulting gelation properties. The length of the alkali chain, the position of the chiral units, the substitution groups, the peptide sequences, in addition to the arrangement or the number of the hydrogen bonds, may influence the properties of organogels [45].

The structures can be formed from amphiphilic compounds that can self-assemble in different microstructures, such as micellar and bilayer phases, which may accumulate in different lyotropic mesophases of larger scales (hexagonal, cubic or lamellar liquid crystalline phases) at high concentrations [63, 64]. The formation of longer structures provides viscoelastic behavior or a gel-like solution. These viscoelastic materials, created through the assembly of structurants, are described in the literature as supramolecular organogels [65]. The formation mechanism of these structures is based on the arrangement of monomeric units that bind through non-covalent bonds, such as van der Waals, hydrogen bonds, electrostatic interactions and π-π or τ-stacking [65, 66].

Two different types of packing models were discovered in non-polar and polar solvents. In non-polar solvents, an interdigitated stacking model was preferred in the self-organized 3D micromorphology of the gels, due to inverse strong hydrogen bonds and weak π-π stacking interactions. Conversely, in polar solvents, the structurant is automatically assembled in a 3D nanostructure through a stacking model due to strong π-π stacking interactions and weak hydrogen bonds [67].

In general, there are a limited number of biocompatible components known to structure lipids through the assembly of molecules [46, 68, 69].

The extensive applications of organogels are of great importance to design and study new structurants and to characterize and determine the practical applications for organogels. It is known that some organogels with excellent mechanical and optical properties are more useful for practical applications [45].

Current knowledge on assembly behavior and subsequent gel formation and the effect of salts was applied to induce the formation of networks in hydrophobic environments using an unsaturated fatty acid, oleic acid and sodium acetate, in sunflower oil. Oleic acid alone did not provide gelation in the oil, but the addition of sodium oleate induced gel formation. In mixtures containing oleic acid, reverse micellar structures are present.

The participation of the sodium oleate in the original spherical micelles of oleic acid change the geometry and assembly behavior of the structurant. At higher concentrations of sodium oleate, lamellar crystal structures are formed. The assembly is theoretically driven largely by hydrophilic interactions between the hydrophilic head groups, as the strength of the interactions increased by the addition of small amounts of water. For water concentrations of more than 2%, the assembly was inhibited [32].

Vegetable waxes provide an ideal feature for organogels, which is more similar to the features provided by saturated and trans fats but show stability problems over prolonged periods. There is currently a clear lack of knowledge regarding the identification of ideal proportions of ceramides, fatty acids, cholesterols, phytosterols, in terms of oil structuring, crystal morphology and consequent suitability for applications in foods. Although carbohydrate-based gelatins were extensively studied, there are very few studies in the field of organogels [26].

4. Emerging Crystallization Technologies

The success of organogels in recent years in food applications continues to stimulate the interest of researchers in this area and provides a broader view [26].

A gel system is prepared by holding the solvent in the gelator matrix, stimulated by pH, heat, light, magnetic field or ultrasound [47]. Supramolecular gels that have their structuring initiated by physical (including sonication) and mechanical (mechanical force) stimuli exhibit noncovalent interactions between the structurant molecules and show dynamic and reversible properties controlled by the stimuli, while structurants cause instant and *in situ* gelatinization in organic solvents or water with different modes and structuring results, but can lead to dynamic changes in the microscopic morphology, optical properties, etc. [45].

During the solvent-organogel transition process, changes in the conformation of the structure of the molecule can occur in thixotropic or ultrasonic organogels, but it is difficult to study these changes with the existing techniques and without crystallization data [45].

A novel low molecular weight, photoresistant, organogel, based on an azobenzene derivative and which can achieve the reversibly gel-solvent transition by the heat/cold process or visible/UV light irradiation, was designed and successfully synthesized. The structurant has a good gelation ability in organic solvents ranging from non-polar to polar solvents due to hydrogen bonds and π-π stacking [45].

The reversible control of the properties of the organogel through the application of heat and mechanical stimuli is often followed by transformations in the phase, morphological structure, rheological and spectroscopic properties, which makes these organogels suitable to be used in the field of drug release and adaptive materials, among other applications [45].

Conclusion

This study carried out a comprehensive review on the possibilities of modification of the crystallization of lipid phases, to subsidize the knowledge of the crystallographic, microstructural and kinetic phenomena involved in the processes of structuring of oils and fats. Different emulsifiers, used in low concentrations, besides the application of ultrasound technology, are proven effective in the processes of lipid modification and represent a highly feasible option, in economic and process terms, to modulate the crystallization properties of industrial oils and fats.

In addition, the use of organogels in food applications as substitutes for trans and saturated fatty acids is highly feasible since structured vegetable oils are generally used to replace fats. Compared with conventional technological processes for the production of technical fats for food applications, the production of organogels is technologically simple, economically accessible and inexpensive. Several oils with majority composition in unsaturated fatty acids can be immobilized. Structuring agents are used in small proportions, being commercially available and safe for consumption.

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Conflict of Interest

The authors state that there is no conflict of interest.

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CAPÍTULO 2

POTENTIAL APPLICATION OF LIPID ORGANOGELS FOR FOOD INDUSTRY

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Potential Application of Lipid Organogels for Food Industry

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ABSTRACT: Controversial issues regarding the role of *trans* fatty acids in food have led to progressive changes in the legislation of several countries to include more information for consumers. In response, the industries decided to gradually replace *trans* fat in various products with the development of fatty bases of equivalent functionality and economic viability to partially hydrogenated fats, causing, however, a substantial increase in the content of saturated fatty acids in foods. Today, the lipid science aims to define alternatives to a problem that is widely discussed by health organizations worldwide: limit the saturated fat content in food available to the population. In this context, organogels have been indicated as a viable alternative to obtain semi-solid fats with reduced content of saturated fatty acids and compatible properties for food application. The objective of this review was to present the studies that address the lipid organogels as an alternative for food application.

Keywords: organogels, structuring agents, saturated fatty acids, lipid bases.

List of abbreviations

EU - European Union

FDA - Food and Drug Administration

FHCO - Hard fat of cottonseed oil

FHCrO - Hard fat of crambe oil

FHPKO - Hard fat of palm kernel oil

FHPO - Hard fat of palm oil

FHSO - Hard fat of soybean oil

GRAS - Generally Recognized as Safe

SFAs - Saturated fatty acids

TAG - Triacylglycerol

TFAs - Trans fatty acids

1. Lipids

Natural oils and fats are basically made up of triacylglycerol (TAG) molecules from a non-random distribution of fatty acids in the glycerol molecule. Therefore, TAG molecules are constituted of three molecules of fatty acids esterified to the molecule of glycerol.

The number of esterified fatty acids classifies these lipid groups as monoacylglycerols, diacylglycerols or TAGs (O'Brien, 2008). Monoacylglycerols and diacylglycerols may be present in oils and fats in smaller amounts, along with phospholipids, sterols, terpenes, fatty alcohols, fat-soluble vitamins, and other substances. These minor lipids are found in human body as components of cell membranes and in varying amounts in food. The main sources of dietary lipids are meats, dairy products, fishes, oils and fats, which can be commercially available in the form of kitchen oil, frying oils and fats, butter, margarine, vegetable creams and special fats (shortenings), incorporated into the manufacture of processed products such as bread, cake, biscuit, chocolate, ice cream and mayonnaise (Garcia, 2015).

For food technology, lipids considered as oils (liquid at room temperature) or fats (solid at room temperature), whose main difference is the melting point. This physical property is a reflection of their particular composition in fatty acids and their position as esterified to the molecule of glycerol (Oetterer, D'Arce, & Spoto, 2006).

The fatty acids that naturally occur in plants have an even number of carbon atoms in an unbranched chain between 12 and 24 carbons (Scrimgeour, 2005). Each fatty acid can occupy different positions in the molecule (*sn-1*, *sn-2* or *sn-3*), allowing a great diversity of combinations for the TAG structure (O'Brien, 2008).

Saturated fatty acids (SFAs) contain only single bonds between carbon atoms (Sanderson & Nichols, 2002). SFAs with more than 24 carbon atoms rarely occur in edible vegetable oils, but are found in the waxes, esterified by a monovalent primary

alcohol (Oetterer et al., 2006). Unsaturated fatty acids may have *cis* or *trans* double bonds, which result in nonlinear and linear molecules, respectively. These factors directly influence the physicochemical properties of lipids, such as the melting point, which is directly related to the number of unsaturation and geometric isomerism (O'Brien, 2008).

Trans fatty acids (TFAs) are geometric and positional isomers of natural unsaturated fatty acids. In this configuration, two hydrogen atoms attached to the carbon atoms that form the double bond are located on opposite sides of the carbon chain, creating a linear and more rigid molecule. Due to their structural characteristics, *trans* fatty acids have a higher melting point when compared with their corresponding *cis* isomer (Sanderson & Nichols, 2002).

Lipids are important components of the diet, in both nutritional and technological aspects, as they ensure physical, chemical, sensory and nutritional characteristics of foods (Garcia, 2015), since they provide consistency and specific melting characteristics to the products containing them, and act as a heat transfer means, such as in conventional frying processes and carriers of fat-soluble vitamins, essential fatty acids, steroid hormone precursors, taste and aroma. In addition, lipids affect the structure, stability, storage quality, sensory and visual characteristics of foods (O'Brien, 2008).

1.1. Lipids: nutritional and regulatory aspects

Today, consumers have become increasingly aware of the relationship between diet and health. Then, the demand for a balanced diet and functional foods that promote specific benefits has increased more and more. Healthy food products can be characterized by several attributes: low to moderate content of sodium, sugar and fat, and significantly reduced calorie density when compared to their conventional products (Palzer, 2009).

Saturated fat is the main dietary cause of elevated plasma cholesterol, whose reduction in diet is globally supported to reduce the risk of cardiovascular disease (Wassell, Bonwick, Smith, Almiron-Roig, & Young, 2010). TFAs are included among dietary lipids that act as risk factors for coronary artery disease, modulating the synthesis of cholesterol and its fractions and acting on eicosanoids. Several studies

have shown a direct relationship between *trans* isomers and increased risk of vascular diseases (Mensink, 2005).

In view of the harmful effects of SFAs and TFAs on health, actions have been taken to encourage industries to increase food health by changing conventional raw materials (Santos et al., 2013).

Since 2006, the Food and Drug Administration (FDA) has required foods containing *trans* fats in their formulation to state their stated levels in the nutritional information of the products. In 2015, it recommended through a resolution that partially hydrogenated TFA-rich fats should be removed from processed food products in up to three years, as they are not considered safe for food. Therefore, TFAs were also excluded from the Generally Recognized as Safe (GRAS) classification for human consumption (FDA, 2016).

In European Union (EU), each country has the autonomy to set limits and recommendations, since there is no standard legislation for the EU. However, the European Food Safety Authority warns about the increased risk of cardiovascular disease for consumption exceeding 2% of the total energy value of TFA (Tobergte & Curtis, 2013).

Based on these considerations, the knowledge field of lipid technology aims to develop alternatives to reduce the amount of fats with SFA and *trans* in foods, and reduce the calorie intake associated with the lipid content in processed products.

2. Conventional lipid modification processes

Vegetable oils that are rich in unsaturated fatty acids do not have the required physical properties for application, limiting their use to their unaltered forms, in terms of consistency and oxidative stability, due to their particular composition in fatty acids and TAGs. In conventional lipid modification processes, the basic structure of the oils and fats can be redesigned depending on the desired plasticity profile and the intrinsic characteristics of raw materials, using techniques such as hydrogenation, interesterification or fractionation (O'Brien, 2008).

Partial hydrogenation of vegetable oils has been used for decades to improve the plasticity and oxidative stability of industrial oils and fats. Then, partially

hydrogenated vegetable oils were commonly used in the production of margarines, confectionery and toppings. However, partial hydrogenation results in the production of TFA, which has a harmful effect on cell membrane integrity and the production of biologically active metabolites derived from essential fatty acids (Mensink, 2005; Stender & Dyerberg, 2004; Wassell et al., 2010).

The harmful effects of TFA on the lipid profile and, consequently, on the increased risk of cardiovascular diseases are well known (Zevenbergen et al., 2009). In this context, historical controversial issues regarding the role of TFAs in food have led to progressive changes in the legislation of several countries to include more information for consumers.

In response, the industries decided to gradually replace *trans* fat in various products with the development of fatty bases of equivalent functionality and economic viability to partially hydrogenated fats (Ribeiro, Grimaldi, Gioielli, & Gonçalves, 2009; Ribeiro, Leite, De Moura, Grimaldi, & Gonçalves, 2007). The demand for TFA-free fat also encouraged the development of studies and processes of oil and fat modification, especially promoting the techniques of fractionation and interesterification (Garcia, 2015).

In this scenario, interesterification proved to be a useful technique to change the melting profile and consistency of lipid mixtures. The process consists in rearranging the distribution of fatty acids in the TAGs without alterations to the profile of fatty acids. The process can be conducted either chemically or enzymatically (Pokorný & Schmidt, 2011).

Chemical interesterification uses catalysts such as sodium methoxide and temperatures of 90 to 110°C to promote a random distribution of fatty acids among the three glycerol positions (Teles Dos Santos, Gerbaud, & Le Roux, 2014). The main question about non-specific interesterification processes is the development of isomers with SFA at the *sn-2* glycerol position, due to the non-specificity of the reaction; for this reason several studies have been conducted to analyze a possible negative nutritional effect of fat obtained by this method (Aliciane, Domingues, Paula, Ribeiro, & Chiu, 2015; Christophe, 2005; Karupaiah & Sundram, 2007). Increasing the amount of SFA in the central position of the TAG molecule, such as incorporating palmitic acid at *sn-*

2, allows a greater absorption of this type of fatty acid by the body, leading to a greater atherogenic potential, already recognized in laboratory animals (Hunter, 2001).

The enzymatic interesterification process uses microbial lipases as catalysts of the reaction. This process reduces energy consumption due to the mild conditions of the reaction and the continuous production from the use of immobilized enzymes. Specific lipases can also be used at *sn-1* and *sn-3*, with unaltered fatty acids at the central *sn-2* position. Despite the advantages of enzymatic interesterification (milder conditions of the reaction and regiospecificity), chemical interesterification is a low-cost method because of the catalyst used in the process, it is much faster and promotes easy change of scale (Teles Dos Santos et al., 2014). Lipase-catalyzed interesterification processes are applied mainly to high value-added products, such as cocoa butter substitute and equivalent products (Senanayake & Shahidi, 2005).

The use of blends, that is, mixtures of fats of different physical properties and fractionation are also additional alternatives to obtain fatty bases of proper physical properties and plasticity for use in several products, although with limited potential due to the chemical composition of raw materials and phase incompatibility issues (Reyes-Hernández, Dibildox-Alvarado, Charó-Alonso, & Toro-Vazquez, 2007).

However, all these technological alternatives, used together or alone, do not reduce the content of SFA in lipid formulations for industrial application. In most cases, achieving properties of crystallization, thermal resistance and consistency that are typical of technical fats also requires a significant increase in the proportion of SFA in lipid compositions, which has been a major challenge for the field of oils and fats (Menaa, Menaa, Trétton, & Menaa, 2013).

In this sense, the reduction of saturated fat content in processed foods is an immediate issue for the food industry. A satisfactory performance of food formulations with lower SFA content depends on important aspects that determine their technological viability, especially including sensory acceptance and stability during and after processing. In addition, physical and functional characteristics such as texture, plasticity, spreadability, cream formation and aeration properties should be taken into account in the development of new formulations for foods with reduced saturated fat (Chung, Degner, & McClements, 2014).

3. Lipid crystallization mechanism

The structuring of lipid phases determines important food properties: (i) consistency and plasticity of fat-rich products during the production and storage stages; (ii) sensory properties, such as a melting sensation in the mouth; (iii) physical stability regarding the formation or sedimentation of crystals, oil exudation and coalescence of particles and emulsions; (iv) visual appearance (Foubert, Dewettinck, Van de Walle, Dijkstra, & Quinn, 2007).

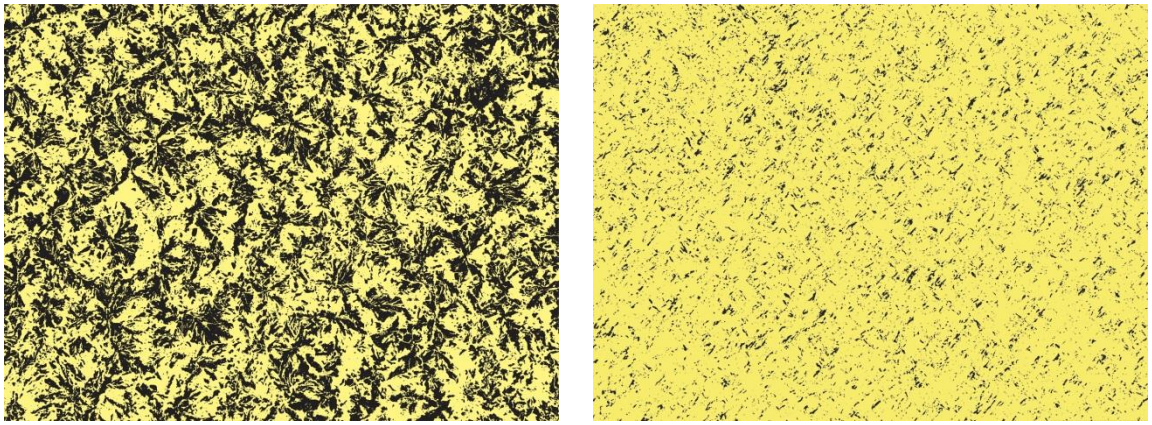
The size and shape of the structuring agents and their interactions will determine the structure of the final product, and consequently its physical properties. The structuring process of triacylglycerol matrices can be classified according to their mechanisms, as conventional or non-conventional (Pernetti, Vanmalssen, Kalnin, & Floter, 2007).

3.1. Conventional structuring

The macroscopic properties of lipids in foods are particularly influenced by the microstructure of fats. Therefore, it is important to consider the effects of the microstructure for subsequent analysis of the macroscopic properties. The lipid crystallization behavior has important implications, mainly in the industrial processing of products whose physical characteristics depend largely on fat crystals, such as chocolates, margarines and lipid-based products. Crystal formation growth rates and the polymorphic transformations are important as they help determine the functionality and application of oils and fats (Sato, 2001).

Plastic fats have different levels and forms of structure, which influence the macroscopic properties and ensure the typical characteristics of the lipid material (Figure 1A). When a complex mixture of TAGs is submitted to cooling, the limited solubility of molecules of higher melting point leads to nucleation events, generating small crystals that grow and interact with each other through non-covalent forces, developing a three-dimensional continuous crystal lattice. After completion of crystallization, the crystals aggregate and form agglomerates, which in turn constitute larger structures, from weak bonds, leading to a final macroscopic network, characterizing the process of conventional structuring of lipid systems based on TAGs (Tang & Marangoni, 2007). The structure of fats originated after a crystalline network

of TAGs allows different configurations that change the rheological and thermal behavior of the material (Marangoni & Narine, 2002).



A **B**

Figure 1. Conventional (A) and non-conventional (B) lipid crystallization mechanism.

Polymorphism can be defined as the ability to present different cell structures resulting from various molecular arrangements. Long-chain compounds, such as fatty acids and their esters, may exist in differentiated crystalline forms (Lawler & Dimick, 2002). In lipids, three specific types of subcells predominate, which refer to polymorphs α, β' and β, according to the current polymorphic nomenclature. The α form is metastable, of hexagonal chain arrangement. The β' form presents intermediate stability and perpendicular orthorhombic arrangement, while the β form presents greater stability and parallel triclinic arrangement. The melting temperature increases as stability grows, as a result of the differences in molecular arrangement density (Martini, Awad, & Marangoni, 2006).

The crystal structure of fats is important for the formulation of lipid-based products in general, since each crystal presents unique properties of plasticity, texture, solubility and aeration. Fats with crystals in the β' form present greater functionality, as they are softer, and ensure good aeration and creaminess properties. Then, the β' form is the polymorph of interest for the production of foods of emulsified lipid phase, for example margarines and icing (O'Brien, 2008).

3.2. Non-conventional structuring: the organogel technology

In recent years, new raw materials and technological processes have been studied as potential alternatives for the structuring of lipid-based materials aiming to reduce the content of SFA and TFA in processed foods (Garcia, Gandra, & Barrera-Arellano, 2013; Marangoni & Garti, 2011). For this reason, the recent scientific literature describes a potential alternative: the use of several components of a structuring action, of natural or synthetic origin and added to lipid matrices, which can act at the molecular or submicron level. These molecular agents would act on the structuring process of lipid systems as a whole, modulating properties such as thermal behavior, polymorphic stability and microstructure. Similarly, the effects of these modifiers at the macroscopic level, such as visual appearance, rheology and consistency have also been studied (Rogers et al., 2014; Smith, Bhaggan, Talbot, & Van Malssen, 2011).

In non-conventional structuring, lipid systems made up of unsaturated TAGs, such as liquid or semi-solid vegetable oils, can be structured as gels, creating continuous networks of small molecules that bond in liquid crystals, micelles or fibrillar networks formed from aggregates of micelles, developing inverse bilayer structures in the form of sticks (Pernetti et al., 2007) (Figure 1B).

This particular type of structuring are called organogels, which are viscoelastic materials made up of structuring agents and an apolar liquid phase (organic compound), which distinguishes it from other gels that are basically made up of water-soluble compounds. They are semi-solid systems, where an oil phase is trapped by a self-sustaining three-dimensional network of the structuring agent (Dassanayake, Kodali, Ueno, & Sato, 2009; Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Rogers, Smith, Wright, & Marangoni, 2007).

The continuous phase of these organogels is lipid-based, presenting the physical characteristics of hydrogels, which have an aqueous continuous phase (Marangoni & Garti, 2011). The most frequently used structural agents include fatty acids, fatty alcohols, mixtures of fatty acids and fatty alcohols, mixtures of phytosterols/orizanols, sorbitan monostearate, mixtures of lecithin, sorbitan tristearate and waxes (Rogers, Wright, & Marangoni, 2009). The mixture of ingredients may have a synergistic effect on the structuring potential of oils when compared to the use of pure materials (Pernetti et al., 2007).

This technology is feasible in comparison to conventional lipid modification technologies, as it does not cause any chemical changes in the structure of fatty acids and TAGs with unaltered nutritional characteristics of the oil, especially the contents of unsaturated fatty acids and natural regiospecific distribution, without increasing the content of SFA (Sundram, Karupaiah, & Hayes, 2007).

The use of organogels in food products is a very attractive alternative, as these compounds can ensure characteristics such as consistency and plasticity with absence of TFA, and a significant reduction of the SFA content, resulting in products of strong nutritional and technological appeal (Rogers, Wrightb, & Marangoni, 2009).

Organogels can be obtained using different methods, with varied gel properties. Organogel formation occurs through crystalline particles, crystalline and polymeric fibers, among others. These methods vary according to the structuring agent used and the process conditions to which the raw materials are submitted (Co & Marangoni, 2012).

The organogels can present different structures, made up of the most diverse organic compounds. The two most important of these structures are the dispersion of solids in a liquid phase (small inert particles, crystallized solids, drops) or specific mechanisms such as self-support (commonly observed in low molecular weight structuring agents). Both of them create three-dimensional networks that can trap a liquid phase. The size and shape of this structure and its interactions are directly related to the physical characteristics and properties of the structuring agents (Pernetti et al., 2007). These structuring agents allow the combination of two distinct phases in a quasi-homogeneous state. The specific components, used alone or together, as well as their interactions, determine the structure of the final product, and consequently its consistency and plasticity properties (Cerdeira, Martini, Candal, & Herrera, 2006).

According to the mechanism related to the structuring of TAGs, the structuring agents can be classified in two groups – based on the crystalline particles and systems of self-assembly (or self-association). The crystalline particles are associated with the classical phenomenon of nucleation, growth and stabilization of the crystal lattice; while in the self-assembly systems, the structuring is promoted by a mechanism of molecular self-organization of its components in the organic phase. Van der Waals covalent electrostatic interactions and hydrogen bonds are examples of forces of intermolecular

interaction of the structuring agents that form the three-dimensional gel network. Components of similar molecular and chemical structures often present positive interactions regarding the phenomena of dispersed particles and self-association (Dassanayake, Kodali, & Ueno, 2011; Siraj et al., 2015).

In this context, different materials have been evaluated, such as trisaturated TAGs, partial acylglycerols (free fatty acids, monoacylglycerols and diacylglycerols), waxes, fatty alcohols, phospholipids, phytosterols, and different classes of emulsifiers. However, the effects of these modifiers on various lipid systems, as well as their interactions, have not been fully clarified in the literature. In addition, these modifiers may present differentiated effects according to the oil or fat to which they are added and according to the concentration at which they are incorporated into the lipid systems (Bot et al., 2011; Ribeiro et al., 2015; Smith et al., 2011). Hydroxy fatty acids, fatty acids, fatty alcohols, mixtures of fatty acids and alcohols, mixtures of phytosterols/orizanols, sorbitan monostearate, waxes, and mixtures of lecithin and sorbitan tristearate present great potential for food application (Hughes et al., 2009).

4. Structuring agents

Structuring agents are recognized as high and low molar mass compounds, considered low molecular weight molecules of less than 3000Da. They can be used to trap liquid oils through the formation of self-sustained three-dimensional crystal lattice that provides a structure (Rogers et al., 2009).

To guarantee technological effectiveness, the lipid structure should present a chemical composition and physical characteristics that allow it to be compatible with the material to be structured, so that it will help enhance the effects on the crystallization pattern of the formed lipid systems (Oliveira, Ribeiro, & Kieckbusch, 2015).

A structuring agent is efficient when used in low concentrations to achieve the desired result, since there will be an increase in the cost of the final product. In addition, the effects caused by these additives at high concentrations in the body are not fully known (Co & Marangoni, 2012). An alternative for the replacement of SFA and TFA in food involves the combination of several strategies, such as trapping gelled oil within

emulsions and the selection of proper structuring agents, in order to achieve the desired functional properties (Wang, Gravelle, Blake, & Marangoni, 2016a).

Elliger, Guadagni and Dunlap (1972) were the first authors to mention the potential oil structuring with 12-hydroxystearic acid in a study on peanut butter thickening. Considering a retrospective related to the development of organogels, a variety of publications and studies describe different types of structuring agents with applications in the pharmaceutical, cosmetic, petrochemical and food industries (Hughes et al., 2009; Kumar & Katare, 2005; Pernetti et al., 2007; Terech & Weiss, 1997). The literature also describes organogels structured with alkanes (Abdallah & Weiss, 2000); 12-hydroxystearic acid (Rogers, 2009); ricinelaidic acid (Wright & Marangoni, 2006); fatty alcohols (Daniel & Rajasekharan, 2003; Gandolfo, Bot, & Flöter, 2004); plant sterols (Bot & Agterof, 2006); lecithins (Scartazzini & Luisi, 1988); mixtures of lecithins (Murdan, Gregoriadis, & Florence, 1999), mono- and diacylglycerols (Da Pieve, Calligaris, Co, Nicoli, & Marangoni, 2010; Ojijo et al., 2004); waxes and was esters (Dassanayake et al., 2009; Toro-Vazquez et al., 2007); mixtures of phytosterols/orizanols, sorbitan monostearate and tristearate (Hughes et al., 2009); polymers (Gravelle, Barbut, & Marangoni, 2012); proteins (Mezzenga, 2011); and ceramides (Rogers, Wright, & Marangoni, 2011).

The combined use of structuring agents may have a synergistic effect on the structuring potential of oils when compared to the use of isolated materials, an approach that has been proposed in recent studies on structured lipid systems (Pernetti et al., 2007; Siraj et al., 2015).

5. Materials with potential for food organogel composition

In the context of Food Science, the main interest associated with the development and characterization of organogels is in the structuring of edible oils. In this approach, the structuring agents should gelate unsaturated oils at cooling and ambient temperatures, allowing applications in processed foods. The use of lipid bases and various structuring agents, and their combinations, for the composition of organogels should consider the following criteria: i) use of lipid bases with exceptional characteristics of functionality, stability and availability among commercially available oils and fats; ii) use of structuring agents from renewable materials, included in the

GRAS category for food application; iii) formulations of lipid systems with chemical composition characteristics and crystallization properties that are compatible with the application of lipid-based foods, such as continuous or emulsified phases (Pernetti et al., 2007; Rogers et al., 2014; Siraj et al., 2015).

5.1. Vegetable oils

In the development of organogels for food application, the organic fluid used in structuring is an oil or fat (Pernetti et al., 2007). Then, potential raw materials are soybean oil, high oleic sunflower oil, and palm oil, due to their properties of stability, chemical composition, economic importance, availability and cost.

Soybean oil stands out for its great importance in the global consumption of vegetable oils because of its nutritional qualities, uninterrupted supply, expressive economic value and high functionality, representing a raw material of particular interest for the production of special fats. It is mainly comprised of polyunsaturated fatty acids, with significant concentrations of oleic acids (23.5%), linoleic acids (54.6%), and α-linolenic acids (8.3%), making it particularly interesting for lipid formulations of reduced saturated fatty acids content (O'Brien, 2008).

High oleic sunflower oil was developed by Russian researchers using chemical mutagenesis and selective sunflower (*Helianthus annus*) crosses aiming to obtain a seed variety that is stable to the climate conditions and with a high content of oleic acid. The typical composition of high oleic sunflower oil is 3-5% palmitic acid, 2-6% stearic acid, 75-88% oleic acid, and less than 1% linolenic acid, which ensures oxidative stability 10 times higher than soybean, canola and regular sunflower oils. In addition, the regiospecific distribution of high oleic sunflower oil is differentiated, with a high proportion of linoleic acid at *sn-2*, which also justifies its high stability to the oxidation process (Grompone, 2005). High oleic sunflower oil, considered a premium raw material, is generally used in food applications that require the use of liquid oil with exceptional oxidative stability. It has a neutral flavor and aroma, due to its high potential for application in foods, cosmetics and pharmaceutical products (Gunstone, 2005). These attributes make the high oleic sunflower oil a high quality liquid lipid source to obtain organogels for food application.

The fast expansion of world production of palm oil seen in the last three decades attracted the attention of the oil and fat industry. Today, the production of palm oil is the vegetable oil cultivation of highest productivity and lowest associated production cost. The wide range of processed or semi-processed products for food application includes their different fractions, known as palm olein and stearin. About 50% of the fatty acids present in palm oil are saturated and about 50% are unsaturated, a balance that determines its technological applicability as a semi-solid lipid base. In addition, palm oil is distinguished from other vegetable oils because it presents a high content of palmitic acid, ensuring differentiated crystallization characteristics to this raw material (O'Brien, 2008).

Olive oil stands out for its health benefits, but also for its sensory properties that contribute to the taste when used properly during cooking (Buckland & González, 2010). Olive oil is the product obtained only from the fruits of the olive tree (*Olea europaea* L.), excluding oils obtained by means of solvents and any mixture of other oils. Virgin olive oil is the product obtained from the fruit of the olive tree only by mechanical or other physical processes, under thermal conditions that do not change the olive oil, and which has not undergone other treatments besides washing with water, decantation, centrifugation and filtration, according to the International Olive Council (IOC, 2013). Virgin olive oil is of great economic importance for the Mediterranean countries, with Spain being the largest producer in the world. Currently, new producers such as Georgia, Saudi Arabia, India and Botswana are entering the olive oil market (Vossen, 2013).

5.2. Hard fats

A high-potential low-cost option for structuring lipid phases are fully hydrogenated vegetable oils, called hard fats. Hard fats are obtained when all double bonds of the fatty acids are saturated in the process of catalytic hydrogenation of liquid oils, according to the process conditions. Although hard fats are low-cost industrial products, they are considered as relatively new materials, because they replaced partially hydrogenated fat when using hydrogenation plants, which had their use significantly reduced after the implementation of worldwide legislations on *trans* fat elimination from processed foods. They are currently used as ingredients in formulated

lipid bases, particularly to obtain interesterified fats (Ribeiro, Basso, & Kieckbusch, 2013).

These components are considered system models in terms of fatty acid and TAG composition, which are important determinants of the structuring and modifying effect of the crystallization processes of continuous or emulsified lipid phases (Omonov, Bouzidi, & Narine, 2010). The presence of these hard fats as additives change the crystalline habit and the crystallization behavior, reducing the crystallization induction period and acting as crystallization germs (Oliveira, 2011).

Specific hard fats from a particular oil source have a unique and differentiated triacylglycerol profile, which characterize these materials as inducers of particular polymorphic habits. After cooling an added hard fat lipid mixture, its trisaturated TAGs of high melting point (65-75°C) promote the formation of crystallization cores to coordinate a highly structured crystal lattice from the liquid system (Pernetti et al., 2007). In particular, hard fats from palm and soybean oils are characterized by polymorphic habits β' and β, respectively, properties of crystallinity that direct them to different applications in lipid-based foods (Ribeiro, Basso, dos Santos, et al., 2013).

In addition to the function as primary crystallization agents, hard fats change the physical properties of continuous fatty systems, allowing several adaptations related to the development of organogels, a property that has justified a number of studies on the conventional structuring of TAGs (Smith et al., 2011; Wassell et al., 2010). Hard fats of canola and soybean oils were studied for liquid oil structuring (Omonov et al., 2010). A systematic study was conducted on the nanostructure of mixtures made up of canola oil hard fat and high oleic sunflower oil (Acevedo & Marangoni, 2010). The rheological and crystallization properties were evaluated in fatty mixtures structured with hard fats from palm stearin and canola oil of low and high content of erucic acid (Zárubová, Filip, Kšandová, Šmidrkal, & Piska, 2010). Cocoa butter structuring was evaluated by incorporating hard fats from palm kernel oil (FHPKO), palm oil (FHPO), cottonseed oil (FHCO), soybean oil (FHSO), and crambe oil (FHCrO). Hard fats FHPO, FHCO, FHSO e FHCrO proved to be effective additives to modulate the physical properties of CB. Major changes on the physical properties of CB were performed by the FHSO. FHPKO was found unsatisfactory as a modifier of the CB (Ribeiro, Basso, & Kieckbusch, 2013).

5.3. Emulsifiers

Other components recently described in the scientific literature with potential to structure complex lipid matrices are the emulsifiers (Rogers et al., 2009; Siraj et al., 2015).

Sorbitan monostearate is a non-ionic hydrophobic emulsifying surfactant, often used in combination with polysorbates for cakes, fillings and creamy toppings, promoting volume increase and softness, and presenting high potential for modifying the crystallization properties in lipid systems (Marangoni & Narine, 2002). It also has the ability to develop viscous dispersions in organic solvents and edible oils through the self-assembly mechanism (Co & Marangoni, 2012).

With these emulsifiers, gelation involves the formation of high stability tubular vesicles, in which sorbitan monostearate molecules would be arranged in inverse bilayers in the tubules. Studies on the structuring of lipid phases using sorbitan monostearate are recent and relatively scarce in the literature. In general, organogels obtained by incorporing sorbitan monostearate into liquid oils are thermoreversible and melt at 40-45°C, a range typically observed for most fatty bases for industrial applications (Hwang, Singh, Winkler-Moser, Bakota, & Liu, 2014; Pernetti et al., 2007; Smith et al., 2011). The organogels obtained through the use of sorbitan monostearate are opaque, semi-solid, thermoreversible, and stable at room temperature for weeks. Such organogels have their properties affected in the presence of additives, such as hydrophilic surfactants and nonionic surfactants, which increase their stability and change their microstructure (Dassanayake et al., 2011).

Zhao et al. (2013) have shown that in whipped cream formulated with organogels structured from sorbitan monostearate, this compound can generate lattices of small crystals, presenting good texture and viscosity properties. Organogels of olive oil and sorbitan monostearate were characterized by Shah, Sagiri, Behera, Pal, & Pramanik (2013). Singh et al. (2015) obtained organogels based on sesame oil and sorbitan monostearate for topical applications in the pharmaceutical industry.

Monoacylglycerols are lipid molecules that have only one fatty acid esterified to the glycerol molecule, which may vary in chain size and degree of unsaturation (Chen & Terentjev, 2010). The structuring of vegetable oils by monoacylglycerols occurs through self-assembly, formation of micelles or inverse lamellar phases while cooling the formed system, that is, the molecules of monoacylglycerols can be structured as

oil-in-water emulsions trapping the oil phase. In hydrophobic medium (in this case, a vegetable oil), the hydrophilic groups present in the monoacylglycerols constitute the membrane of the micelles forming a lipid bilayer stabilized by hydrogen bonds. The organization of the hydrophilic heads inside the bilayer promotes elasticity and, consequently, the gelation of oil systems containing monoacylglycerols, as these are organized as larger platelets forming a continuous three-dimensional network that can trap the liquid oil through capillary forces (Lopez-Martínez, Charó-Alonso, Marangoni, & Toro-Vazquez, 2015; Valoppi et al., 2016; Wang, Gravelle, Blake, & Marangoni, 2016b).

The effect of the addition of 10% monoacylglycerols was evaluated in different oils (castor oil, cod liver oil, corn oil, extra virgin olive oil, sunflower oil, peanut oil, and mixtures of medium chain triacylglycerols). A difference was observed in the physical properties of organogel formulation; however, all organogels presented β polymorphism, regardless of the oil type used as organic phase (Valoppi et al., 2016). Lupi Gabriele and Cindio (2012) evaluated the rheological and microstructural characteristics of emulsions obtained from organogels prepared by lipid phase containing mixtures of olive oil and cocoa butter, using a mixture of monoacylglycerols and diacylglycerols as structuring agents. Toro-vazquez et al. (2013) investigated the effect of different monoacylglycerols on thermal properties, microstructure and consistency of organogelified emulsions developed with candelilla wax and safflower oil.

Lecithin can be obtained from oilseeds such as soybean, sunflower seeds, and rapeseed, consisting of a byproduct of vegetable oil refining (O'Brien, 2008; Van Nieuwenhuyzen & Tomás, 2008). The presence of phosphatidylcholine (16-26%), phosphatidyl ethanolamine (14-20%), phosphatidylinositol (10-14%), phytoglycolipids (13%), and phosphatidylserine (4%) characterize conventional soy lecithin (Attia et al., 2009).

Lecithin is widely used as an emulsifier in the food, cosmetic, pharmaceuticals and biotechnology industries (Shchipunov & Schmiedel, 1996). The emulsifying property of lecithin is attributed to phospholipids, which consist of a glycerol esterified with two fatty acids and a phosphate group or phosphate grouping and different nitrogenous bases (Arnold et al., 2013).

In oil structuring, lecithins with a phospholipid content above 95% are more efficient, as they favor the formation of micelles, generating aggregates with entangled microstructures and consequent oil trapped in the liquid phase (Kumar & Katare, 2005).

Lecithin organogels was first described by Scartazzini and Luisi (1988); and phospholipids with other structuring agents have been used in promising drug products (Kumar & Katare, 2005).

5.4. Waxes

Lipids present on the surface of leaves, stems and fruits have a very different structure from intracellular lipids and play a very important role in the protection of the plants from loss and absorption of water, gases and volatile biological compounds (Pokorný & Schmidt, 2011). Most surface lipids are waxes that present a long chain fatty acid esterified with a long chain alcohol, and can be classified according to their origin: animal (beeswax), vegetable (carnauba, candelilla, sunflower wax, among others) and mineral (petroleum wax) (Damodaran, Parkin, & Fennema, 2010).

Examples of vegetable waxes include carnauba (*Copernica cerifera*), known as “queen of waxes,” ouricury (*Syagrus coronata*, *Cocos coronata*, *Attalea excelsa*), candelilla (*Euphorbia cerifera*, *E. antisiphilitica*, *Pedilanthus pavenis*), rice (*Oryza sativa*), sunflower (*Helianthus annuus*), and sugarcane. Waxes are widely used in the food, pharmaceutical and chemical industries, and, for this reason, they involve high economic interest (Rocha, 2012).

The use of vegetable waxes as structuring agents in lipid systems offers the benefit of using commercially available low-cost food grade additives (Kuznesof, 2005). In recent years, the potential of wax as a structuring agent has become an alternative technique for oil structuring, and different edible oil structuring systems have been intensively studied (Marangoni & Garti, 2011).

As materials derived from natural sources, waxes have different compositions and physical behaviors, which are unique to each material. Most wax esters, in their natural form, contain small amounts of sterols, esters, fatty alcohols, fatty acids, and resinous matter. The esters of vegetable waxes, after refining, basically contain esters of fatty acids and fatty alcohols of different chain lengths (Dassanayake et al., 2009).

Candelilla wax is derived from leaves of a small shrub from the *Euphorbiaceae* family, found in the north region of Mexico and the southwest region of the United States (Kuznesof, 2005). In the United States, candelilla wax was approved as a food additive by the FDA, recognized as a safe (GRAS) food ingredient for human diet (FDA, 2016).

When studying the composition of candelilla wax, Warth (1948) observed that the content of hydrocarbons can account for 50-51% of the composition, the main ones are: hentriacontane ($C_{31}H_{64}$) and tritriacontane ($C_{33}H_{68}$). A more recent study conducted by Morales-Rueda et al. (2009) showed that the main component of candelilla wax is hentriacontane (content of approximately 80%), with other alkanes observed with an odd number of carbons, such as nonacosane (C_{29}, 4.2%) and tritriacontane (C_{33}, 8.0%); triterpene alcohols were also identified (7.4%) of molecular formula $C_{30}H_{49}OH$ (germanicol, lupeol or moretenol), and 1.6% of other unidentified compounds.

The use of candelilla wax as a structuring agent is technically feasible, as promising characteristics in a three-dimensional network with candelilla wax organogel in sunflower oil showed high hardness at 25ºC. At the concentration of 3% candelilla wax, the organogels did not present phase separation up to three months at room temperature, presenting consistency of potential use in the food industry (Toro-Vazquez et al., 2007). Studies on the thermomechanical properties of candelilla wax in safflower oil reported that it is possible to gelate triolein-rich lipid matrices (Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, & Toro-Vazquez, 2009).

Several types of waxes were studied to understand the factors that affect the structuring ability, including many vegetable waxes, which were evaluated for the structuring ability of the soybean oil and compared with hydrogenated vegetable oils, petroleum waxes and non-edible commercial gelling agents, for example, copolymer and polyamide wax. A high degree of purity of the structuring agent is not always necessary for better gelling, but a suitable combination of the various components in a structuring agent can provide good results of candelilla wax gelation in soybean oil (Hwang, Kim, Singh, Winkler-Moser, & Liu, 2012). However, Blake, Co and Marangoni (2014) reported that critical concentrations for the formation of organogels of canola oil with rice bran wax, sunflower wax, candelilla wax and carnauba wax are 1, 1, 2 and

4% , respectively, suggesting that rice bran wax and sunflower wax are more efficient structuring agents.

Rocha et al. (2013) evaluated the potential for organogel formation using sugar cane wax and its hot ethanol soluble and insoluble fractions, which presented the ability to form organogels with static crystallization at 5°C at the studied concentrations of 1, 2, 3 and 4% m/m.

6. Organogel applications in the food industry

The application of organogels in foods has been studied for some years and includes the stabilization of water-in-oil emulsions and a means of controlled release of pharmaceutical and nutraceutical products. Applications in the food industry include the potential use of structuring agents to minimize the migration of liquid oil into food, such as chocolate filling, margarine, baking products like biscuits and cookies, puff pastry, and spreads, and to structure edible oils, reducing the use of SFA and TFA (Hughes et al., 2009; Rogers et al., 2009). Organogel emulsions are most suitable for emulsified foods such as margarine, yogurt, processed and bar cheeses, mayonnaise and sauces (Moschakis, Panagiotopoulou, & Katsanidis, 2016). Siraj et al. (2015), in a detailed study on organogel applications in processed foods, highlight the potential of these systems for the transportation of nutraceutical components, emulsions of reduced calorie content, creams for toppings and fillings, spreads, lipid bases for baking products, comminuted meat products, among others (Table 1).

Table 1. Applications of organogels in food products

Application	Year	Authors	Organic phase	Structures
Frankfurters	2012	A. K. Zetzl, A. G. Marangoni, S. Barbut	Canola, soybean and flaxseed oil	Ethylcellulose
Ice Cream	2013	D. C. Zulim Botega, A. G. Marangoni, A. K. Smith, H. D. Goff	High-oleic sunflower oil	Rice bran, candelilla, or carnauba wax
Ice Cream	2013	D. C. Zulim Botega, A. G. Marangoni, A. K. Smith, H. D. Goff	High-oleic sunflower oil	Rice bran wax
Margarine	2013	H. Hwang, M. Singh, E. L. Bakota, J. K. Winkler-Moser, S. Kim, S. X. Liu	Soybean oil	Sunflower, rice bran and candelilla wax

Sweet Breads	2013	S. Calligaris, L. Manzocco, F. Valoppi, M. C. Nicoli	Sunflower oil	Monoglyceride
Margarines	2014	H. Hwang, M. Singh, J. K. Winkler-Moser, E. L. Bakota, S. X. Liu	Soybean, almond, canola, corn, flaxseed, grapeseed, olive, peanut, pumpkin seed, safflower, sesame, sunflower, walnut oil	Sunflower wax
Spreads, Chocolate Paste and Cakes	2014	A. R. Patel, P. S. Rajarethinem, A. Gredowska,O. Turhan, A. Lesaffer, W. H. De Vos, D. V. de Walle, K. Dewettincka	Sunflower and rapeseed oil	Shellac
Butter and Margarine	2015	E. Yılmaz, M. Ogutcu	Virgin olive and hazelnut oil	Beeswax and sunflower wax
Cookies	2015	A. Jang, W. Bae, H. Hwang, H. G. Lee, S. Lee	Canola oil	Candelilla wax
Cookies	2016	B. Mert, I. Demirkesen	Sunflower oil	Carnauba and candelilla wax
Cookies	2016	H. Hwang, M. Singh, S. Lee	Olive, soybean and flaxseed oil	Sunflower, rice bran, beeswax, and candelilla wax
Sandwich Cookie Cream	2016	R. Tanti, S. Barbut, A. G. Marangoni	Canola oil	Hydroxypropyl methylcellulose and methylcellulose
Cream cheese	2016	H. L.Bemer, M. Limbaugh, E. D.Cramer, W. J. Harper, F. Maleky	Soybean, high-oleic sunflower oil	Rice bran wax or ethylcellulose
Frankfurter	2016	E. Panagiotopoulou, T. Moschakis, E. Katsanidis	Sunflower oil	Phytosterol and ɣ-oryzanol
Frankfurters	2016	S. Barbut, J. Wood, A.G. Marangoni	Canola oil	Ethylcellulose and sorbitan monostearate

The selection of structuring agents to manufacture food products should be judicious and take into account the possible applications of this structured material (Rocha, 2012). Highly effective structuring agents, when used in low concentrations, can replace a large amount of lipid raw materials containing *trans* or saturated fats (Hwang et al., 2012).

The mechanical properties of ethylcellulose (10%) in vegetable oils (canola, soybean and flaxseed) were evaluated, as well as their potential to reduce SFA in sausages. The resulting organogels maintained the fatty acid profile of the vegetable oil, but with a solid structure. There was no significant difference in hardness when

compared to the product obtained with standard fat, indicating the potential of ethylcellulose organogel to replace SFA in a variety of food products that should keep their texture properties (Zetzl, Marangoni, & Barbut, 2012).

For the production of frankfurter sausages with partial replacement of bacon, organogel emulsions of sunflower oil were developed with γ-orizanol and phytosterols. No differences were detected in pH values, oxidation and texture profile of sausages due to the incorporation of lipid gels. Bacon could be partially replaced with organogels without significantly affecting the physical, chemical and sensory properties of the product (Moschakis et al., 2016).

Organogels were developed to replace the lipid phase in ice creams, in order to reduce the content of SFA. Blends with 10% wax (candelilla, rice or carnauba), 90% high oleic sunflower oil and glycerol monooleate were evaluated as emulsifier. Improvements were observed in the quality of the ice cream produced with rice bran wax when compared to ice cream produced only with high oleic sunflower oil; then, the organogel obtained with rice bran wax presented the potential to replace saturated fat in ice cream. However, a high fat concentration (15%) and the glycerol monooleate emulsifier seem to be required to achieve a better ice cream structure when rice bran wax organogel is used as the source of fat, to create a structure that resist to melting in ice cream (Zulim Botega, Marangoni, Smith, & Goff, 2013a, 2013b; Zulim Botega, 2012).

Sweet breads were produced by replacing the standard lipid fraction with organogels obtained from mixing sunflower oil and palm oil structured with Myverol™ saturated monoacylglycerols in order to reduce the amount of SFA in the formulations. The crystallization ability of the monoacylglycerols allowed bread formation to be similar to the control, with 81% reduction of SFA, showing this type of emulsifier promotes system structuring and the interaction of the various components of the formulation, increasing the lipid-starch interaction (Calligaris, Manzocco, Valoppi, & Nicoli, 2013).

Organogels obtained from waxes (sunflower, rice bran, and candelilla) and soybean oil were tested for incorporation in margarine. The candelilla wax organogel presented phase separation in the emulsified form. The rice bran wax showed good hardness as organogel, but low hardness for margarine application. Sunflower wax

contributed to greater firmness of the organogel and the margarine samples among the evaluated vegetable waxes. Margarines prepared from organogels containing 3% sunflower wax showed greater firmness than commercial spreads, demonstrating the feasibility of organogels, rich in polyunsaturated fatty acids for the production of margarine and spreads (Hwang et al., 2014).

Margarines with reduced SFA content of 17.3 to 36.6% compared to commercial margarines were produced with soybean oil and high oleic sunflower oil, respectively. Oil structuring was accomplished through the incorporation of candelilla wax, monoacylglycerols and interesterified fat. The margarines with organogel presented better emulsion stability at the evaluated temperatures when compared to commercial margarines (Chaves, 2014).

Margarines produced with organogels obtained from hazelnut oil and olive oil and structured with beeswax were developed and submitted to sensory testing, where consumers were asked to indicate their intention to purchase if the product was for sale. This study showed that 57 and 43% of consumers would definitely buy these margarines, but 12 and 25% of consumers would definitely not buy the product formulated with hazelnut oil and olive oil, respectively, due to their sensory characteristics (Yilmaz & Ogutcu, 2015).

Canola oil organogels structured with candelilla wax were prepared and used to replace fat in the production of biscuits with a high level of unsaturated fatty acids. The incorporation of candelilla wax (3% and 6%) in canola oil produced solid organogels, but the hardness of the organogels was lower in comparison to conventional fat at room temperature. In organogel biscuits, the content of unsaturated fatty acids increased about 90 to 92% and the level of SFA was reduced to approximately 8 to 10%, demonstrating the effectiveness of the organogel to replace fat in biscuits, obtaining a product rich in unsaturated fatty acids and consequently with low SFA content and absence of TFA in relation to standard fat (Jang, Bae, Hwang, Lee, & Lee, 2015).

The potential application of organogel from carnauba wax and candelilla wax was evaluated for the replacement of fats in cookies. The incorporation of 2.5 and 5% of the waxes in sunflower oil resulted in better-looking soft cookies, but at a consistency lower than that obtained with standard fat. The analysis of lipid composition showed

that the organogels presented higher levels of unsaturated fatty acids when compared to conventional fat, indicating their potential as a healthier alternative for application in baking products (Mert & Demirkesen, 2016).

To understand the effects of different types of waxes, organogels were prepared with sunflower wax, rice bran wax, beeswax and candelilla wax and employed in the formulation of cookies. In order to investigate the effects of different vegetable oils on the properties of cookies, olive oil, soybean oil and flaxseed oils were used, represented mainly by oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3), respectively. The highest firmness of organogel was obtained with sunflower wax and linseed oil. The properties of cookies, such as hardness and lipid phase melting behavior, were significantly affected by the incorporation of the organogels. However, the fracturability of cookies containing organogels were not significantly affected by the different structuring agents and oils. Cookies obtained with organogels showed similar properties to standard cookies, indicating high viability of the organogel technology for food application, such as biscuits rich in unsaturated fats (Hwang, Singh, & Lee, 2016).

Organogels with 10% rice bran wax or ethylcellulose in combination with soybean oils and high oleic sunflower were developed to produce cream cheese. The analysis of fatty acid composition showed a 90% reduction in the SFA content of cream cheese with organogel when compared to similar commercial products, presenting a healthier substitute of this product, since the values of hardness, spreadability and viscosity were similar among samples containing rice bran wax, differing only from organogel with ethylcellulose, which showed lower adhesiveness than the commercial products (Bemer, Limbaugh, Cramer, Harper, & Maleky, 2016).

Standard chocolate spreads and spreads prepared by total and partial replacement of palm oil (27%) with shellac organogels were evaluated for viscosity using frequency scan data. Both G’ and G’’ were higher for the standard spread when compared to the spread formulated with organogel. A more solid aspect was observed in both spreads, through higher values of G’ when compared to G’’. However, G’ for the spreads containing organoel presented greater frequency dependence, that is, a positive slope, showing a different pattern of the standard spread curve, which results in a comparatively lower plastic viscosity, indicating a weaker gel structure in the organogel-based spread. However, standard spread and the spread formulated with organogel showed no exudation when stored at 30°C for more than 4 weeks. The

values of G* along the curve were higher for the standard spread when compared to the spread formulated with organogel, a property that can be correlated with the large difference in the values of the solid fat contents at 20ºC (21.4% for the spread and 11.3% for organogel spread) (Patel et al., 2014).

7. Conclusion

In conclusion, the use of organogels in food applications as potential substitutes for SFA and TFA is highly feasible, since vegetable oils with small structural properties are generally used to replace conventional fats. In addition, worldwide public health bodies are somewhat focused on reducing the number of deaths caused by cardiovascular diseases, and the reduction of SFA and TFA in foods is an important strategy. In comparison to the conventional technological processes for the production of technical fats for food applications, the production of organogels is a technologically simple, economically accessible low-cost method. In addition, various lipid raw materials can be used, the immobilized phase (vegetable oil) may vary according to geographic region, availability and cost. Structuring agents are used in small proportions, are commercially available, safe for consumption and affordable. Finally, organogels can be used in a range of foods with promising results, promoting an effective reduction of SFA and TFA.

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CAPÍTULO 3

CHOCOLATE SPREADS: CHARACTERIZATION FOR REFORMULATION

Artigo Submetido

Food and Function

Royal Society of Chemistry

Chocolate spreads: characterization for reformulation

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ABSTRACT

Chocolate spreads consist of vegetable fat, sugar, soy lecithin, powdered milk, cocoa powder and/or hazelnut paste. The objective of this study was to characterize commercial chocolate spreads and their respective lipid phases, aiming at strategies to reformulate this product category. Six different commercial chocolate spreads were evaluated through the characterization of their lipid phase in fatty acid composition (FAC), solid fat content (SFC), crystallization kinetics, thermal behavior, polymorphism and microstructure. The physical-chemical characterization of the commercial spreads was performed on lipid content (LC), oil exudation, particle size distribution, rheological properties, hardness and spreadability. The LC of the spreads ranged from 17.66 to 24.90%. Saturated fatty acids (SFA) content ranged from 19.31 to 35.05%; unsaturated fatty acids (UFA) ranged from 64.80 to 80.24% and trans fatty acids presented 0.44% as the maximum concentration, showing the absence of partially

hydrogenated fats. The crystallization induction time (IT) of the lipid phases ranged from 16.5 to 71 minutes; and the maximum solids content (SCmax) ranged from 2.17 to 5.35%. IT of the lipid phases of the spreads were similar to the parameters of the isothermal characterization of palm oil samples. Melting and crystallization curves showed two different regions, reflecting the different triacylglycerol classes present in the lipid phase of the chocolate spreads. The crystalline lipid phase of all spreads was characterized by β' polymorphism. Some fats showed typical spherulitic crystals of palm oil. Other fats did not show individualized crystals under the analysis conditions (25°C), since the onset crystallization temperature occurred between 14.99 and 18.30°C. The hardness ranged from 1.34 to 3.01 Kg and the spreadability from 1.10 and 2.78 Kg.s. All spreads showed particle size between 12.01 to 34.80 μm in d 0.5 and d 0.9, respectively. Regarding rheological parameters, the value of G' (elastic modulus) and higher than G'' (viscous modulus) was observed, indicating properties of solid materials. Thus, chocolate spreads presented a predominant elastic behavior, with no oil exudation. This characterization is fundamental for the reformulation of chocolate spreads with improved nutritional composition.

Keywords: spreads; chocolate; physical-chemical composition; fatty acids; lipid content; crystallization; rheological properties.

INTRODUCTION

Chocolate spreads are spreadable creams with a basic composition of palm oil, sugar, cocoa powder and soy lecithin [1]. Chocolate spreads are dispersions of cocoa powder and sugar particles in a continuous oil medium, with a considerable proportion of solid fat. Chocolate spreads have specific rheological properties since they behave as a solid that avoids the sedimentation of dispersed particles and phase separation (liquid oil), but also present high spreadability [2].

Chocolate spreads are very popular due to their taste and high nutritional and energetic value. The popularity of these foods is mainly associated with their potential to induce sensory pleasure and positive emotions [3]. The use of chocolate spreads is not restricted to retail sales, also being applied in the bakery industry for confectionery, fillings and toppings [1].

Chocolate spreads are usually stored at room temperature, but when kept under refrigeration they require the same spreadability. Moreover, spreads must withstand temperature changes during storage. The lipid phase in chocolate spreads presents high propensity to exudation and oxidation since the fat used has a liquid phase, and the technological process for obtaining these products includes milling the ingredients. Thus, all trap systems for fats must be efficient so oil is effectively retained in the crystalline network [4].

Chocolate spreads are low moisture foods, which makes the reduction of lipid content (LC) by increasing the water content in the formulation impossible, because if the fat content decreases, the ratio of dry ingredients in the spread increases. Therefore, the plastic properties of lipids with different crystallization properties must be explored to reduce the saturated fatty acids (SFA) content [5].

Chocolate spreads are fat-based foods whose physical properties depend on their crystalline structure. Consequently, the oils and fats used in the formulation have significant effects on the product quality [6]. The typical fat content of chocolate spreads is approximately 30%, thus characterizing them as continuous fat systems where sugar and other particles are dispersed in fat phase, which has a great influence on the sensorial behavior, providing creaminess and softness. Typically, β-polymorphic fats form large crystals and provide desirable snap in chocolate products, while β’ fats form small crystals and promotes a softness feeling during tasting [4].

Solid fat (partially hydrogenated vegetable oils or natural fats with high SFA contents) are used for formulations of chocolate spreads. Furthermore, lipid stabilizers (high melting

point triacylglycerols based on fully hydrogenated oils) are usually incorporated into the formulation to prevent oil exudation at storage, distribution and commercialization temperatures [2].

Palm oil and its stearines, with high SFA content, are partially solid at room temperature, and are among the most widely used raw lipid materials for the formulation of chocolate spreads [7,8]. Palm oil presents high resistance to oxidation due to its high-saturated fatty acids content, especially palmitic acid (about 40%). The blood profile of fatty acids reflects the largest source of fat consumed; therefore, diets high in palmitic acid raise the level of this type of SFA in the blood [9]. Diets with high amounts of palm oil significantly increase LDL-C and total cholesterol, in addition to triggering atherosclerosis [10]. Furthermore, palm oil has the highest potential for formation of 3-monochloro-1,2-propanediol (3-MCPD) fatty acid esters, which are known to be harmful to health due to high diacylglycerols concentrations, fundamental precursors for the formation of 3-MCPD esters and related compounds [11].

The major challenge in the development of new fat-based products is to mimic the sensory properties of the original fat, which depend on the crystalline lipid microstructure [12]. Structural factors such as triacylglycerols, crystalline elements, and microstructures determine the macroscopic rheological properties of the lipid system [13]. Information on the rheological behavior and thermal properties (crystallization and melting behavior) of the lipid phase present in food products can provide better quality control at each processing step [14].

Saturated fat is the main dietary cause of elevated plasma cholesterol, and its reduction in diet is globally supported to reduce the risk of cardiovascular diseases [15]. Given the harmful effects of SFA and trans fats to health, actions were taken to direct industries toward increasing the healthiness of foods by changing the conventionally used raw materials [10].

Several food commissions and coronary heart disease prevention groups worldwide have been studying ways to provide consumers with information about the composition of foods

through clearer nutrition claims [16]; thus, reformulating lipid-based foods is crucial so consumers can have healthier product options.

In the literature, some researchers have recently studied the total or partial substitution of the lipid phase of chocolate spreads and the use of alternative lipid bases with reduced SFA and trans fat levels in the formulation [2,5,7,17].

The objective of this study was to characterize different commercial chocolate spreads by determining LC, oil exudation content, particle size distribution, rheological properties, hardness and spreadability; and their respective lipid phases regarding fatty acid composition (FAC), solid fat content, crystallization kinetics, thermal behavior, polymorphism and microstructure, aiming at strategies to reformulate the lipid phase of this type of product.

MATERIAL AND METHODS

Materials

Six different commercially available chocolate spreads were analyzed, being purchased in markets of Campinas – SP, Brazil, from February to April of 2016. Samples were designated as A, B, C, D, E, and F.

Characterization of spreads

The lipid phase was extracted by defatting the chocolate spreads via cold solvent extraction according to the ISO 17189 (2003) method, applicable to spreads containing cocoa and milk solids. The lipid phase of the spreads was evaluated through the characterization of FAC, solid fat content, crystallization kinetics, thermal behavior, polymorphism and microstructure. The physical-chemical characterization of the spreads was performed on LC, oil exudation content, particle size distribution, rheological properties, hardness and spreadability. All analyses were performed in triplicate.

Characterization of the lipid phase of spreads

Composition in fatty acids. Composition in fatty acids was determined in a gas chromatography device with capillary column, after esterification using the Hartman & Lago method[18]. Fatty acid methyl esters were separated according to the AOCS Ce 1f-96 method on [19] Agilent DB-23 column (50% cyanopropyl-methylpolysiloxane). Dimensions: 60 m, ϕ 0.25 mm internal, 0.25 μm film. Analysis conditions: 110°C for 5 minutes as initial oven temperature, followed by a 5°C increase per minute until reaching 215°C, and 215°C for 24 minutes; 280°C detector temperature; 250°C injector temperature; helium as carrier gas; split ratio was 1:50; injected volume was 1.0 μL. The qualitative composition was determined by comparing the peak retention times with those of the respective fatty acid standards.

Solid Fat Content (SFC). Solid fat content was determined using a Bruker pc120 Minispec Nuclear Magnetic Resonance Spectrometer with the aid of high precision dry baths (0–70°C) Tcon 2000 (Duratech, USA). AOCS method Cd 16b-93: direct method, serial reading of samples, with tempering for the lipid phases [19].

Crystallization kinetics. Crystallization kinetics was obtained by melting the fats (100°C for 15 minutes and for 1 h at 70°C) for total destruction of the crystalline history and the solid fat content was monitored at 25°C (± 0.5°C) on a Mq20 NMR Bruker Analyzer Nuclear Magnetic Resonance Spectrometer [20]. Data was automatically acquired every 1 minute for 1 hour. The characterization of the crystallization kinetics was performed according to the induction time (IT) in relation to the formation of crystals and to the maximum solids content (SFCmax). The IT is obtained graphically and reflects the time required for a stable critical-sized core to be formed in the liquid phase [21].

Thermal behavior. The thermal analysis of the fats was performed in a TA Q2000 differential scanning calorimeter coupled to the RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing system used was Universal V4.7A (TA Instruments, Waters LLC, New Castle). Analysis conditions were: ~10 mg in sample mass

[19]; temperature between -60 and 100°C for the modified AOCS Cj 1-94 method, with a 5°C/min ramp (crystallization and melting). The following parameters were used to evaluate the results: onset crystallization and melting temperature (T_{oc} and T_{om}), peak crystallization and melting temperatures (T_{fc} and T_{fm}), crystallization and melting enthalpies (ΔH_C and ΔH_M) and final crystallization and melting temperature and (T_{fc} and T_{fm}) [21].

Polymorphism. Polymorphism was determined by X-ray diffraction, according to procedures of Sawalha et al[22]. The analyses were conducted in a Philips diffractometer device (PW 1710), using the Bragg-Bretano (θ:2θ) geometry with Cu-k radiationα (λ = 1.54056Å, 40 KV voltage and 30 mA current). The measurements were obtained with 0.02° steps in 2θ and 2 seconds acquisition time, with *scans* from 15 to 30° (scale 2θ) at 25°C. Crystalline forms were identified from the characteristic short spacings (SS) of the crystals.

Microstructure. The microstructure of the fats was evaluated by microscopy under polarized light. A drop of fat was placed on a glass slide with the aid of a capillary tube, the slide was covered with a cover slip and kept at 25°C for 24 hours prior to analysis. The microstructure of the crystals was evaluated using a polarized light microscope (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). Images were captured by the Image Pro-Plus 7.0 software (Media Cybernetics, USA) in four different visual fields of each slide for each fat and the mean particle diameter result was expressed by the mean and standard deviation of these values [23,24]. Fats were analyzed at 25°C using 20x magnification. Evaluated parameters were number of crystalline elements, mean density, mean diameter of the crystals, agglomerated crystals and mean diameter of the individual crystals.

Characterization of spreads

Lipid content (LC). LC was measured by cold solvent extraction according to ISO 17189 (2003), applicable to spreads containing cocoa and milk solids.

Hardness. Hardness was determined using a texturometer (TA-XTi2, Stable Microsystems, England) controlled by a microcomputer. For the analyses, 30 mL of the spreads were placed in 50 mL beakers and conditioned in a BOD incubator at 5°C for 24 h. A compression/extrusion test was performed using an acrylic cylindrical probe, 25 mm in diameter and 35 mm in length, with 1.0 mm/s velocity, and a 15 mm fixed probe penetration distance. The value considered was the maximum force obtained [25].

Spreadability. The spreadability of the samples was evaluated in a texturometer (TA-XTi2, Stable Microsystems, England), using the TA.XTPlus Application Study (Spreadability/Softness of Margarine) method developed specifically for spreads, creams, margarines and similar products. A conical *probe* with 90° angle, 63 mm distance, 10.0 mm/s pre-test velocity, 3.0 mm/s test velocity and 10.0 mm/s post-test velocity was used. The following parameters were obtained: shear work (kgs) and firmness (kg)[26].

Particle size distribution analysis. Particle size distribution of the spreads was determined using the MasterSizer Laser Diffraction Particle Size Analyzer, equipped with the Hydro 2000S Sample Presentation Unit (1.590 refractive index) (Malvern Instrument Ltd., Malvern, England). Pre-dispersed spreads (0.4g in 10 mL of sunflower oil) at room temperature (25°C) were added directly to the sampling unit until a 15% obscuration value was obtained. Samples were dispersed with ultrasonic bath aid for 2 minutes to ensure that the particles were individually dispersed and suspended, and then kept under stirring. The particle size distribution was expressed as mean volume diameter D [4.3], median D (0.5) and mode. The parameters D (0.1) and D (0.9) were also evaluated, which represent the diameter values below which 10 and 90% of the accumulated distribution (in volume) are located, respectively [27]. The Span index was used as indicative of the amplitude of particle size distribution, it was calculated by equation 1.

$$Span = \frac{D(0.9) - D(0.1)}{D(0.5)} \text{ (Equation 1)}$$

Rheological properties. Rheological analyses were performed according to proposed methodology Rocha et al. [25] using a controlled voltage rheometer (Physica MCR 301, Anton Paar, Germany). The geometry used were stainless steel parallel plates of rough surface (50 mm in diameter and 200μm gap). Temperature was controlled using a Peltier system. Frequency (f) of 1Hz and 1% deformation within the linear viscoelasticity range were used. Spreads were analyzed by voltage and frequency scans to evaluate their mechanical resistance and behavior against different observation times, respectively. Voltage scans were obtained from 0.1 to 10 Pa (f = 1 Hz) at 25°C. Frequency scans were obtained between 0.01 and 10 Hz, within the linear viscoelasticity range, using the same method as the voltage scans. The following parameters were determined: elastic modulus (G'), viscous modulus (G''), complex modulus (G*), phase angle (δ) and complex viscosity (η*).

Oil exudation content. The spreads were heated to 70°C for complete melting of the crystals and placed on graduated beakers (50 mL), following, they were stored in an incubator at 3°C for 1 hour, for crystallization of the lipid phase. The beakers were then stored in an oven at 38°C for 60 days to simulate high storage temperatures [28]. During this period, the exudate oil was monitored (mL of exudate oil), being measured according to equation 2:

$$Exudate\ Oil(\%) = \frac{Exudate\ oil\ (mL)}{Total\ initial\ spread\ (mL)} X\ 100 \text{ (Equation 2)}$$

Statistical analysis

The objective results were evaluated using the Statistica 8.0 software (Statsoft, USA) [29] to calculate the regression coefficient, probability (p-value) and analysis of variance (ANOVA), considering a 5% significance level. The same software was used to compare the means and to compare the commercial spreads using Tukey's test.

RESULTS AND DISCUSSION

Lipid phase of chocolate spreads

Lipid content (LC)

The LC of commercial chocolate spreads ranged from 17.66 to 24.90%, as shown in Table 1. Fats A and F had the lowest lipids percentage, 17.66 and 18.37%, respectively, with no difference considering the 5% significance level, followed by fats E (20.99%), B (22.51%) and D (23.08%), which were similar to the 5% significance level. Fat C presented the highest LC (24.90%) among all fats, showing no significant difference (at 5% significance), only of the fat D. The LC found for the different products were low when compared to formulations evaluated in recently published studies on the composition chocolate spreads, in which LC between 30% and 40% were found [5,30,31]. 22% of palm oil has been used in the standard formulation of chocolate spread and additional 12% of hazelnut paste – composed by 65% of total fat [2,32]. Total fat content of the spreads was also calculated according to the nutritional information provided in the labels of the spreads (Table 2), ranging from 28.00 to 36.50%.

Composition in fatty acids

Table 3 shows the FAC of the commercial spreads. SFA composition contents ranged between 19.31 and 35.05% and the unsaturated fatty acids (UFA) between 64.80 and 80.24%, with absence of partially hydrogenated fats, since trans fatty acids had a 0.44% maximum concentration.

Fats A and F presented a similar fatty acid profile, presenting 27.99 and 21.58% palmitic acid (C16:0), 5.03 and 4.91% stearic acid (C18:0), 53.94 and 57.93% oleic acid (C18:1) and 10.24 and 10.15% linoleic acid (C18:2), respectively. Fats B and D also showed equivalent composition, with 25.55 and 26.66% palmitic acid (C16:0), 5.61 and 5.72% stearic acid (C18:0), 23.15 and 22.45% oleic acid (C18:1) and 42.49 and 42.16% of linoleic acid (C18:2), respectively. Fats C and E were distinguished by the levels of oleic acid (C18:1), 31.31 and 53.93%, and linoleic acid (C18:2), 44.44 and 19.07%, respectively.

Palmitic (C16:0) (from 12.06 to 27.99%) and stearic (C18:0) (from 4.91 to 6.13%) acids were the major FAC present in the spreads; however, fats presented 64.80 to 80.24% UFA variation; and oleic acids (C18:1) (from 22.45 to 57.93%) and linoleic acids (C18:2) (from 10.15 to 44.44%) were the major fatty acids. The FAC of the lipid phase of spreads may affect the hardness and spreadability of lipid-based spreads due to the characteristic melting range of the different classes of triacylglycerols associated with this composition.

Table 4 shows the commercial denomination of the chocolate spreads. Spreads A, C, and E are referred to as hazelnut and cocoa cream, and spread F simply as hazelnut cream, but all contain hazelnut and cocoa powder as ingredients. Spreads B and D are called "chocolate flavored cream" and in fact do not present hazelnut in the ingredients list, only cocoa powder. According to Spigno et al. [32] hazelnut oil contains 6.6 to 8.3% palmitic acid (C16:0), 1.8 to 3.8% stearic acid (C18:0), 75.7 to 80.7% oleic acid (C18:1) and 10.1 to 13.8% linoleic acid (C18:2) [33], which should also be considered in the identification of the composition of the lipid phase of spreads.

Palm and soybean oils contain about 39.3 to 47.5% and 8.0 to 13.5% palmitic acid (C16:0), 3.5 to 6.0% and 2.0 to 5.5% stearic acid (C18:0), 36.0 to 44.0% and 17.0% to 30.0% oleic acid (C18:1) and 9.0 to 12.0% and 48.0 to 59.0% linoleic acid (C18:2), respectively [5,34]. Correlating the fatty acids composition of the spreads (Table 3), of hazelnut oil [33], the list of commercial spreads ingredients (Table 4) and information from Codex Alimentarius[34], the spreads A and F have a characteristic composition of the oil palm and hazelnut blend. Spreads B and D show typical blend composition of palm oil and soybean oil. Spreads C and E indicate the characteristic composition of a soybean, palm and hazelnut oil blend, with different proportions of these raw materials. Spreads A, B, C and E emphasize soy lecithin as an emulsifier on the label; spread D reports an unspecified emulsifier and spread F presents sunflower lecithin in its ingredient list (Table 4).

Solid Fat Content (SFC)

Fig. 1 shows the solids profiles determined for the lipid phases of chocolate spreads between 10 and 45°C. Fat A, with a higher amount of SFA in its composition (Table 3), showed higher thermal resistance, mainly at 10°C (12.01% solids), but total melting at body temperature. Fat F showed FAC and solids profile similar to that of fat A, but the higher UFA content was associated with higher SFC at the same temperature (10.77%), but greater plasticity as temperature increased. The solids profile indicates that the lipid phases do not have only palm oil in their composition, since the typical SFC of this oil should be around 45.3% at cooling temperature (10°C), 21.5% at 20°C, 13.0% at 25°C, 7.2% at 30°C, 3.2% at 35°C and practically null above 40°C; this suggests the presence of at least 50% of some refined vegetable oil, such as canola oil or soybean oil, according to the fatty acids composition profiles found in this study [35,36] or contain any interesterificated fat in its composition. Moreover, interesterificated fat blends present SFC directly related to concentrations of liquid oil and fully hydrogenated oil used; however, SFC reduction at all temperatures evaluated indicates greater plasticity [37,38].

Fats B and D showed a very close profile of solids and fatty acids composition (Table 3); however, fat D presented higher SFC at all evaluated temperatures, probably due to the different compositions in triacylglycerol classes.

Fats C and E presented low SFC at refrigeration temperature (10°C) due to the possible presence of hazelnut oil, in addition to the expressive amount of vegetable oil in its composition at higher proportions. Similar behaviors are found in studies on the substitution of palm oil by oleogels in chocolate spreads , where SFC was considerably lower for the reformulated spreads at all evaluated temperatures due to the decrease in AGS (palm oil) content, substituted by UFA (vegetable oils) [2,31].

SFC at different temperatures describes the melting profiles of the lipid bases, thus qualifying the spread for possible industrial applications. At low temperatures (from 4 to 10°C), SFC values typify the spreadability of refrigerated spreads. At room temperature (from 20 to 22°C), at least 10% solid fat is required to ensure resistance to oil exudation and stability of the spread. At temperatures between 30 and 35°C, general use fats like palm oil are distinguished by melting with concomitant flavor release; and the SFC provides an estimate of the sensory attributes during tasting [20]. Body temperature (37.5°C) is critical for the sensory quality of lipid-based products. Saturated fat content must be under 5% at this temperature range to minimize a possible waxy sensation[36,37]. Fats A, C, E and F showed no solids at body temperature, possibly due to the high UFA concentration, especially C18:1 oleic acid (Table 3); while fats B and D showed higher SFC at this temperature, 0.36 and 1.12%, respectively, however, with no effect on the perception of waxy sensations.

Crystallization kinetics

Table 1 shows the IT and the SFCmax of the lipid phases of the chocolate spreads. IT ranged from 16.5 to 71 minutes and the SFCmax ranged from 2.17 to 5.35%. Fats A and F presented slower crystallization with 71.0 and 66.0 minutes for IT, respectively, showing no considering the 5% significance level, followed by fats B (26.5 minutes), C (30.5 minutes), D (16.5 minutes) and E (25.5 minutes), which are similar to the 5% significance level.

SFCmax values of fats A, C, E and F did not present difference at the 5% significance level. Fats B and D presented a difference at 5% significance and higher SFCmax values (4.29 and 5.35%, respectively), possibly due to the high SFA concentration when compared to the other fats.

IT values of the lipid phases of the spreads were similar to the isothermal characterization parameters of palm oil samples (from 26 to 52 minutes)[36]; however, SFCmax values found were

lower than those reported (from 7.9 to 10.9%) by the same authors, caused by the presence of UFA rich oils in the fats evaluated in this study.

Thermal behavior

Table 5 and Fig. 2 present the thermal behavior of the lipid phases of spreads. The melting and crystallization curves can be subdivided into different regions, reflecting the different triacylglycerol classes present in the lipid phase.

Regarding the thermal behavior during crystallization, fat A showed lower T_{oc}, probably due to the high concentration of high-melting point triacylglycerols. Other fats showed similar crystallization behavior. Crystallization curves showed two broad and overlapping peaks (representing fractions of triacylglycerols of lower and higher melting point), which are associated to the presence of such different triacylglycerol classes [5,39].

Such different thermal behavior for sample A in the crystallization and melting events corroborates the results for solids profiles, in which the fat showed a lower plasticity when compared to the others. The other fats showed compatible and similar melting parameters, according to the results obtained for solids profiles and crystallization kinetics.

Thermal behavior parameters indicated that the lipid phases of the spreads present a first melting peak related to more unsaturated triacylglycerols; however, unlike the others, fats A and F did not present the second melting peak, indicating higher levels of lower point triacylglycerols, corroborating the higher IT values in crystallization kinetics (Table 1).

Polymorphism

Fig. 3 shows the X-ray diffractograms of the lipid phases of the spreads. Crystalline forms are characterized by specific SS. The characteristic SS correspond to 4.15 Å for α, 3.8 and 4.2 Å for β' and 4.6 Å for β, and are used to determine the relative proportion and type of polymorphs present in lipid bases [40].

We found SS equal to 4.2 and 3.8 Å, for all lipid phases, thus characterizing the β' polymorphic form. Similarly, Basso et al. [40] reported SS equal 4.2 and 3.8 Å for palm oil. Corroborating different literature studies which characterize palm oil as β', the presence of stable crystals in the β' form in palm oil occurs due to their diverse fatty acids composition and, particularly, the greater amount of palmitic acid [40].

In the lipid phase of spreads, crystals should be stabilized in the β' form to favor the spreadability and creaminess of the products [20]. Fats A, B, D and F showed more well-defined peaks due to the higher levels of SFA (Table 3), especially palmitic acid, which explains the higher crystallinity of these fats. These results corroborate the parameters of crystallization kinetics since these fats also have higher solids content at equilibrium (Table 1).

Microstructure

The lipid composition and crystallization conditions have influence on the crystal shape, and different polymorphic forms and crystalline morphologies are possible. Crystals are aggregated into larger structures forming a network, which characterizes the fat's microstructural level. The concept of microstructure includes information on the state, quantity, shape, size, spatial relationship and interaction between all components of the crystal network, having a large influence on the macroscopic properties of fats [42–45].

Triacylglycerols usually crystallize as spherulites, which correspond to the aggregation of crystalline lamellae, growing radially from the same central cores and can develop ramifications during crystalline ripening [46]. Eventually, depending on the cooling conditions or even the characteristic melting profile of each fat, triacylglycerols may also crystallize into other morphologies, such as needles and discs [44].

Table 6 shows the number of crystalline elements, mean density, mean D of the crystals (μm), agglomerated crystals (%) and mean D the individual crystals (μm) of the lipid bases of

the spreads after static isothermal crystallization at 25°C for 24 h. Images of Fig. 4 are shown at 20x magnification.

The crystals of fat A showed spherulitic crystals typical of palm oil [30,31]. The spherulites observed in the fats corroborate the polymorphic form β', which provides a soft texture for food products [47]. The other fats did not show individualized crystals under the analysis conditions, since T_{oc} occurred between 14.99 and 18.30°C (Table 5). We observed a combination of fat crystals, as well as regions containing liquid oil, in which smaller crystalline dimensions were observed with large proportion of the liquid phase. The microstructure corroborates the SFA content (Table 3).

Fats B, D and F showed higher number of crystalline elements, which is directly related to higher SFA content and higher SFCmax at 25°C (Table 1). Fats A, D and E showed more well-defined crystals (Fig. 4), probably due to the high mean density of fats.

The mean diameter of fats was under 30 μm. Therefore, spreads should not present a sandy sensation in the mouth resulting from the lipid phase[48]. The number of crystalline elements was consistent with the total enthalpy values of crystallization for all fats (Table 5), since the larger the crystalline area, the higher the energy required for the crystallization of the fat [49].

Characterization of chocolate spreads

Hardness and spreadability

The hardness and spreadability values of the chocolate spreads are shown in Table 1. Hardness ranged from 1.34 to 3.01 Kg and spreadability from 1.10 and 2.78 Kg.s. Hardness values has been reported for chocolate spreads formulated with oleogels ranging from 2.42 to 3.37 N after 30 days of storage at 20°C [17]. Spreads C and E showed lower values for hardness and spreadability (1.34 and 1.56 kg; 1.10 and 1.13 Kg.s, respectively), thus corroborating the lower UFA content (Table 3).

Spreads B, D and F showed greater spreadability, compatible with their higher UFA and SFC levels at 25°C. These samples showed a higher number of crystalline elements and smaller crystal diameter (Table 6), in accordance to typical characteristics of more cohesive and harder crystalline networks [21].

For spread A, relatively low hardness and spreadability values (1.90 Kg and 1.36 Kg.s, respectively) were observed, in contrast to its fatty acids and solids profile composition. These characteristics may be negative in lipid applications where hardness and texture are important but may be beneficial in technological applications that require increased flow for spreads [5].

In general, hardness and spreadability parameters of spreads can be associated to UFA contents, formation of small crystals of fat dispersed in a high proportion of liquid oil, promoting the formation of less cohesive crystalline networks.

Particle size and size distribution

Chocolate spreads are refined for a particle size under 30 μm [48] and the final particle size has a critical influence on rheological and sensory properties [50]. Table 1 presents the solid particle size distribution parameters of the spreads, including Span index, d 0.5, which corresponds to the maximum diameter for 50% of particle distribution.

Lipid particles greater than 30 μm can cause a sandy texture in the mouth and particles smaller than 20 μm are substantially smooth and creamy[48]. Span index values did not present significant differences at 5% significance, ranging from 2.45 to 3.31 μm. The size and distribution of chocolate particles has been evaluated and found 11.63, 7.64, 5.93 and 5.16 μm d 0.5 for the times 15 and 30 minutes, 4 and 8 hours of refining, respectively, and 46.41, 30.67, 15.50 and 11.75 μm d 0.9 for the times 15 and 30 minutes, 4 and 8 hours of refining, respectively [51].

All spreads showed particle size under 12.01 and 34.80 μm in d 0.5 (μm) and d 0.9 (μm), respectively; therefore, the evaluated chocolate spreads probably present low sensory

perception of sandy sensation, since the particle size distribution direct influences this characteristic and rheological properties in chocolates [50].

The crystals detected in this analysis are probably sugar or cocoa solids crystals, with dimensions similar to the maximum diameter of the lipid crystals according to the microstructure analysis, thus characterizing high uniformity spreads with regard to the particle size of the different components.

The optimization of the particle size distribution in chocolate-based products must consider the sensory sensibility of the palate. Particle size affects both viscosity and hardness, and a chocolate milled to a maximum size of 20 μm will have a higher creaminess than one milled to 30 μm. Particle size distribution is fundamental in the fluidity of the product, but it is generally restricted to experience-based empirical knowledge [48].

Frequency scanning

Fig. 5 presents the frequency sweep analyses to investigate the deformation behavior of chocolate spreads within the linear viscoelasticity region. All spreads showed similar behavior, finding higher G' values than G'' values, indicating solid material behavior. Thus, spreads showed a predominant elastic behavior, as in the results observed l [2,17,31].

Spreads B and D spreads showed the highest G' and G'' values, corroborating the results for SFCmax of the lipid phase at 25°C, hardness, spreadability, d 0.5 (Table 1) and AGS content (Table 3). The lowest G' and G'' values were observed for spreads A and E, thus justifying the reduced SFCmax at 25°C, d 0.5 and number of crystalline elements values, also explaining the low hardness and spreadability values (Table 1).

Spreads B, D and F showed a greater number of crystalline elements (Table 6), which is directly related to the high G' and G'' and with the high hardness, spreadability and SFCmax at 25°C values (Table 1). Spreads A, D and E showed more well-defined crystals (Fig. 4), probably due to the high fat crystalline density of the spreads.

Oil exudation content

There was no liquid oil exudation in any of the spreads, which may be associated to the following stabilizing properties: SFCmax at 25°C, hardness, spreadability, particle size and distribution, β' polymorphism, mean crystal density during the stabilization period at 25°C for 60 days and to the solid material behavior, since all presented G' greater than G''.

CONCLUSIONS

The chocolate spreads analyzed in this study showed a characteristic stability, since no liquid oil exudation occurred during the stabilization period at 25°C for 60 days, a result that can be associated to other properties of the spreads, such as SFCmax at 25°C, hardness, spreadability, particle size and distribution, β' polymorphism, mean crystal density and solid material behavior, since all presented G' greater than G''. In addition, β' polymorphism, in addition to particle size under 34.80 μm in d 0.9, present in the chocolate spreads of this study provides particles that are not sensorially perceptible. Hardness and spreadability were adequate for spreadable products; however, they could present lower AGS levels (from 19.31 to 35.05%) to meet the demand of consumers seeking healthier foods.

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Table 1. Lipid content, hardness, spreadability, particle size and distribution of commercial chocolate spreads and IT and SFCmax of their lipid phases.

Samples	LC (%)	IT (minutes)	SFCmáx (%)	Hardness (Kg)	Spreadability (Kg.s)	*Span* (µm)	d 0.5 (µm)	d 0.9 (µm)
A	17.66 ± 0.17 ^{a}	71.00±11.31 ^{a}	2.41±0.49 ^{a}	1.90±0.21 ^{abc}	1.36±0.11 ^{ab}	3.31±1.06 ^{a}	8.50±0.76 ^{a}	28.15±3.78 ^{a}
B	22.51 ± 0.99 ^{b}	26.50±10.61 ^{b}	4.29+0.37 ^{b}	3.01±0.43 ^{d}	2.78±0.61 ^{c}	2.72±0.60 ^{a}	12.03±0.88 ^{d}	35.02±5.99 ^{a}
C	24.90 ± 0.99 ^{c}	30.50±7.78 ^{b}	2.20±0.01 ^{a}	1.34±0.44 ^{a}	1.10±0.31 ^{a}	2.45±0.19 ^{a}	8.48±0.24 ^{a}	23.89±1.84 ^{a}
D	23.08 ± 0.34 ^{bc}	16.50±7.78 ^{b}	5.35±0.88 ^{c}	2.36±0.52 ^{bc}	2.18±0.47 ^{bc}	2.65±0.19 ^{a}	10.9±0.21 ^{cd}	31.86±2.25 ^{a}
E	20.99 ± 0.84 ^{b}	25.50±4.95 ^{b}	2.17±0.03 ^{a}	1.56±0.36 ^{ab}	1.13±0.24 ^{a}	2.71±0.26 ^{a}	9.84±0.51 ^{bc}	29.66±3.53 ^{a}
F	18.37 ± 1.11 ^{a}	66.00±3.00 ^{a}	2.55±0.06 ^{a}	2.52±0.33 ^{cd}	1.90±0.26 ^{abc}	3.05±0.05 ^{a}	9.07±0.32 ^{ab}	30.43±1.27 ^{a}

LC: lipid content; **IT:** Induction time; **SFCmax:** Maximum solids content.

Table 2. Nutritional information of commercial spreads.

NUTRITIONAL INFORMATION												
	A 20 g serving (1 tablespoon)		**B** 20 g serving (1 tablespoon)		**C** 20 g serving (1 tablespoon)		**D** 25 g serving (1 tablespoon)		**E** 20 g serving (1 tablespoon)		**F** 20 g serving (1 tablespoon)	
	Amount per serving	**% DV (*)**	**Amount per serving**	**% DV (*)**	**Amount per serving**	**% DV (*)**	**Amount per serving**	**% DV (*)**	**Amount per serving**	**% DV (*)**	**Amount per serving**	**% DV (*)**
Energy value	107 Kcal ou 446 KJ	5	112 Kcal ou 470 KJ	6	108 Kcal ou 454 KJ	5	133 Kcal ou 558 KJ	7	111 Kcal ou 466 KJ	6	106 Kcal ou 442 KJ	5
Carbohydrates	12 g	4	11 g	4	12 g	4	17 g	6	11 g	4	13 g	4
Proteins	1,3 g	2	0,6 g	1	0,7 g	1	0 g	0	0,9 g	1	0,8 g	1
Total Fat	6,2 g	11	7,3 g	13	6,6 g	12	7 g	13	6,9 g	13	5,6 g	9
Saturated fat	2,1 g	10	2,6 g	12	1,1 g	5	1,8 g	8	0,9 g	4	2,3 g	12
Trans fats	0 g	**	0 g	**	0 g	**	0 g	**	0 g	**	0 g	**
Dietary fiber	0,6 g	2	0,5 g	2	0 g	0	0 g	0	0,6 g	2	0,7 g	3
Sodium	8,4 mg	1	37 mg	2	12 mg	1	27 mg	1	7,8 mg	0	9,0 mg	0
(*)% Reference daily values are based on a 2,000 kcal or 8,400 KJ diet. Personal daily values may be higher or lower depending on one's energy needs. (**) Daily value not established.												

Table 3. Fatty acid composition of lipid phases of commercial chocolate spreads.

Samples	A	B	C	D	E	F
Fatty Acids (%)						
Capric C10:0	0.04±0.01	0.09±0.01	n.d.	n.d.	n.d.	n.d.
Lauric C12:0	0.51±0.30	0.16±0.02	0.07±0.01	0.12±0.05	0.52±0.70	0.84±0.12
Myristic C14:0	0.80±0.08	1.01±0.01	0.19±0.01	0.85±0.10	0.58±0.23	0.77±0.05
Pentadecyl C15:0	0.05±0.00	0.06±0.01	0.03±0.01	0.04±0.01	0.06±0.01	0.07±0.01
Palmitic C16:0	27.99±0.93	25.55±0.08	12.06±0.14	26.66±1.44	15.06±1.19	21.58±0.57
Palmitoleico C16:1	0.19±0.01	0.41±0.01	0.11±0.00	0.38±0.02	0.30±0.05	0.22±0.01
Margarico C17:0	0.10±0.00	0.11±0.03	0.10±0.01	0.10±0.03	0.13±0.01	0.08±0.02
Heptadecenoic C17:1	0.04±0.00	0.05±0.00	0.05±0.00	0.05±0.01	0.13±0.06	0.06±0.01
Stearic C18:0	5.03±0.09	5.61±0.37	5.57±0.14	5.72±0.67	6.13±0.37	4.91±0.20
Oleic C18:1	53.94±0.44	23.15±1.30	31.31±1.53	22.45±0.84	53.93±9.13	57.93±2.09
Elacidico C18:1t	0.13±0.01	0.12±0.00	0.44±0.02	0.13±0.00	0.24±0.09	0.16±0.09
Linoleic C18:2	10.24±0.78	42.49±0.87	44.44±1.35	42.16±1.18	19.07±7.45	10.15±2.20
Linolenic C18:3	0.21±0.07	0.43±0.09	4.13±0.26	0.54±0.26	1.36±0.34	1.75±0.64
Arachidic C20:0	0.36±0.01	0.35±0.04	0.46±0.02	0.33±0.03	0.77±0.12	0.50±0.03
Gadoleic C20:1	0.18±0.00	0.11±0.01	0.20±0.00	0.11±0.02	0.69±0.25	0.55±0.02
Behenic C22:0	0.09±0.01	0.16±0.02	0.64±0.07	0.24±0.08	0.70±0.09	0.20±0.01
Lignoceric C24:0	0.08±0.02	0.12±0.03	0.19±0.01	0.16±0.01	0.30±0.12	0.25±0.13
Totals						
Unsaturated	64.80±1.30	66.64±2.28	80.24±3.16	65.69±2.33	75.48±17.27	70.66±4.97
Saturated	35.05±1.46	33.22±0.61	19.31±0.43	34.22±2.41	24.25±2.85	29.20±1.15
Trans	0.13±0.01	0.12±0.00	0.44±0.02	0.13±0.00	0.24±0.09	0.16±0.09
ω6:ω9	0.19	1.83	1.42	1.88	0.35	0.17

n.d.: not detected

Table 4. Sales denomination and list of ingredients of commercial spreads

Samples	Sales Name	Ingredients
A	Hazelnut and cocoa cream	Sugar, vegetable oil (palm), hazelnuts (13%), partially defatted cocoa powder (7.4%), skimmed milk powder (5.6%), whey powder, soy lecithin emulsifier, flavoring. Milk constituents 8.7%.
B	Chocolate flavored cream	Sugar, vegetable fat, whey permeate, cocoa powder, whole milk powder, cocoa mass, salt, soy lecithin emulsifiers and polyglycerol polyricinoleate and flavoring.
C	Hazelnut and cocoa cream	Sugar, soybean oil, vegetable fat, whey powder, hazelnuts, cocoa powder, skimmed milk powder, soy lecithin emulsifier, artificial chocolate flavor.
D	Chocolate flavored cream	Sugar, refined vegetable oils, whey powder, cocoa powder, modified starch, vitamin A, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6, folic acid, vitamin B12, calcium, iron, zinc, emulsifier.
E	Hazelnut and cocoa cream	Sugar, vegetable oil, hazelnut paste, cocoa powder, skimmed milk powder, whey powder, fractionated vegetable fat, soy lecithin emulsifier, INS 322 and flavoring.
F	Hazelnut cream	Sugar, vegetable oils (palm), hazelnut (13%), cocoa powder, skimmed milk powder, whey powder, lactose, emulsifier sunflower lecithin and flavoring.

Table 5. Thermal behavior of the lipid phases of commercial chocolate spreads

Crystallization									
Samples	**Peak 1**				**Peak 2**				
	T_{oc} (ºC)	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**T_{oc} (ºC)**	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**ΔH_{total} (J/g)**
A	8.62	-7.77	-40.61	28.83	-43.65	-49.83	-58.35	6.65	35.48
B	16.59	12.29	1.25	2.54	-0.14	-10.67	-46.39	21.02	23.56
C	17.44	8.62	-5.36	1.34	-5.36	-16.67	-54.81	11.20	12.54
D	18.30	15.38	-0.46	4.69	-1.20	-11.21	-47.82	23.53	28.22
E	17.15	8.81	-8.83	1.42	-8.71	-18.40	-35.94	4.81	6.23
F	14.99	11.20	-0.67	3.25	-3.02	-11.80	-37.12	12.56	15.81
Melting									
Samples	**Peak 1**				**Peak 2**				
	T_{om} (ºC)	**T_{pm} (ºC)**	**T_{fm} (ºC)**	**ΔH_m (J/g)**	**T_{om} (ºC)**	**T_{pm} (ºC)**	**T_{fm} (ºC)**	**ΔH_m (J/g)**	**ΔH_{total} (J/g)**
A	-22.07	4.89	38.19	56.65	n.d.	n.d.	n.d.	n.d.	56.65
B	-41.25	0.91	15.49	43.74	17.73	36.49	49.54	5.93	49.67
C	-40.29	-22.94	8.14	54.63	12.18	30.22	42.76	2.18	56.81

D	-41.03	0.12	14.41	42.70	16.65	37.72	52.83	8.63	51.33
E	-35.81	-18.27	7.58	47.94	11.80	30.42	42.39	3.23	51.17
F	-30.47	-2.51	48.73	61.23	n.d.	n.d.	n.d.	n.d.	61.23

T_{oc} and T_{om}: onset crystallization and melting temperature; **T_{pc} and T_{pm}** peak crystallization and melting temperature; **T_{fc} and T_{fm}:** final crystallization and melting temperature; **ΔH_c, ΔH_m and ΔH_{total}:** crystallization, melting and total enthalpies; **n.d.:** not detected.

Table 6. Microstructure of lipid phase of commercial chocolate spreads

Samples	Crystalline elements	Mean density	Mean D crystals (µm)	Agglomerated crystals (%)
A	1049.67±494.26	62.66±37.72	8.37±2.27	72.21±17.28
B	26470.00±13814.43	16.21±1.94	3.74±0.30	53.20±4.65
C	1774.67±1221.95	36.28±17.07	5.53±1.48	65.74±14.35
D	4965.67±3584.09	39.93±4.59	6.06±0.80	66.49±4.79
E	96.33±78.62	76.87±19.92	9.77±2.28	67.05±25.91
F	25550.00±7345.67	14.70±0.90	2.69±0.39	37.44±8.08

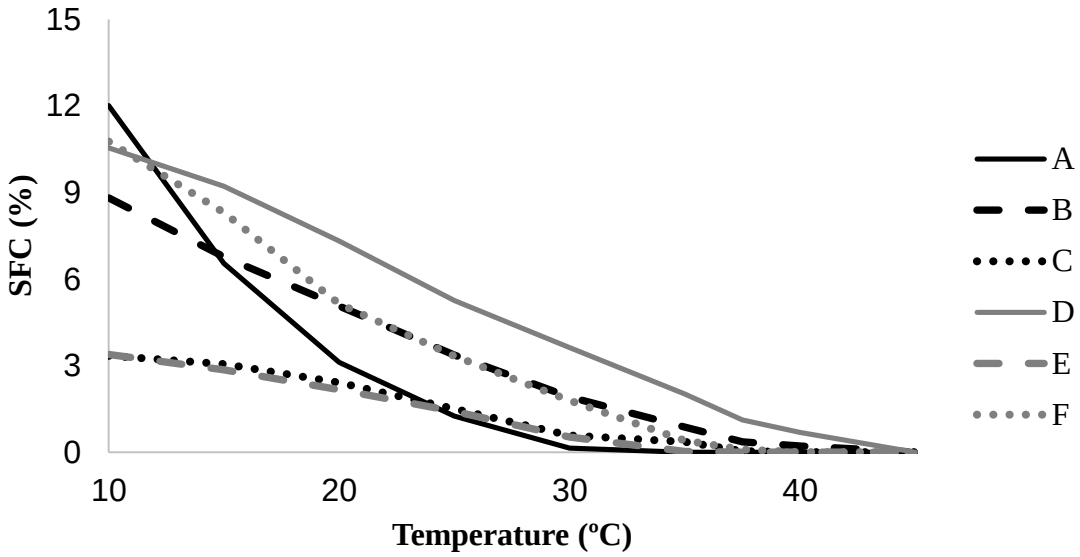


Figure 1. Fat solids profile of commercial chocolate spreads.

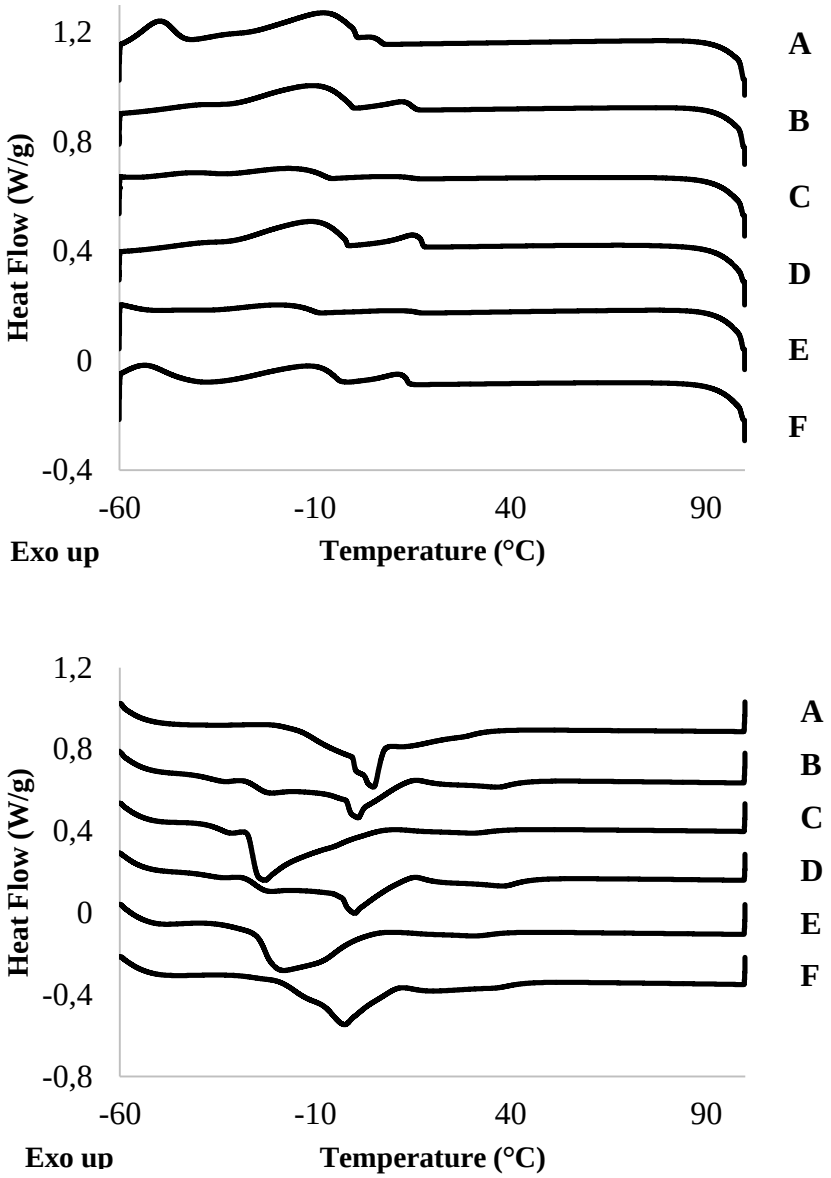


Figure 2. Fusion and crystallization curves of the lipid phase of commercial chocolate spreads.

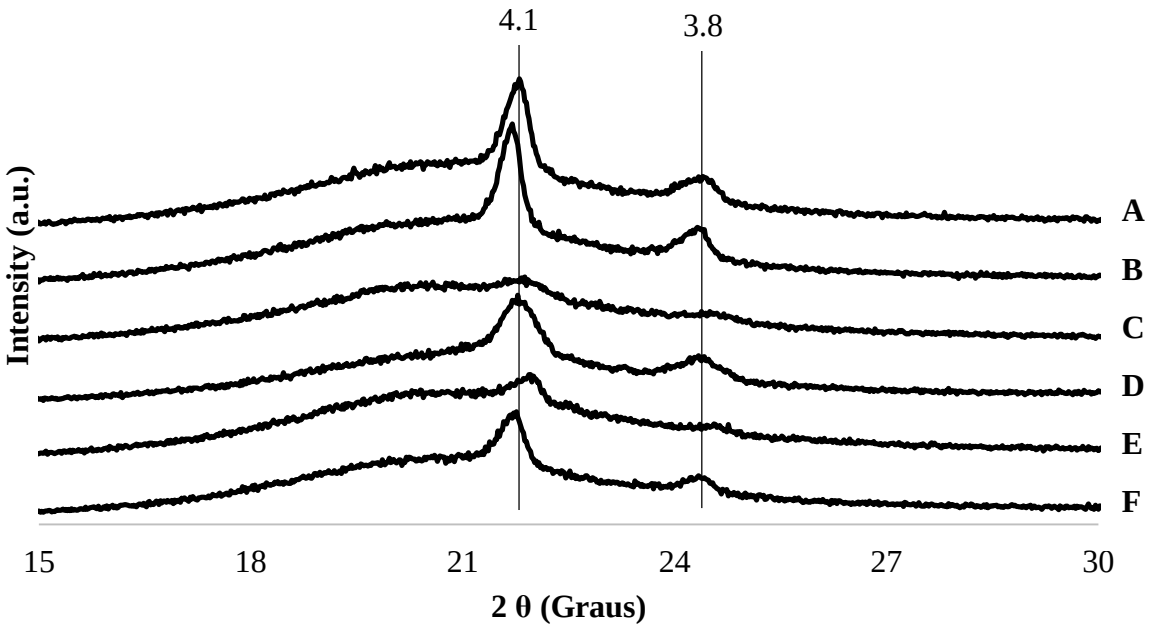


Figure 3. Lipid phase diffractograms of commercial chocolate spreads.

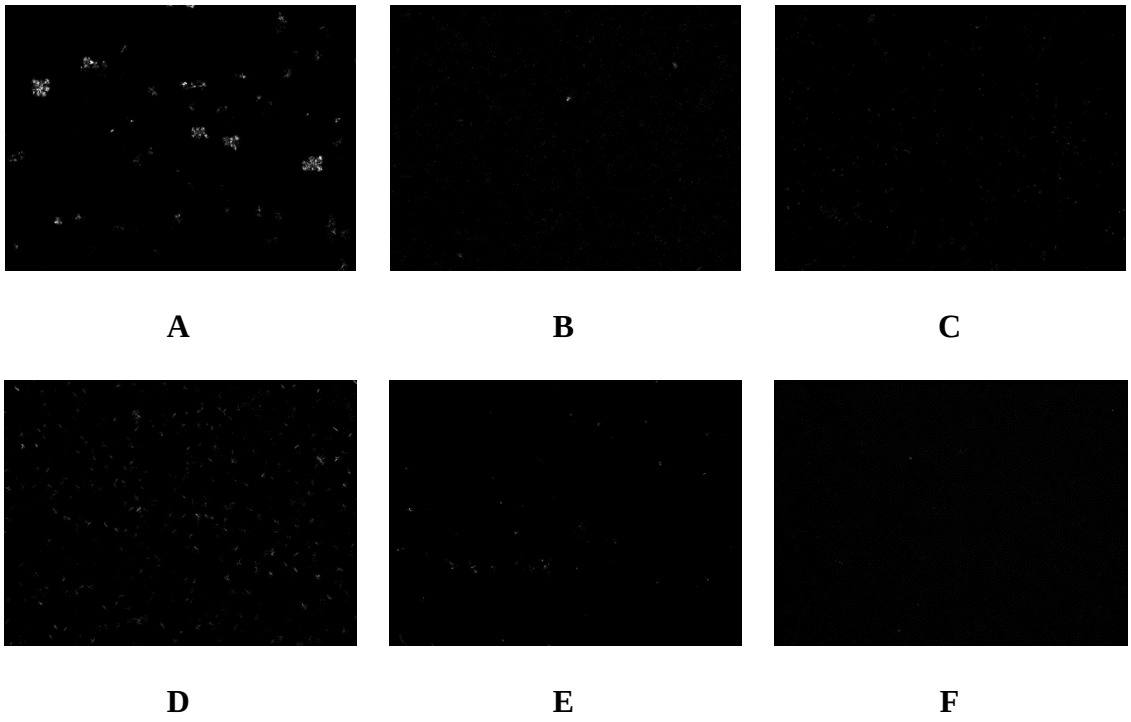
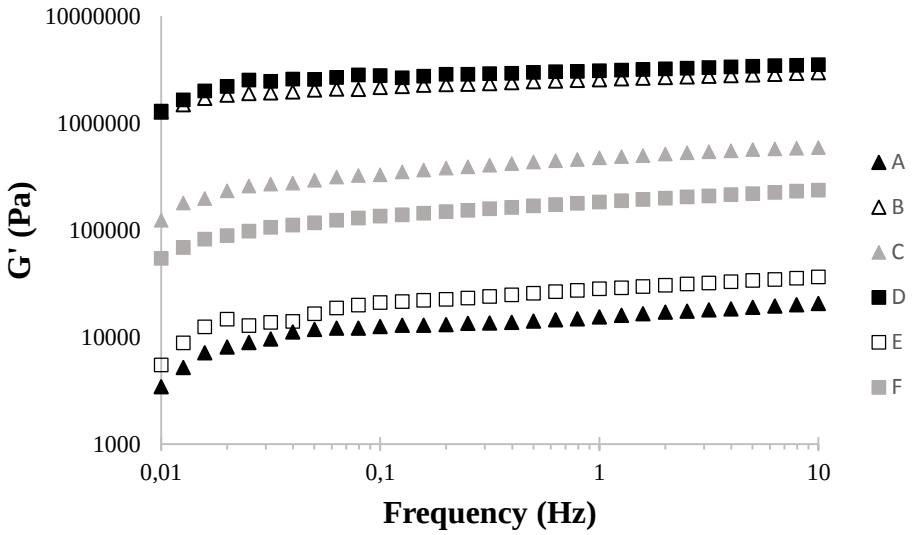
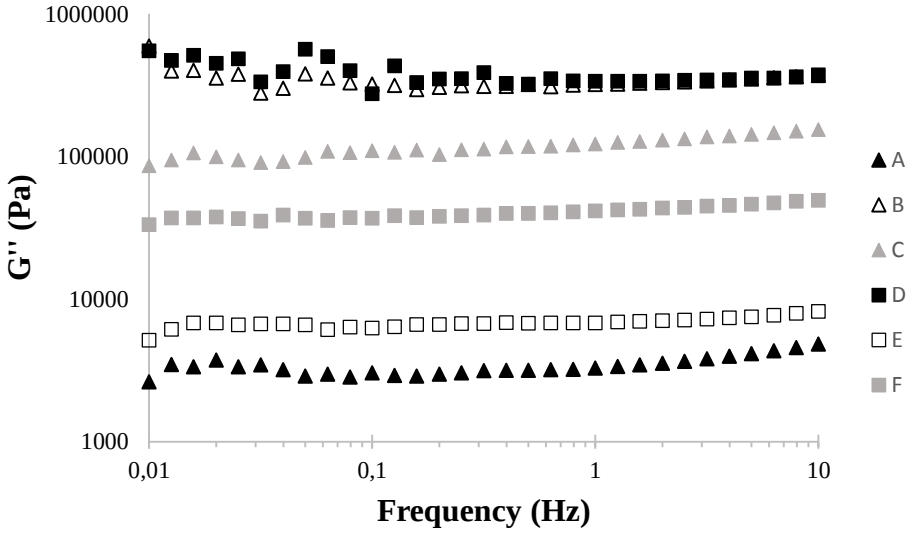


Figure 4. Microstructure of the lipid phase of commercial chocolate spreads at 20x magnification and 25°C.



(a)



(b)

Figure 5. G’ (a) and G’’ (b) of the frequency scanning of commercial chocolate spreads.

CAPÍTULO 4

FOOD GRADE STRUCTURING AGENTS FOR OLEOGELS: A PHYSICOCHEMICAL STUDY

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Food grade structuring agents for oleogels: a physicochemical study

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ABSTRACT

Structural agents can be used to form oleogels through three-dimensional network mechanisms of the self-assembly or crystalline particle types. Oleogels are viscoelastic materials composed of structuring agents and a non-polar liquid phase, making them semi-solid systems. The oil phase is immobilized by a three-dimensional network, self-assembly by the structurant. From an industry standpoint, the use of oleogels in food products is a very attractive option. They provide foods with characteristics typical of conventional fats, such as consistency and plasticity, without the use of *trans* fatty acids and while significantly reducing saturated fatty acid content. A structurant is considered efficient when its use in low concentrations allows for stable oleogels to be obtained. The objective of this study was an extensive physicochemical characterization of food-grade structurants with potential use in the development of oleogels. The following structurants were characterized in terms of their fatty acids composition, solid content (SC), crystallization kinetics, thermal behavior during melting and crystallization, polymorphism, and microstructure: candelilla wax (CW), fully hydrogenated oils from soybean

(HS), crambe (HC) and palm (HP), monoacylglycerols (M), and sorbitan monostearate (S). All structurants exhibited similar properties, such as high saturated fatty acid concentration, high solids at the analyzed temperature, low crystallization induction time, high thermal resistance, β' preferential polymorphism, as well as uniform parameters in regards to crystalline morphology and dimensions.

Keywords: structuring agents, saturated fatty acids, lipid bases, solid content.

1. INTRODUCTION

Lipid structures can have high or low molar mass. Molecules with less than 3000 Da are considered to have low molecular weight [1]. They are used for immobilizing liquid oils by forming self-assembly three-dimensional crystalline lattice, which provide structure [2].

Oleogels are viscoelastic materials composed of structuring agents and a non-polar liquid phase (equivalent to the organic compound). This makes them different from other gels, which are mostly formed by water-soluble compounds. Thus, oleogels are semi-solid systems, in which the oily phase is immobilized by the structurant's self-assembly three-dimensional network [3–5].

To ensure technological effectiveness, the lipid structurant must possess certain chemical and physical characteristics, including a thermal behavior, polymorphism and microstructure compatible with the lipid phase one intends to structure. In this way, the structurant can contribute to intensify the effects of crystallization or gelation in different lipid systems [6].

A structurant is considered efficient when its use in low concentrations is sufficient to obtain stable oleogels, at a production cost compatible with the targeted application. In fact, the effects of high concentrations of these additives over the human health are yet to be fully elucidated [7].

The literature has multiple examples of oleogels, structured using 12-hydroxystearic acid [8], plant sterols [9], lecithins [10], monoacylglycerols and diacylglycerols, mixtures of lecithin with sorbitan esters [11, 12], fatty acids and alcohols [13], waxes and wax esters [4, 14–18], as well as fully hydrogenated oils (also known as hardfats) [19–22].

In recent years, the potential of waxes as structurants has led to these materials being used in alternative techniques for the structuring of oils, and on this basis various edible oil-structuring systems have been intensively researched [14, 23–25].

Candelilla wax (CW) is derived from leaves of *Euphorbiaceae* family, found in northern Mexico and southwestern United States [26]. In the United States, CW has been approved as a food additive by the Food and Drug Administration (FDA), being classified as a GRAS ("Generally Recognized as Safe") food ingredient [27]. CW applications are limited only by its characteristic waxy aftertaste, as can be experienced in margarine formulated with this compound [28–31].

Emulsifiers have also been described in the scientific literature as components with the potential to structure complex lipid matrices [2, 32]. Sorbitan monostearate (S) is a hydrophobic non-ionic surfactant emulsifier, often used in combination with polysorbates in cakes, fillings and creamy toppings. It promotes increase in volume and softness, and has a considerable potential for modifying crystallization properties in lipid systems [33]. In addition, the self-assembly mechanism allows it to form viscous dispersions in organic solvents and edible oils [7, 34–36].

Fully hydrogenated vegetable oils, known as hardfats, are an interesting option for structuring lipid phases. These materials are considered model systems in terms of the fatty acids and triacylglycerols compositions. Both components are important factors of the structuring and modifying effects of crystallization processes, in continuous or emulsified lipid phases [37].

In addition to their function as primary crystallization agents, hardfats modify the physical properties of continuous fatty systems, allowing for several adaptations related to the development of oleogels. This modifying feature has justified a series of studies on the conventional structuring of triacylglycerols [19, 38–40].

The objective of this study was the extensive physicochemical characterization of food-grade structurants with potential use in the development of oleogels. Characteristics of fatty acid composition, solid content (SC), crystallization kinetics, melting and crystallization behavior, polymorphism and microstructure were described.

2. MATERIAL AND METHODS

2.1. Material

The evaluated structurants were: CW (Light Special Candelilla REAL®), supplied by Multiceras SA, García – NL, México; fully hydrogenated vegetable oils (hardfats), obtained from palm oil (HP), soybean oil (HS) (SGS Agricultura e Indústria Ltda, Brazil) and crambe oils (HC) (Cargill, Itumbiara – GO, Brazil); M Grindsted Crystallizer 100, supplied by DuPont do Brasil SA; and S, produced by Sigma Aldrich, St. Louis, Missouri, USA.

2.2. Physicochemical characterization

Fatty acid composition. The analysis of fatty acid composition was performed in a gas chromatograph with capillary column, after esterification using the Hartman and Lago method [41]. Fatty acid methyl esters were separated according to the AOCS Ce 1f-96 method using the Agilent [42] DB-23 column (50% cyanopropyl-methylpolysiloxane), dimensions: 60 m, f int: 0.25 mm, 0.25 mm film. Analysis conditions: oven temperature 110 °C – 5 min, 110–215 °C (5°C/min), 215 °C – 24 min; detector temperature: 280 °C; injector temperature: 250 °C;

drag gas was helium; split ratio: 1:50; injection volume: 1.0 μL. Qualitative composition was determined by comparing peak retention times with standards for the respective fatty acids.

Solid content (SC). SC was determined using a Bruker pc120 Minispec Nuclear Magnetic Resonance Spectrometer with the aid of high precision dry baths (0–70 °C) using a TCON 2000 (Duratech, USA) dry bath system, according to AOCS method Cd 16b-93: direct method, serial reading of samples, with tempering for the structurants [42].

Crystallization kinetics. Obtained by stabilizing the structurants (100ºC for 15 min, 1 h at 70ºC), with SC measured at 25ºC (± 0.5ºC) in a Mq20 NMR AnalyzerBruker Nuclear Magnetic Resonance Spectrometer [43]. The data were automatically acquired, with one sampling per minute during 1 hour. Crystallization kinetics were characterized per the induction period (τSC) – referring to the beginning of crystal formation and obtaining of the maximum solids content (SCmax) [34, 44].

Thermal behavior. Structurant thermal analysis was performed in a TA Q2000 differential scanning calorimeter (DSC) coupled to the RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing system used was the Universal V4.7A (TA Instruments, Waters LLC, New Castle). Analysis conditions were: ~10 mg mass; modified AOCS Cj 1-94 method [42]; temperature between –60 and 100 °C, with a 5 °C/min ramp (crystallization and melting). The following parameters were used to evaluate the results: onset crystallization and melting temperatures (T_{OC} and T_{OM}), peak crystallization and melting temperatures (T_{PC} and T_{PM}), crystallization and melting enthalpies (Δ_{CH} and Δ_{MH}), and final crystallization and melting temperatures (T_{FC} and T_{FM}) [44, 45].

Polymorphism. Polymorphism was determined by X-ray diffraction, according to the procedures of Sawalha et al. [46]. The analyses were conducted in a Philips diffractometer (PW 1710), using Bragg–Bretano geometry (θ:2θ) with Cu-k radiation (λ = 1.54056 Å, 40 KV voltage and 30 mA current). The measurements were obtained at 0.02-degree steps in 2θ and

acquisition time of 2 seconds, with scans of 15 to 30° (2θ scale) at 25 °C. Identification of crystalline forms was based on crystals' characteristic short spacings (SS) [42].

Microstructure. Structurant microstructure was evaluated by polarized light microscopy. With the aid of a capillary tube, a drop of structurant was placed on a glass slide. A cover slip was placed over the slide, which was then kept at 5 °C for 24 hours and 25 °C for another 24 hours. Structurants were analyzed at 25 °C using 20× magnification. The microstructure of the crystals was evaluated using a polarized light microscope (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). Images were captured using Image Pro-Plus 7.0 software (Media Cybernetics, USA), with four different visual fields for each fat sample slide. Mean particle diameter results were expressed as the mean and standard deviation of these four measurements [47, 48].

3. RESULTS AND DISCUSSION

3.1. Fatty acids composition

As a basis for comparison, we used the CW composition obtained by Doan et al. (2017) by High Performance Liquid Chromatography coupled to Evaporative Light Scattering Detector (HPLC-ELSD), given that CW was not chemically characterized in this study. According to the authors, hydrocarbons (72%) are the main component of CW, followed by wax esters (15%), free fatty acids (9%), and free fatty alcohols (2%). The main fraction is composed mostly of n-alkanes with saturated chains of odd numbers of carbons (29 to 33). C31 accounts for 82% of that fraction. Wax esters are mainly composed of saturated fatty acids (C16, C18 and C22) esterified in saturated fatty alcohols (C18, C28 and C30).

The determination of structurants' chemical composition is of great relevance for evaluating their interactions with organic materials. Besides, it helps understand the formation of crystalline networks, as well as assembled or hybrid structures.

Table 1 shows structurants' fatty acids composition. Overall, fatty acid (palmitic, stearic and behenic) contents ranged from 96.73 to 99.81%. Unsaturated fatty acids ranged between 0.19 and 3.27%. No *trans* fatty acids were detected, indicating the efficiency of the hardfats total hydrogenation process. *Trans* fatty acids are considered nutritionally undesirable [50].

Structurants exhibited distinct fatty acid profiles, despite similar levels of saturated and unsaturated fatty acids. HP had 43.09% palmitic acid and 54.11% stearic acid, similar to results obtained by Masuchi et al. (2014) and Oliveira et al. (2015). HS had 10.84% palmitic acid and 87.24% stearic acid, in agreement with previously reported results [6, 21, 52–54]. HC had 7.61% palmitic acid, 47.71% stearic acid and 37.67% behenic acid, also in agreement with other studies evaluating these materials [6, 21, 55, 56]. Among these hardfats, fatty acids with varied in chain sizes (C16:0–C22:0), molecular weights (256.43–340.59 $g \cdot mol^{-1}$) [57] and melting temperatures (59.5–79.9ºC) were predominant [58, 59]. These parameters are important for the evaluation of hardfats' mechanisms and structuring properties. HC in particular stands out as the only hardfat containing high levels of behenic acid (C22:0) for structuring oleogels [56].

The M structurant had 4.44% palmitic acid, 47.82% stearic acid, 4.96% arachidic acid and 41.30% behenic acid, consistent with results obtained by Silva et al., (2018). As can be seen, proportions of stearic (C18:0) and behenic (C22:0) acid were equivalent. The S structurant had 40.29% palmitic acid and 51.13% stearic acid.

Palmitic (C16:0), stearic (C18:0) and behenic (C22:0) acids were the main saturated fatty acids in the structurants. Palmitic acid is naturally present in palm oil, in concentrations between 40 and 45%. This is reflected in the observed HP composition. The S structurant also showed a composition typical of a fully hydrogenated palm oil fraction. Stearic acid was predominant in all structurants. This is explained by the fact that structurants' raw materials contain high levels of unsaturated fatty acids such as oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and

erucic (C22:1) (crambe oil). After total hydrogenation, these fatty acids result in stearic (C18: 0) and behenic (C22: 0) acids [53, 60].

Recent studies shows that the effects of different structuring agents' fatty acid composition, chain sizes and molecular weights are dependent on the organic phase used to obtain the oleogel, even when considering equal concentrations of structurants [30, 34, 45].

Saturated fatty acids increase lipids' structuring capabilities and have the important property of acting as a physical barrier against moisture [61]. Lipid materials rich in saturated fatty acids have higher oxidative stability, since they are not susceptible to lipid oxidation [62].

The fatty acid composition of the structurants can directly affect certain characteristics of the lipid bases and the products in which they are applied, such as consistency, spreadability, SC, thermal behavior and rheological properties. These differences in fatty acid composition can produce structured oils with varied nutritional and technological implications [63].

Stearic acid in the body can be transported to the mitochondria by the Carnitine Palmitoyl transferase I enzyme, where it is oxidized [64] and may be transformed into oleic acid (18:1 n-9) by the stearoyl-CoA desaturase protein, known to promote the conversion of saturated fatty acids to monounsaturated [65]. This point to the neutral effects of stearic acid over the body. Behenic acid has been used in several applications because it inhibits the action of pancreatic lipase, reducing calories in oils and fats [66].

Table 1. Composition in fatty acids of the structurants

Samples	HS	HC	HP	M	S
			Fatty Acids		
Capric C10:0	n.d.	0.06±0.00	n.d.	n.d.	n.d.
Lauric C12:0	n.d.	0.42±0.01	0.33±0.02	0.06±0.04	n.d.
Myristic C14:0	0.10±0.01	0.31±0.01	1.08±0.02	0.10±0.00	2.09±0.04

Pentadecylic C15:0	n.d.	n.d.	n.d.	n.d.	0.49±0.02
Palmitic C16:0	10.84±0.12	7.61±0.05	43.09±0.73	4.44±0.05	40.29±0.90
Palmitoleic C16:1	n.d.	n.d.	n.d.	n.d.	0.82±0.01
Margaric C17:0	0.20±0.01	0.09±0.00	0.16±0.04	0.09±0.00	2.00±0.06
Heptadecenoic C17:1	n.d.	n.d.	n.d.	n.d.	0.16±0.00
Stearic C18:0	87.24±0.25	47.71±0.11	54.11±0.78	47.82±0.10	51.13±0.53
Oleic C18:1	0.13±0.11	0.37±0.16	0.40±0.10	0.23±0.02	1.72±0.20
Linoleic C18:2	0.06±0.01	0.07±0.02	0.13±0.07	0.06±0.03	0.56±0.10
Arachidic C20:0	0.73±0.00	4.16±0.02	0.58±0.03	4.96±0.01	0.49±0.00
Behenic C22:0	0.52±0.01	37.67±0.13	0.08±0.00	41.30±0.11	0.24±0.03
Lignoceric C24:0	0.19±0.00	1.51±0.02	0.08±0.00	0.92±0.00	n.d.
		Totals			
Saturated	99.81	99.56	99.47	99.70	96.73
Unsaturated	0.19	0.44	0.53	0.30	3.27

HS: soy hardfat; **HC**: crambe hardfat; **HP**: palm hardfat; **M**: monoacylglycerols; **S**: sorbitan monostearate; **n.d.:** not detected.

3.2. Solids content

SC as a function of temperature provides an indication of the basic physicochemical attributes and technological performance of the structurants. SC values at temperatures below 25 °C serve to qualify hardness parameters, while SC at temperatures between 25 and 30 °C refer to thermal resistance, considering that in this temperature range most lipid bases start melting (Danthine & Deroanne, 2006). Structurants present a different composition than conventional fats, but are useful as lipid bases for productive applications. This justifies the evaluation of their SC at different temperatures.

Figure 1 shows structurants' SC values in the 10 to 70ºC range. All structurants showed high mechanical and thermal resistance. In the 25 to 35ºC temperature range, they presented a SC of 91.16%, due to the high concentrations of saturated fatty acids in these materials (Table 1). Structurant M exhibited the highest thermal and mechanical resistance, followed by structurants HS, CW, HC, HP and S (according to melting temperatures, in decreasing order). In addition, structurants' melting range was related to the presence of higher behenic acid (C22:0) (79.9 °C) [58] and stearic acid (C18:0) (69.6 °C) concentrations [67]. In comparison to others, structurant S presented decreased SC at lower temperatures, probably due to its higher palmitic acid contents (59.5ºC) [59]. Among the saturated fatty acids predominant in the different structurants, palmitic acid has the smallest chain size (Table 1).

This analysis allowed for the determination of each structurant's solid fraction during melting, with the exception of CW and M, which did not melt completely within the evaluated temperature range. Meanwhile, the temperature range for complete melting of the other structuring agents was between 55 and 70ºC, due to the presence of hydrocarbons and wax esters in CW, and high behenic acid (C22:0) contents in M (41.3%). These have a melting point above 70°C.

During crystallization of the lipid phase, materials with homogeneous chemical composition and similar molecular structures are prone to cocrystallization. Thus, the fatty acid composition of the oil to be used in the manufacturing of oleogels is of great importance [68].

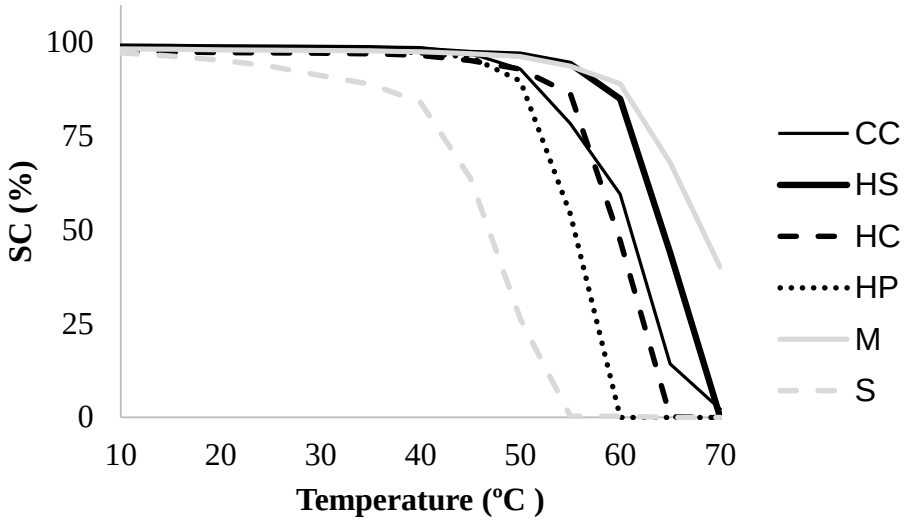


Figure 1. Solid profiles of candelilla wax (CW), soybean oil hardfat (HS), crambe oil hardfat (HC), palm oil hardfat (HP), monoacylglycerols (M), and sorbitan monostearate (S).

3.3. Crystallization kinetics

Table 2 shows structurants' induction time and SCmax when undergoing isothermal crystallization at 25 °C. IT ranged from 0 to 3 minutes, and SCmax from 91.92 to 98.96%. There was no direct relationship between IT and SCmax for any of the structurants, but all structurants became solid at the stabilization temperature.

All structurants presented high SCmax proportions, ranging from 95.73 to 98.96% solids, except for S, with 91.92% solids. This result is consistent with sorbitan monostearate's SC profile and fatty acids composition, and may be associated with the molecule's dissimilarity to the molecules of the other structurants, since the presence of esterified sorbitol makes molecular packaging difficult [68].

IT values for CW, HC and M were lower due to the presence of hydrocarbons and wax esters in CW, and due to the chain size of major fatty acids in the compositions of HC and M. These results are consistent with those reported by Basso et al. (2010), who stated that the monoacylglycerols of behenic acid are probably more effective as crystallization inducers,

promoting greater crystallization stability. This may be due to the presence of longer chains of fatty acids, as compared to those found in high proportions in the monoacylglycerols of HP.

Table 2. Induction time and maximum solids content of structurants

Estruturantes	Induction time (minutes)	Maximum solids (%)
CW	n.d.	98.96
HS	2	98.40
HC	n.d.	95.73
HP	3	98.19
M	n.d.	97.46
S	2	91.92

CW: candelilla wax; **HS:** soybean oil hardfat; **HC:** crambe oil hardfat; **HP:** palm oil hardfat; **M:** monoacylglycerols; **S:** sorbitan monostearate; **n.d.:** not detected.

3.4. Thermal behavior

Thermal behavior of structurants as a function of melting and crystallization parameters is presented in Table 3 and Figure 2. The melting and crystallization curves can be subdivided into different regions, reflecting the general composition of each structurant. The thermal parameters are presented as a function of onset crystallization and melting temperatures (T_{OC} and T_{OM}), peak crystallization and melting temperatures (T_{PC} and T_{PM}), crystallization and melting enthalpies (Δ_{CH} and Δ_{MH}), and final crystallization and melting temperatures (T_{FC} and T_{FM}).

All structurants had a single crystallization peak, except for CW and M, which had two peaks. In the case of CW, the second peak is likely associated with residues from a minor component or from processing, since its Δ_H was only 5.04 J/g, much lower than the others',

259 which ranged from 61.04 J/g (M) to 144.20 J/g (CW). M structurant's more well-defined and
260 pronounced peaks are probably due to the presence of fatty acids of different chain sizes and
261 melting points, notably stearic (C18:0) and behenic (C22:0) acids, related to peaks 2 and 1,
262 respectively.

263 Structurant M presented the highest T_{OC} (74.20ºC), followed by CW, HC, HS, S and HP
264 (65.52, 54.00, 50.34, 49.86 and 45.83ºC, respectively), in accordance with the chemical
265 composition and melting point of each structurant. The presence of hydrocarbons and wax
266 esters in CW, which have a high melting point, and the fatty acid composition of M and HC,
267 with high behenic acid (C22:0) contents, are related to the higher T_{OC} observed values. HS, HP
268 and S had similar or equivalent contents of palmitic (C16:0) and stearic (C18:0) acids,
269 associated with the single crystallization peak. As a function of fatty acids composition, these
270 parameters are also related to T_{PC}, T_{FC} and Δ_{CH}.

271 All structurants had one melting peak, except for HP and M, which showed two melting
272 peaks. In the case of HP, the first peak corresponds to palmitic acid (C16:0) (59.5 °C) [59], and
273 the second to stearic acid (C18:0) (69.6ºC) [67]. Both fatty acids appear in high concentrations
274 in these structurants, and the peaks are related to their respective melting points. In the M
275 structurant, the two more well-defined and prominent peaks were probably due to the presence
276 of fatty acids of different chain sizes and melting points, notably palmitic (C16:0) acid,
277 corresponding to peak 1, and stearic (C18:0) and behenic (C22:0) acids, corresponding to peak
278 2.

279 As in the SC analysis, in the DSC thermal behavior analysis structurants' melting began
280 at approximately 55ºC, with a minimum melting temperature of 53.24ºC (S) and a maximum
281 of 77.45ºC (M).

282 Hardfats play an interesting role in structuring triacylglycerols because of their
283 insolubility (or limited solubility) in polyunsaturated vegetable oils, and their ability to form a

284 solid crystal lattice. The different types of hardfats had similar melting characteristics, including
285 high melting point, high melting enthalpy in comparison to conventional fats, and the ability to
286 generate a matrix which crystallizes at high temperatures, forming a thin dispersion of stable
287 crystals [70–72]. The results obtained for the analyzed hardfats are consistent with those found
288 in the literature, with Δ_{MH} above 110 J/g [19].

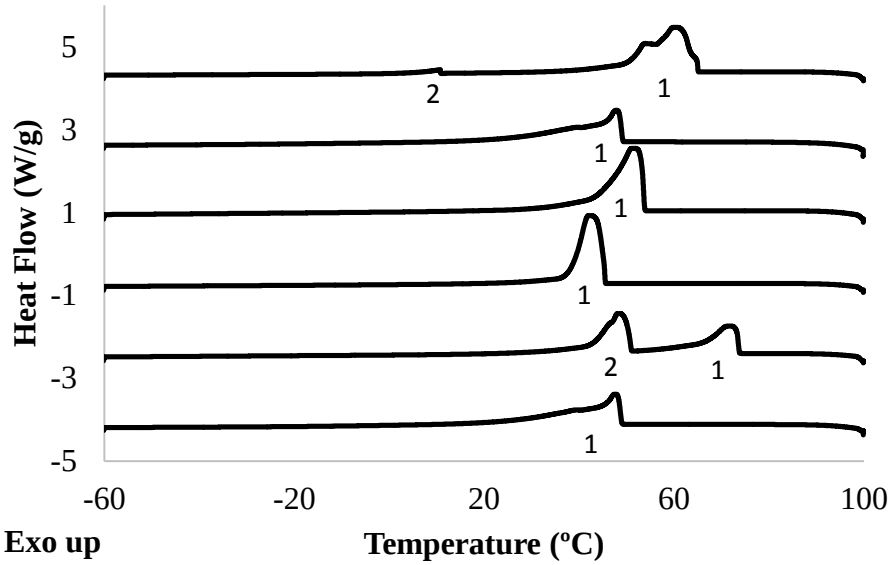
289

290 **Table 3.** Thermal behavior of structurants

Crystallization								
Structurants	**Peak 1**				**Peak 2**			
	T_{oc} (ºC)	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**T_{oc} (ºC)**	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**
CW	65.52	61.81	35.47	144.20	-12.64	-1.98	-1.77	5.04
HS	50.34	48.11	29.40	119.80	n.d.	n.d.	n.d.	n.d.
HC	54.00	52.10	21.14	123.40	n.d.	n.d.	n.d.	n.d.
HP	45.83	42.45	23.70	108.70	n.d.	n.d.	n.d.	n.d.
M	74.20	72.93	51.60	61.04	51.44	48.96	33.80	64.91
S	49.86	47.61	12.14	93.03	n.d.	n.d.	n.d.	n.d.
Fusão								
Structurants	**Peak 1**				**Peak 2**			
	T_{om} (ºC)	**T_{pm} (ºC)**	**T_{fm} (ºC)**	**ΔH_m (J/g)**	**T_{om} (ºC)**	**T_{pm} (ºC)**	**T_{fm} (ºC)**	**ΔH_m (J/g)**
CW	45.22	66.27	85.50	111.60	n.d.	n.d.	n.d.	n.d.
HS	43.95	54.16	57.53	86.21	57.69	64.13	70.64	114.90
HC	46.47	62.06	71.15	90.81	n.d.	n.d.	n.d.	n.d.
HP	44.22	50.20	53.20	29.81	53.20	58.91	65.54	57.05
M	41.18	54.26	57.21	42.91	57.69	77.45	86.22	63.02

S	27.32	53.24	65.36	54.40	n.d.	n.d.	n.d.	n.d.

CW: candelilla wax; **HS:** soybean oil hardfat; **HC:** crambe oil hardfat; **HP:** palm oil hardfat; **M:** monoacylglycerols; **S:** sorbitan monostearate; **T_{oc} and T_{om}**: onset crystallization and melting temperatures; **T_{pc} and T_{pm}** peak crystallization and melting temperatures; **T_{fc} and T_{fm}:** final crystallization and melting temperatures; **ΔH_c and ΔH_m** crystallization and melting enthalpies; **n.d.:** not detected.



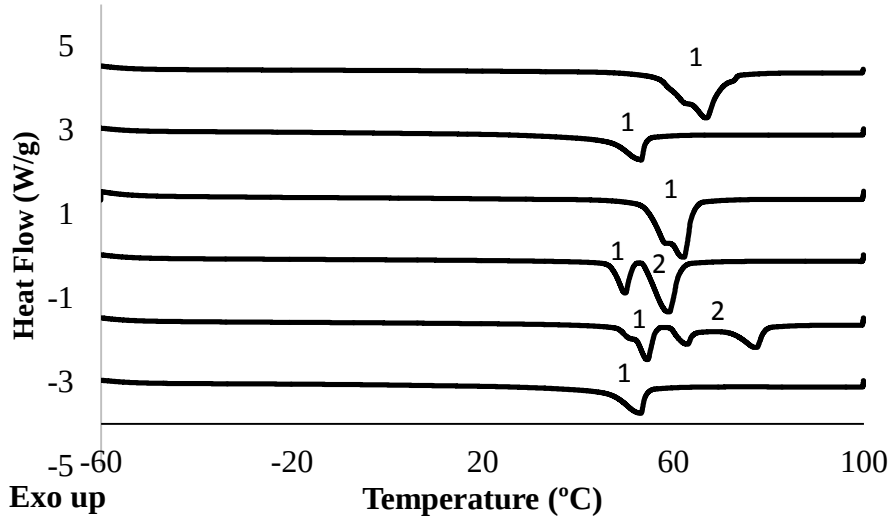


Figure 2. Fusion and crystallization curves of candelilla wax (CW), soybean oil hardfat (HS), crambe oil hardfat (HC), palm oil hardfat (HP), monoacylglycerols (M) and sorbitan monostearate (S).

3.5. Polymorphism

Figure 3 shows diffractograms for the structurants. Crystalline forms are characterized by short spacings (SS) or interplanar distances between fatty acid chains, which can be determined by X-ray diffraction. Characteristic SS values for lipid materials are 4.15 Å for the α-form; 3.8 and 4.2 Å for the β' form, and 4.6 Å for the β form [42, 54]. Short spacings are used to determine the polymorphs present in the structurants.

In this study, a SS of 4.6 Å was verified in the HS and M structurants, which had a β polymorphic form; meanwhile, structurants CW, HS, HC, HP and M had crystals with SS values of 4.2 and 3.8 Å, corresponding to the β' form. S had a SS value of 4.15 Å, corresponding to the crystalline phase α (Table 4), probably due to the presence of esterified sorbitol in the molecule, which hinders its molecular packaging and, consequently, its polymorphic transition.

The more heterogeneous the composition of the structurant, the greater the tendency towards stabilization of the β' polymorphic habit, given the difficult crystalline packaging of the more stable crystalline form [44, 68]. The homogeneity of HS, due to the high proportion (87.24%) of stearic acid (C18:0) in its composition, favors the presence of the β polymorphic habit. This is explained by this structurant's ease of molecular packaging. HC has an heterogeneous fatty acid composition (Table 1), leading to the stabilization of the β' polymorphic form. The HC and HP structurants have a predominance of palmitic (C16:0) and stearic (C18:0) acids. Palmitic acid favors the stabilization of the β' polymorphic form [44]. Ribeiro et al. (2013) found that HP and HS oils are characterized by polymorphic forms β' and β, respectively. These properties of crystallinity make them suitable to different lipid-based food applications.

Although the M structurant has a high concentration of behenic acid (C22:0), which favors the β polymorphic form, its molecular structure is heterogeneous, due to the regiospecific distribution typical of monoacylglycerols.

Tripalmitin and monoacylglycerols in behenic acid act as accelerators of palm oil's crystallization. This is essential for the chocolate industry, in which crystals of the β type are desired [69]. However, in the lipid phase of spreads and margarines, the crystals should preferentially stabilize in the β' form, favoring the product's spreadability [43].

The presence of hardfats as additives modify the crystalline form and alter crystallization behavior, reducing the induction period. In this sense, hardfats act as crystallization inducers [73]. This behavior is related to the physical characteristics and fatty acid composition of the oil from which the hardfat was obtained [72].

Specific hardfats from particular oily sources have unique triacylglycerol profiles, characterizing these materials as inducers of certain polymorphic habits. After cooling a hardfat-added lipid mixture, its high-melting trisaturated triacylglycerols (65–75 °C) promote the formation of crystallization nuclei, allowing for a highly structured crystal lattice to form from the liquid system [74].

Table 4. Polymorphism of structurants

	Short Spacing				
Structurants	**4.6**	**4.2**	**4.15**	**3.8**	**Polymorphic form**
CW	n.d.	4.22	n.d.	3.81	β'
HS	4.59	4.22	n.d.	3.84	β' + β
HC	n.d.	4.24	n.d.	3.80	β'
HP	n.d.	4.19	n.d.	3.79	β'
M	4.59	4.20	n.d.	3.93	β' + β
S	n.d.	n.d.	4.12	n.d.	α

CW: candelilla wax; **HS:** soybean oil hardfat; **HC:** crambe oil hardfat; **HP:** palm oil hardfat; **M:** monoacylglycerols; **S:** sorbitan monostearate; **n.d.:** not detected.

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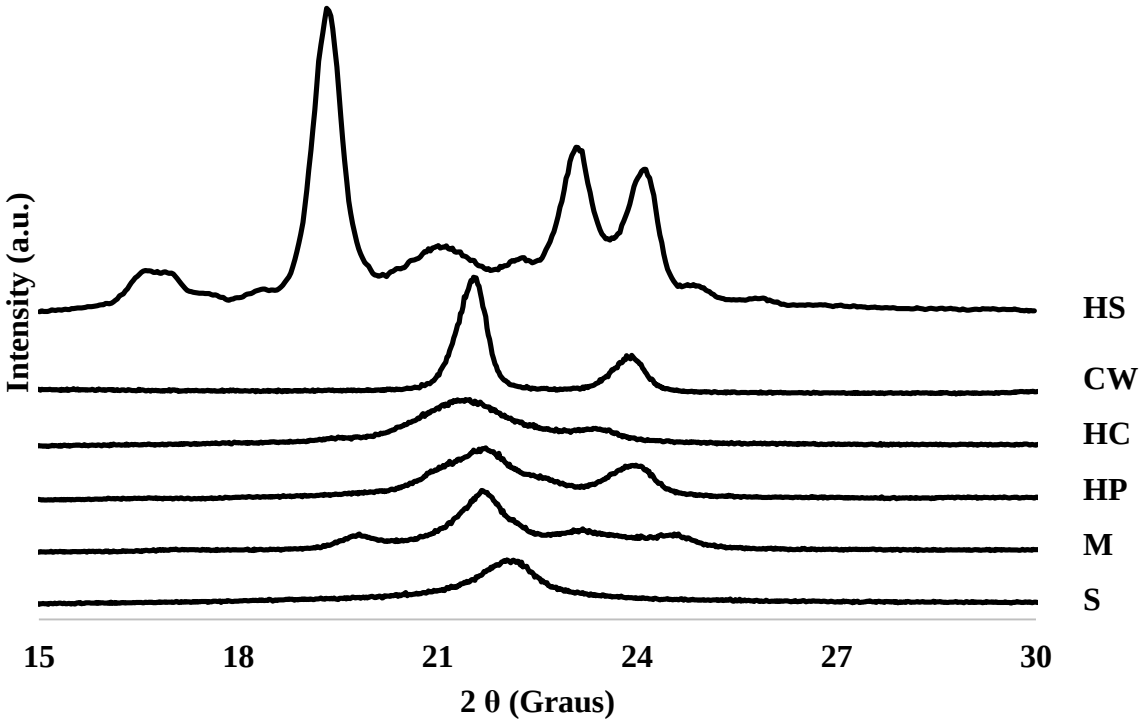

Figure 3. Polymorphism of soybean oil hardfat (HS), candelilla wax (CW), crambe oil hardfat (HC), palm oil hardfat (HP), monoacylglycerols (M), and sorbitan monostearate (S).

3.6. Microstructure

Table 5 shows the number of crystalline elements, mean density, mean D of the crystals (μm), agglomerated crystals (%), and mean D the individual crystals (μm) of structurants, after static isothermal crystallization at 25 °C for 24 hours. Figure 4 shows structurant images obtained using polarized light microscopy with 20x magnification.

It was possible to classify the structurants into two groups, according to their microstructural behavior. Mean diameter crystals ranged from 6.04 (CW) to 8.43 μm (S). HP and S structurants exhibited a greater number of crystalline elements (Table 5). This may be directly related to the structuring of lipid bases at SCmax and 25 ºC (Table 2). HS, HC, M and S structurants presented more well-defined crystals (Figure 4), due to the high mean density of the samples (Table 5). Triacylglycerols, commonly crystallized from melted material, become

crystals of the spherulite type. Crystalline lamellae aggregate and grow radially from the same central nuclei, and may develop ramifications during aging [75]. Eventually, depending on the cooling conditions or even the characteristic melting profile of each structurant, triacylglycerols may also crystallize into other morphologies, such as needles and discs [6].

CW and HP structurants showed the lowest network density and smaller crystal size; in contrast to S, which had elevated mean density as well as mean D crystals. Generally, the size of the crystals is related to the hardness of the material that has been applied to the lipid bases. Smaller crystals are stronger than larger crystals [76].

Table 5. Microstructure of structurants

Structurants	Crystalline elements	Mean density	Mean D crystals (μm)	Agglomerated crystals (%)
CW	29753	17.20	6.04	68.35
HS	23119	29.53	7.08	56.00
HC	19409	27.00	7.02	58.11
HP	45003	13.86	6.97	78.83
M	25859	30.98	7.09	67.55
S	42300	29.95	8.43	79.95

CW: candelilla wax; **HS:** soybean oil hardfat; **HC:** crambe oil hardfat; **HP:** palm oil hardfat; **M:** monoacylglycerols; **S:** sorbitan monostearate.

CW **HS** **HC**

HP **M** **S**

Figure 4. Microstructure of candelilla wax (CW), soybean oil hardfat (HS), crambe oil hardfat (HC), palm oil hardfat (HP), monoacylglycerols (M), and sorbitan monostearate (S).

4. CONCLUSION

All structurants exhibited similar properties, such as high saturated fatty acid concentration, high solids content at the analyzed temperature, low crystallization induction time, high thermal resistance, β' preferential habit, as well as uniform parameters in regards to crystalline morphology and dimensions.

5. ACKNOWLEDGEMENTS

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CAPÍTULO 5

STRUCTURING POTENTIAL AND MECHANISMS OF ORGANOGELS FORMED BY DIFFERENT STRUCTURANTS AND HIGH-OLEIC SUNFLOWER OIL

Artigo Submetido

Grasas y Aceites

Structuring potential and mechanisms of different structurants to form high-oleic sunflower oil organogels

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SUMMARY: The objective of this study was to assess the effect of specific food-grade structurants (Candelilla wax (C), fully hydrogenated palm oil (H), standard soybean lecithin (L), mixtures of Grindsted Crystallizer 100 monoacylglycerols (M) and sorbitan monostearate (S)) in different mixtures and at different concentrations, on the formation of high-oleic sunflower oil (HOSO) organogels. The saturated fatty acid contents of the structurants were high, except for lecithin. At the concentrations analyzed, organogels formulated with C showed greater hardness; organogels with the structurants H and M showed an intermediate hardness and organogels containing L and S showed lower hardness. The M accelerated accelerated the crystallization process, when combined with the other structurants. The presence of C as a structurant induced the formation of smaller crystals. The combinations with higher hardness and hardness stability were CH, HM, HS, CHS, CMS, HMS and CHMS.

KEYWORDS*: High-oleic sunflower oil; Organogel; Structurants; Structuring mechanisms.*

RESUMEN: El objetivo de este estudio fue evaluar el efecto de estructurantes específicos de grado alimenticio (cera de candelilla (C), aceite de palma completamente hidrogenado (H), lecitina de soja estándar (L), mezclas de monoacilglicerol Grindsted Crystallizer 100 (M) y monoestearato de sorbitán (S)) en diferentes mezclas y en diferentes concentraciones, en la formación de organogeles de aceite de girasol con alto contenido de ácido oleico (HOSO). El contenido de ácidos grasos saturados de los estructurantes fue alto, excepto por la lecitina. A las concentraciones analizadas, los organogeles formulados con C mostraron mayor dureza; Los organogeles con los estructurantes H y M mostraron una dureza intermedia y los organogeles que contenían L y S mostraron una menor dureza. La aceleración M aceleró el proceso de cristalización, cuando se combinó con los otros estructurantes. La presencia de C como estructurante indujo la formación de cristales más pequeños. Las combinaciones con mayor dureza y estabilidad de dureza fueron CH, HM, HS, CHS, CMS, HMS y CHMS.

PALABRAS CLAVE*: Translate; Into; Spanish; And; Order; Alphabetically*

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1. INTRODUCTION

In the context of non-conventional structuring of organic phases, lipid systems composed of unsaturated fatty acids (UFA), such as liquid vegetable oils, can be structured as gels (also colled organogels or oleogels). Such materials are formed by continuous networks of small molecules that assemble into liquid crystals, micelles or fibrillar networks, leading to materials that present similar behavior, but several different structural arrangements as described in literature (Chaves *et al.,* 2019; Pernett*i et al.*, 2007; Sira*j et al.,* 2015). Such materials present viscoelastic behavior ~~materials~~ and are usually composed of a structuring agent~~s~~ and an apolar liquid phase. It differs from hydrogels that are mostly formed by water-soluble compounds (Rogers *et al.,* 2009).

The most commonly used structuring agents are fatty acids, fatty alcohols, mixtures of fatty acids and fatty alcohols, phytosterols, oryzanols, sorbitan monostearate, lecithin mixtures, sorbitan tristearate, waxes and wax esters (Rogers 2009). The use of a mixtures of structuring agents can lead to ~~have~~ a synergistic effect on their oil-gelling potential as compared to pure structurants/organogelators (Pernett*i et al.,* 2007). Another important characteristic is the composition of the lipid phase, especially its polarity. Recent studies indicate that the relation between the polarity of the continuous phase and the structurant plays an important role in the stability of the obtained gels (Hwang *et al.*, 2014).

The organogel technology showed its potential application compared to conventional lipid modification processes, since it does not cause chemical changes in the structure of fatty acids and triacylglycerols (TAG), thereby maintaining the nutritional characteristics of the oil, especially the UFA content and natural stereospecific distribution (Sundram *et al.*, 2007).

The use of organogels in food products is a very attractive application, since these materials can potentially confer characteristics, such as texture and consistency, without increasing the content of *trans* fatty acids and with a significant reduction of the content of saturated fatty acids (SFA) in the ~~end~~ final product, thereby giving it a strong nutritional and technological appeal, specially a *clean labelling* (Rogers 2009).

According to Co and Marangoni (2012), organogels can be formed through crystal particles, crystal fibers and polymeric networks, among others. These methods vary according to the structurant used and the conditions to which the raw materials are exposed during the process (Chaves *et al.*, 2019).

The two most important structures observed for organogels are the dispersion of solids in a liquid phase (small inert particles, crystallized solids or drops) and specific mechanisms such as self-support (commonly observed in low molecular weight structurants). Both form three-dimensional networks that can immobilize a liquid phase. The size and shape of this structure and its interactions directly depend on the physical properties of the structurants (Co & Marangoni 2012; Pernetti *et al.*, 2007; Siraj *et al.*, 2015) and their relationship with the continuous phase. The structurant allows two distinct phases to combine in a quasi-homogeneous state, even modifying their thermal properties.-The high-oleic sunflower oil (HOSO), generally used in food applications that require the use of liquid oil with exceptional oxidative stability, is considered a premium raw material. It has a neutral flavor and aroma, and this characteristic is associated with its high potential for application in foods, cosmetics and drugs (Gunstone 2005).

The potential use of waxes as structurants has become an alternative approach in the structuring of oils, and different edible oil-structurant systems have been intensively studied (Marangoni & Garti 2011). Candelilla wax is approved as a food additive by the Food and Drug Administration (FDA) and is generally recognized as safe (GRAS) and as a food ingredient in the human diet (FDA 2016).

Fully hydrogenated vegetable oils, known as hardfats, are a low-cost option with high potential for structuring lipid phases. Palm oil hardfat, as used in the present study, is characterized by the polymorphic habit β′, which is a crystal property that supports its application in specific lipid-based foods such as margarines (Oliveira *et al.*, 2015).

Emulsifiers are components that have been reported to have a potential to structure complex lipid matrices (Rogers 2009; Sira*j et al.*, 2015). Monoacylglycerols are lipid molecules that have only one fatty acid esterified with glycerol, which can vary in terms of chain size and degree of

unsaturation (Chen & Terentjev 2010). The structuring of vegetable oils by monoacylglycerols occurs by a self-assembly mechanism through the formation of micelles or inverse lamellar phases upon cooling of the formed system (Valoppi *et al.*, 2016).

Sorbitan monostearate is a non-ionic hydrophobic emulsifying surfactant used to modify the crystallization properties of lipid systems (Marangoni & Narine 2002). It can form viscous dispersions in organic solvents and edible oils by self-assembly mechanism (Co & Marangoni 2012). Recent studies on the structuring effect of sorbitan monostearate suggest its use as a potential structuring element in vegetable oils, in addition to a positive interaction with triacylglycerols (Cerqueira *et al.*, 2017).

In the structuring of oils, lecithins with 95% phospholipid contents are more efficient as they favor the formation of micelles, resulting in aggregates with entangled microstructures, with subsequent immobilization of the oil in the liquid phase (Kumar & Katare 2005). Studies with lecithin and sorbitan tristearate showed that when either of these components are used individually in oil at concentrations ranging from 6% to 20%, they do not favor structuring. However, there is a synergistic effect when they are mixed at specific ratios, and firm gels are obtained at ratios ranging from 40:60 to 60:40 (lecithin : sorbitan). The organogel of lecithin and sorbitan tristearate is a new candidate for structuring edible oil without the use of TAGs. The structure is provided by a combination of crystals and weak junctions and can be adjusted by changing processing conditions such as shear rate and cooling rate (Pernetti *et al.*, 2007).

There are reported studies that have screened structurants in different lipid bases for application in food products (Chaves *et al.*, 2018). This information is of great relevance for the understanding and knowledge of the non-conventional structuring of lipid bases for application in food products.

The objective of this study was to assess the effect of specific structurants in binary, ternary or higher mixtures at different concentrations, on the formation of high-oleic sunflower oil organogels. The key parameters considered were fatty acid composition, solid content, crystallization kinetics, microstructure, hardness and stability.

2. MATERIALS AND METHODS

2.1. Materials

The HOSO, used as the organic phase, was provided by Cargill Agrícola S.A., Brazil. The structurants used were Candelilla wax (C) in the form of pellets (Light Special Candelilla REAL®), supplied by Multiceras S.A., Mexico; fully hydrogenated palm oil (H) (hardfat), supplied by Cargill Alimentos Ltda, Brazil; standard soybean lecithin (L), supplied by Bunge Alimentos S.A., Brazil; Grindsted Crystallizer 100 monoacylglycerols (M), supplied by DuPont do Brasil S.A., Brazil; and sorbitan monostearate (S), supplied by Sigma Aldrich, USA.

2.2. Methods

Characterization of raw materials

Fatty acid composition. The structurants H, L, M and S and the HOSO were characterized by analyzing the fatty acid composition in a capillary-column gas chromatograph after esterification using the Hartman and Lago method (Hartman & Lago 1973). The fatty acid methyl esters were separated according to the AOCS Ce 1f-96 method (AOCS 2009) in an Agilent DB-23 column (50% cyanopropyl methylpolysiloxane), having dimensions 60 m, ϕ int: 0.25 mm, 0.25 μm film. Analysis conditions: oven temperature of 110 °C - 5 min, 110 °C – 215 °C (5 °C/min), 215 °C - 24 min; detector temperature: 280 °C; injector temperature: 250 °C; carrier gas: helium; split ratio 1:50; volume injected: 1.0 μL. The qualitative composition was determined by comparing the peak retention times with those of the respective fatty acid standards.

Formulation of organogels

HOSO organogels were formulated at concentrations of 4%, 5% and 6% of C, H, L, M and S as single structurants, the structurantes were also combined at different concentrations (50:50, 33:33:33, 25:25:25:25 and 20:20:20:20:20), and analyzed for hardness and stability.

Samples at 6% were prepared using the same combinations and analyzed for induction time and maximum solids fat content (MaxSFC) (Cornell 2002).

Organogels were prepared by mixing the HOSO and the structurants, the oil was heated at a temperature above the melting point of the structurants (80 °C), the structurants were added and the samples stirred for 3 minutes for complete homogenization (visually assessed), samples were cooled at the storage temperature (5 °C) as previously described at literature (Rocha *et al.*, 2013; Stahl *et al.*, 2018), with modifications.

Assessment of organogels

Solid fat content (SFC). It was determined using a Bruker pc120 Minispec Nuclear Magnetic Resonance (NMR) Spectrometer with the aid of Tcon 2000 high precision dry baths (0 - 70 °C) (Duratech, USA). AOCS Cd 16b-93 method: direct method, reading of samples in series, with tempering for the organogels (AOCS 2009).

Crystallization kinetics. This was determined by initial tempering of the organogels (100 °C for 15 min, 1 h at 70 °C) and the solids content was monitored at 25 °C (± 0.5 °C) on an Mq20 NMR Analyzer Bruker Nuclear Magnetic Resonance Spectrometer (Wassell & Young 2007). The data was automatically acquired, after every minute, for 1 hour. The crystallization kinetics was characterized according to the induction period related to the formation of solids and maximum solid fat content (SFCmax) (Campos 2005; Stahl *et al.*, 2018).

Microstructure. The microstructure of the organogels was analyzed by polarized light microscopy. With the aid of a capillary tube, a drop of organogel was placed on a glass slide kept at controlled temperature, which was then covered with a coverslip. The microstructure of the crystals was analyzed using a polarized light microscope (Model BX53, Olympus America Inc., USA) coupled to a digital video camera (Olympus America Inc., USA). The images were captured and quantified using Image Pro-Plus 7.0 (Media Cybernetics, USA) in four different visual fields of each slide for each organogel. Mean particle diameter values were expressed as means and

standard deviations (Toro-Vazquez *et al.,* 2013). The organogels were stabilized at 25 °C for 24 hours and analyzed using a 20x magnification.

Crystal density was measured using the ratio among solid crystals (white pixels) and liquid (black pixels) the resulting value was called solid crystal density (SC%) and it was calculated according to the equation 1.

$$SC\% = \frac{\text{number of white pixels}}{\text{total amount of pixels}} * 100 \quad \text{Equation 1}$$

Hardness. Hardness analysis (compression/extrusion) was performed using a microcomputer-controlled texture analyzer (TA-XTi2, Stable Microsystems, England) (Rocha *et al.,* 2013). For the analysis, 30 mL of organogel was placed in 50 mL beakers and incubated in a B.O.D. incubator at 5 °C for 24 h. A compression/extrusion test was performed using an acrylic cylindrical probe, 25 mm in diameter and 35 mm in length, at a velocity of 1.0 mm/s, and a fixed distance of 15 mm for penetration of the probe. The value considered was the mean of the maximum force obtained in three different measurements.

Stability of organogels. The organogels were stabilized for 24 hours at 25°C. All organogels were monitored for stability, with observation and measurement of phase separation or liquid oil exudation, using a graduated glass beaker allowing to grade the samples from 0, all liquid oil separation up to 100, no phase separation. In addition, stability was qualitatively analyzed by the *tilt-test*, samples were tilted and the self-standing ability of the samples was assessed visually and depending on the appearance of the samples was described as gel or liquid. Organogel samples that did not flow were named as *gel*, materials that slowly flowed were named as *thickened liquid* and the materials that immediately flowed were named as *liquid* (Rocha *et al.,* 2013).

Statistical analysis

191 The objective results were evaluated using the Statistica 8.0 software (Statsoft, USA)(STAT
192 SOFT 2007) to calculate the regression coefficient, probability (p-value) and analysis of variance
193 (ANOVA), considering a 5% significance level.
194
195 **3. RESULTS AND DISCUSSION**

196 **3.1. Fatty acid composition of raw materials**

197 To discuss the results, the general composition of C obtained by Doan *et al.*, (2017) was used
198 as a reference, since it was not chemically characterized in this study. According to the authors,
199 the main components of C are hydrocarbons (72%), followed by wax esters (15%), free fatty acids
200 (9%) and free fatty alcohols (2%). The major fraction is composed mainly of n-alkanes having
201 saturated chains with odd numbers of carbons (29 to 33 carbons), where C31 represents 82% of
202 that fraction. The wax esters are composed predominantly of SFA, having 17% C16 and 31%
203 C30, esterified with saturated fatty alcohols having even number of carbons (24 to 34 carbons),
204 with C28, C30 and C32 representing 11%, 42% and 14% of the fraction, respectively.

205 The knowledge of the chemical composition of the structurants is of great relevance to assess
206 the interactions with organic materials and to understand the structuring mechanisms. Table 2
207 shows the fatty acid composition of the structurants (H, L, M and S) and HOSO. The SFA contents
208 of H, L, M, S and HOSO were 99.47%, 22.13%, 99.70%, 96.73% and 8.82%, respectively,
209 indicating their predominance in the composition of the structurants, except for lecithin. The UFA
210 contents of the structurants ranged from 0.53 to 77.87% and HOSO showed a value of 91.18%.

211 The structurants showed different fatty acid profiles, despite the similarity of the total amount
212 of SFA and UFA. H showed 43.09% of palmitic (C16:0) and 54.11% of stearic, which was similar
213 to the results obtained by Masuch*i et al.*, (2014) and Oliveir*a et al.*, (2015). L showed 16.37% of
214 palmitic (C16:0), 18.81% of oleic (C18:1) and 52.40% of linoleic (C18:2), in agreement with the
215 lecithins analyzed by Fernande*s et al.*, (2012). M showed 47.82% of stearic (C18:0) and 41.30%
216 of behenic (C22:0), as reported by Silva *et al.*, (2018). S showed 40.29% of palmitic (C16:0) and

51.13% of stearic (C18:0). HOSO showed 80.08% of oleic (C18:1) and 10.34% of linoleic (C18:2).

The SFA increases the ability to structure lipids and has the important barrier property against moisture and liquid oil retention (O'Brien 2009). The fatty acid composition of the structurants can directly affect some characteristics of the lipid bases and the respective processed products, such as hardness, spreadability, solid content, thermal properties and rheological properties. Differences in fatty acid composition can lead to organogels with different technological, nutritional and stability characteristics.

3.2. Hardness

The consistency of a material is its resistance against a permanent deformation. The magnitude of consistency measures the mechanical strength of a structure as a result of the intermolecular forces between the components of the material (Walstra 2003). The hardness values of the organogels at the concentrations of 4%, 5% and 6% are shown in Table 1.

At the concentrations used in this study, the organogels containing C showed higher hardness (15.19 N to 30.08 N), for pure structurants (just one organogelator) and for the combinations. This is in accordance with the results described in previous studies (Doa*n et al.*, 2015; Rocha *et al.*, 2013).

The structurants H and M showed intermediate hardness values (0.35 to 1.44 N), consistent with the high SFA content of these structurants, which is 99.47% and 99.70%, respectively.

The structurants L and S showed lower hardness values (0.10 to 0.15 N) at the concentrations used in this study. The hydrophilic groups of the emulsifiers (L and S) exert repulsive forces due to the chemical composition and number of hydrophilic moieties as reported by Sangwal (2007), forming a less cohesive structure. Organogels formulated with L, M, S and CHM, and other organogels containing L did not show higher hardness values for higher concentrations of the structurants, which indicates that L, in addition to providing low hardness (0.14 N at 5%), had a

242 negative effect when in combination with other structurants, resulting in low hardness values
243 (maximum of 0.81 N at 5% of HLM). This behavior can possibly be attributed to a eutectic effect,
244 which results in a decrease of the melting point and consistency of the mixture compared to the
245 individual components (Goln *et al.*, 2011), or a reduction of the interfacial tension of the network
246 caused by the presence of lecithin, the reported effect can have a negative impact on lipid
247 applications where physical properties such as hardness and texture is important. On the other
248 hand it can have a positive effect in applications where the technological objective is to increase
249 the fluidity (Manzocc*o et al.*, 2014), such as in lubricating agents or plasticizers.

250 The organogels containing C and H, and all organogels without L (except for CHM and
251 CHMS) showed higher hardness values at higher concentration of the structurants, indicating that
252 though S alone leads to lower hardness values for the organogels (0.15 N at 6%), it shows a
253 synergistic effect when combined with other structurants and can result in increased hardness.
254 However, its maximum value is 5.59 N at 6% of HS, which is much lower than the values obtained
255 for organogels with C alone (30.08 N). This effect can be due to intermolecular interactions
256 between the TAGs of H (for which the main fatty acid is stearic acid (C18:0; 54.11%)) and long
257 chain fatty acids present in the structurants M and S (47.82% and 51.13% , respectively), followed
258 by formation of the crystal lattice. The stearic acid chains (C18:0) may have been adsorbed or
259 physically incorporated into the surface of the crystal lattice, creating an occluded structure,
260 trapping the oil, as seen in the pickering effect for emulsions (Dickinson 2012).

261 Among the binary combinations of structurants, the maximum hardness was observed for CH,
262 CS and HS at a concentration of 6% (10.95 N, 4.88 N and 5.59 N, respectively). The ternary
263 mixtures showed better results for HMS, CHS and CMS at the concentration of 6% (3.23, 3.51
264 and 5.58 N, respectively). All mixtures with more than 3 structurants showed hardness values
265 below 0.29 N at 6% (CHLMS), except for the organogel CHMS, which showed values of 1.55 N,
266 1.83 N and 1.37 N at concentrations of 4%, 5% and 6%, respectively, which supports the
267 hypothesis of the negative effect of the structurant L.

3.3. Visual stability

Table 1 shows visual results for the organogels after 24 hours of stabilization at 25ºC. The container that had the gel was tilted and the organogels that did not flow due to gravity were considered 100% stable (Cerqueir*a et al.*, 2017). The organogels obtained with C, H, M, CH, CM, CS, CHM, CHS and HLM showed 100% stability at all concentrations. The organogels CL, HL, CHL, HLS, CHLM and CHLS were unstable at lower concentrations (4% of structurants) and the organogels L, S, CLM, CLS, LMS, CLMS, HLMS and CHLMS were unstable at all concentrations.

3.4. Solid fat content (SFC)

Figure 1 shows SFC values for organogels at temperatures ranging from 10 to 70°C, grouped as A: single structurants, B: binary mixtures, C: ternary mixtures, and D: mixtures with 4 or 5 structurants.

Group A organogels showed SFC values ranging from 1.57% to 6.83% at 10°C and the lowest values were obtained for L and S (1.83% and 4.55%, respectively), which indicates that these structurants present limited applications for food applications, since the SFC is low at ~~the~~ refrigeration temperature; M showed higher SFC at 60°C. Group B showed a standard behavior for binary mixtures, ranging from 4% to 6% of solids at 10ºC, except for organogel LS, with less than 1% of solids at the same temperature, thus remaining in fluid state. The binary mixtures with high SFA contents, (Table 2; HM, CM, CH and HS) showed higher thermal resistance and hardness; LS was the only (binary) organogel with no SFC at body temperature (35-37°C).

Group C organogels showed lower thermal resistance compared to group B, as observed by the slopes of the lines in Figure 1 (C). SML and HSL showed lower solid contents for temperatures ranging from 10°C to 30 °C, while other organogels had SFC values between 4% and 6%, approximately.

All group D organogels showed similar behavior throughout the analyzed temperature range. During the crystallization of the lipid phase, materials with similar chemical composition and molecular structure are more likely to co-crystallize (Marangoni 2005). This is exemplified by the predominance of palmitic (C16:0) and stearic (C18:0) acids in the composition of the structurants H and S (Table 2).

3.5. Crystallization kinetics

The crystallization kinetics of the organogels samples (6% w/w) was analyzed under isothermal conditions (25ºC). Table 1 shows the induction time (IT) and the maximum solid fat content (SFCmax) of the organogels. The IT ranged from 0 to 14 minutes and the SFCmax from 0.56% to 5.43%.

The maximum IT was 20 minutes for the CHLS organogel with SFCmax of 1.49%, but the IT was 10, 6, 7 and 8 minutes for organogels with a combination of 4 structurants (CHLM, CHMS, CLMS and HLMS, respectively). This indicates that M, when combined with the other structurants, has an accelerating effect at the beginning of the crystallization process, meaning that such material acts as a crystallization seed (Metin & Hartel 2005).

The LS organogel had a SFCmax of 1.71%, but HLS organogel did not have a SFC above 0.56%, probably due to the presence of L, which is rich in UFA, has a low-melting point, and is liquid at room temperature (Table 2).

The CH, CM, HM, CHM and HMS organogels showed the highest values of SFCmax (4.84%, 5.14%, 5.43%, 4.86% and 4.83%, respectively), possibly due to the high concentration in SFA of the structurants used in these organogels (Table 2). The IT for these organogels was 6, 4, 5, 5 and 5 minutes. There is an effect related to the composition of C, the main components of which are hydrocarbons (72%), which are composed mainly of n-alkanes with saturated chains (C31 represents 82% of this fraction), and esters, which are composed mainly of SFA (with 17% and 31% of C16 and C30, respectively) esterified with saturated fatty alcohols (24 to 34 carbons; C28,

C30 and C32 represent 11%, 42% and 14% of the fraction, respectively). This effect is similar to the one observed by Bass*o et al.,* (2010) for behenic acid, since they are saturated long chain components with high melting points (generally higher than 70 °C), meaning that the physical interactions are really important for organogelation mechanism.

3.5. Microstructure of organogels

The concept of microstructure includes the state, amount, shape, size, spatial relationship and interaction between all the components of a crystal network and has a great impact on the macroscopic properties of fats (Ribeir*o et al.,* 2015).

TAGs crystallized from the melt are generally spherulite crystals, which correspond to the aggregation of crystal lamellae that grow radially from the same central nuclei and can develop branches during crystal maturation (Rousset 2002). Eventually, depending on the cooling conditions or even the melting profile of each sample, TAGs can also crystallize with different geometries, such as needles and discs (Oliveira *et al.,* 2015).

Organogels can be structured to form a three-dimensional network, where the solvent is trapped inside the structuring matrix, avoiding the migration of oils. The network is stabilized by weak interactions between the chains, such as hydrogen bonds and van der Waals forces (Pirner *et al.,* 2016). Although it is already known that organogels are formed through weak intermolecular interactions between the structurants associated with the formation of three-dimensional networks (Steed 2011), the type of interactions that are necessary are still poorly understood (Nikiforidis *et al.,* 2015).

Table 3 shows the average number of crystal elements, mean density, mean D of the crystals (μm), agglomerated crystals (%) and mean D of individual crystals (μm) of the organogels after static isothermal stabilization at 25 °C for 24 hours. Figure 2 shows images of the microstructure of the organogels at a magnification of 20x, after stabilization at 25 °C for 24 hours.

The M, HL, CHM, CLS and CLMS organogels showed higher number of crystals (Table 3). The mean diameter of the crystals ranged from 1.38 (C) to 12.66 (HLMS) μm for samples C and HLMS respectively, indicating that the presence of C as a structurant induces the formation of smaller crystals. The size of the crystals is related to the hardness of the lipid bases (Table 1) and smaller crystals are usually present at stronger organogels comparing with larger crystals (Hwang *et al.*, 2012).

It was possible to observe crystal morphologies and regions that contain liquid oil. Small, dispersed crystals were observed with a greater presence of the liquid phase in all organogels, except for L, LS, SH and MS (Figure 2). Probably the temperature used in the analysis was too high for the sample or the presence of the structurants L and S, together or individually, had a negative influence on the formation of the three-dimensional network, it can also explains the reduced hardness that was observed for the same samples.

The C, CH, CL and CHMS organogels showed the lowest network density (SC%) and smaller crystal size. Conversely, L, S, CHL, CLM and HLMS organogels showed high mean density and mean D of the crystals. This might be due to the presence of the structurants L and S, since the number of crystal elements was 37 for L due to the low SFC at 25°C and the high UFA content, which is consistent with the results obtained for hardness, visual stability, SFC and crystallization kinetics.

The mean diameter of the organogels was less than 30 μm, which is a fundamental parameter to minimize the sandy/waxy mouth sensation resulting from the lipid phase (Beckett 2008).

3.6. Structuring mechanisms

Based on the information of composition, physical and microscopic characteristics it is possible to make a hypothesis about the organogel formation mechanism based on the behavior of the structurants as previously reported on the literature.

The behavior of C can be attributed to its chemical composition, which mainly includes hydrocarbons (72%) and wax esters (15%) (Doan *et al.,* 2017), resulting in the self-assembly crystallization mechanism. The microplates are formed by the parallel alignment of n-alkanes and very long chain fatty acids that interlace to develop a porous three-dimensional network that physically immobilizes the liquid phase (Doan *et al.,* 2015). The mean diameter of the crystals of C was 1.38 μm, meaning the formation of smaller crystals, such behavior leads to greater hardness of the lipid base (Hwang *et al.,* 2012). The increase of the concentration of C in organogels produced harder organogels, for single structurants and the combinations, meaning that the hardness is concentration dependent (Doan *et al.,* 2015; Rocha *et al.,* 2013).

Organogels made with C, H and S indicated that there is a positive interaction between the structurants, and the hardness increased with increased concentrations. This effect can be due to intermolecular interactions between the TAGs of H, the chains of stearic acid (C18:0) may have been adsorbed or physically incorporated into the surface of the crystal lattice, creating an occluded structure and imprisoning the oil, as seen in the pickering effect for emulsions (Dickinson 2012), such behavior is particularly related to material polarity as reported by Hwang et al., (2015), similar polarities produced more stable, hard and organized networks.

H had particles that were able to form a dense crystal lattice with spherulite crystals. Studies show that spherulite crystals can modulate the crystallization process by acting as preferential nuclei or crystallites (templates) and modify the nucleation rate of the crystal lattice, providing greater thermal stability. The structurants C, H and S showed that the system was more efficient due to the co-crystallization process and that this process is conducted by C (Silv*a et al.,* 2018; Stah*l et al.,* 2018).

L had a negative impact as structurant, probably due to the fact that its composition contains mostly UFA, in addition to its ability to decrease the interfacial tension and its similar polarity with HOSO in terms of fatty acid composition (Table 2) (Hwang 2005)**.** This negative behavior

390 of L as structurant may have occurred due to the different interactions between L and HOSO, and
391 high presence of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids in L.

392 Basso *et al.*, (2010) reported that behenic acid monoacylglycerols are probably more effective
393 in inducing crystallization due to the presence of a longer fatty acid chain which promotes greater
394 stability in the crystallization process as compared the fatty acid chains in the monoacylglycerols
395 of fully hydrogenated palm oil.

396 In this study it was possible to verify the effect of high contents of saturated fatty acids and
397 alcohols. Considering the alkanes present in C and the SFCmax values for some organogels, this
398 effect is similar to the one observed by Basso *et al.*, (2010) for systems structured with behenic
399 acid, since they are long chain saturated components.

400 The structurants H, M and S showed similar stearic acid composition (C18:0) and this SFA
401 may have positively interacted with the oleic acid (C18:1) of HOSO due to the similar chain size.
402 The presence of the stearic acid of S favors co-crystallization of these structurants due to the
403 triacylglycerols of H and the structural affinity through incorporation on the surface of the
404 crystals, limiting subsequent agglomerations. We also observed a positive interaction when S and
405 C were used in combination, probably due to the size and similarity of the molecules (Murdan *et*
406 *al.*, 1999; Rogers 2009; Stahl *et al.*, 2018).

407 S increases the consistency of lipid matrices due to the formation of a characteristic tubular
408 network. According to Murdan, Gregoriadis and Florence (1999), cooling leads to insolubility of
409 S molecules, leading to the formation of tubules that aggregate progressively and to a structure
410 that surrounds the liquid oil, thus forming a gel that prevents movement of the crystals by the
411 crystallization of TAGs in the medium, with better mechanical resistance of the lipid bases
412 formed. Shah, Sagiri, Behera, Pal and Pramanik (2013) found that tubular structures form a three-
413 dimensional network only when there are interactions between S and lipid molecules, promoting
414 the stability of lipid systems, similar to our observations in this study.

4. CONCLUSION

The formation of organogels of high-oleic sunflower oil, structured with candelilla wax, fully hydrogenated palm oil, monoacylglycerols and sorbitan monostearate, was effective for the parameters assessed for single structurants, binary mixtures, ternary mixtures and even quaternary mixtures, especially at high concentrations. However, the presence of standard soybean lecithin, alone or combined with any other structurant, regardless of the concentration used, did not promote an efficient formation of the organogel structure. The structurants analyzed show a fatty acid composition with high concentration of SFA, except for standard soybean lecithin. Some combinations of the organogels of high-oleic sunflower oil provided better results in terms of hardness and visual stability (CH, HM, HS, CHS, CMS, HMS and CHMS) at all concentrations used. These structuring results for high-oleic sunflower oil with low cost structurants confirm that the analyzed organogels are lipid bases with high potential to be used as fat substitutes in industrial processes to meet the demand of consumers looking for healthier foods.

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TABLES

Table 1. Possible matrix combinations containing five structurants at a 6% concentration, hardness, stability, induction time and maximum solids content of the organogels.

Structurant	Hardness (N)			Stability (%)			Induction time (Minutes)	Maximum solids fat content (%)
	4%	5%	6%	4%	5%	6%		
C	15.19±0.31	24.03±1.25	30.08±1.07	100.00	100.00	100.00	3	5.47
H	0.35±0.05	0.92±0.04	1.14±0.06	100.00	100.00	100.00	15	6.28
L	0.11±0.00	0.14±0.00	0.10±0.01	0.00	4.00	10.00	n.d.	1.81
M	1.44±0.35	0.96±0.07	0.99±0.05	100.00	100.00	100.00	2	6.40
S	0.13±0.01	0.12±0.01	0.15±0.01	40.00	60.00	70.00	4	3.73
CH	2.20±0.19	6.51±0.63	10.95±0.54	100.00	100.00	100.00	6	4.84
CL	0.14±0.01	0.20±0.04	0.28±0.02	90.00	99.00	100.00	7	3.13
CM	0.19±0.03	0.31±0.07	0.57±0.04	100.00	100.00	100.00	4	5.14
CS	1.52±0.10	2.90±0.21	4.88±0.05	100.00	100.00	100.00	4	3.54

HL	0.15±0.03	0.25±0.03	0.12±0.02	0.00	98.00	99.00	11	1.21
HM	1.31±0.07	3.28±0.12	3.21±0.20	100.00	100.00	100.00	5	5.43
HS	1.08±0.07	2.06±0.16	5.59±0.06	99.00	99.00	99.00	8	1.66
LM	0.33±0.01	0.49±0.01	0.44±0.02	100.00	100.00	100.00	4	3.96
LS	0.11±0.02	0.12±0.03	0.10±0.00	0.00	0.00	0.00	14	1.71
MS	0.43±0.01	0.47±0.05	0.93±0.03	99.00	100.00	100.00	3	3.15
CHL	0.20±0.04	0.28±0.03	0.16±0.02	50.00	100.00	100.00	9	3.57
CHM	0.23±0.01	0.81±0.09	0.40±0.14	100.00	100.00	100.00	5	4.86
CHS	0.81±0.20	2.00±0.02	3.51±0.45	100.00	100.00	100.00	8	2.28
CLM	0.24±0.15	0.10±0.02	0.12±0.01	0.00	0.00	0.00	6	3.64
CLS	0.12±0.02	0.10±0.02	0.11±0.01	0.00	0.00	0.00	6	2.49
CMS	1.47±0.14	3.01±0.17	5.58±0.21	99.00	99.00	100.00	6	3.47
HLM	0.39±0.06	0.81±0.03	0.33±0.03	100.00	100.00	100.00	7	4.14
HLS	0.25±0.05	0.17±0.02	0.20±0.04	0.00	98.00	99.00	n.d.	0.56

HMS	0.64±0.07	1.16±0.14	3.23±0.09	99.00	99.00	100.00	5	4.83
LMS	0.12±0.02	0.12±0.06	0.24±0.02	0.00	0.00	0.00	9	1.76
CHLM	0.18±0.02	0.21±0.01	0.17±0.02	0.00	100.00	100.00	10	3.96
CHLS	0.18±0.01	0.13±0.01	0.16±0.02	0.00	99.00	100.00	20	1.49
CHMS	1.55±0.22	1.83±0.05	1.37±0.19	99.00	99.00	99.00	6	3.74
CLMS	0.12±0.03	0.13±0.03	0.21±0.02	0.00	50.00	65.00	7	2.23
HLMS	0.24±0.10	0.22±0.04	0.08±0.02	0.00	50.00	60.00	8	2.08
CHLMS	0.15±0.01	0.15±0.05	0.29±0.03	0.00	70.00	75.00	8	1.86

548 **C:** candelilla wax; **H:** palm oil hardfat; **L:** soybean lecithin, **M:** Grindsted Crystallizer 100 monoacylglycerols; **S:** sorbitan monostearate; and **n.d.:** not detected.

549 **Table 2.** Fatty acid composition of the structurants and the lipid base

Fatty acid (%)	H	L	M	S	HOSO
Lauric C12:0	0.33±0.02	n.d.	0.06±0.04	n.d.	0.18±0.07
Myristic C14:0	1.08±0.02	0.16±0.03	0.10±0.00	2.09±0.04	0.20±0.06
Pentadecyl C15:0	n.d.	0.08±0.01	n.d.	0.49±0.02	0.02±0.01
Palmitic C16:0	43.09±0.73	16.37±0.03	4.44±0.05	40.29±0.90	4.09±0.38
Palmitoleic C16:1	n.d.	0.12±0.02	n.d.	0.82±0.01	0.16±0.06
Margaric C17:0	0.16±0.04	0.18±0.02	0.09±0.00	2.00±0.06	0.05±0.01
Heptadecanoic C17:1	n.d.	0.05±0.01	n.d.	0.16±0.00	0.06±0.01
Stearic C18:0	54.11±0.78	4.26±0.15	47.82±0.10	51.13±0.53	2.76±0.10
Oleic C18:1	0.40±0.10	18.81±0.18	0.23±0.02	1.72±0.20	80.08±1.03
Linolelaidic C18:2t	n.d.	n.d.	n.d.	n.d.	0.07±0.00
Linoleic C18:2	0.13±0.07	52.40±0.34	0.06±0.03	0.56±0.10	10.34±0.52
Linolenic C18:3	n.d.	6.37±0.02	n.d.	n.d.	0.17±0.05
Arachidic C20:0	0.58±0.03	0.30±0.00	4.96±0.01	0.49±0.00	0.27±0.01
Gadoleic C20:1	n.d.	0.13±0.00	n.d.	n.d.	0.31±0.03
Behenic C22:0	0.08±0.00	0.47±0.01	41.30±0.11	0.24±0.03	0.89±0.05
Lignoceric C24:0	0.08±0.00	0.31±0.06	0.92±0.00	n.d.	0.36±0.06
Saturated	99.47	22.13	99.70	96.73	8.82
Unsaturated	0.53	77.87	0.30	3.27	91.18

550 **H:** palm oil hardfat; **L:** soybean lecithin, **M:** Grindsted Crystallizer 100 monoacylglycerols; **S:** sorbitan
551 monostearate; **HOSO:** high-oleic sunflower oil and **n.d.:** not detected.
552
553 **Table 3.** Microstructure of the organogels at a magnification of 20x, after stabilization at 25°C for 24
554 hours.

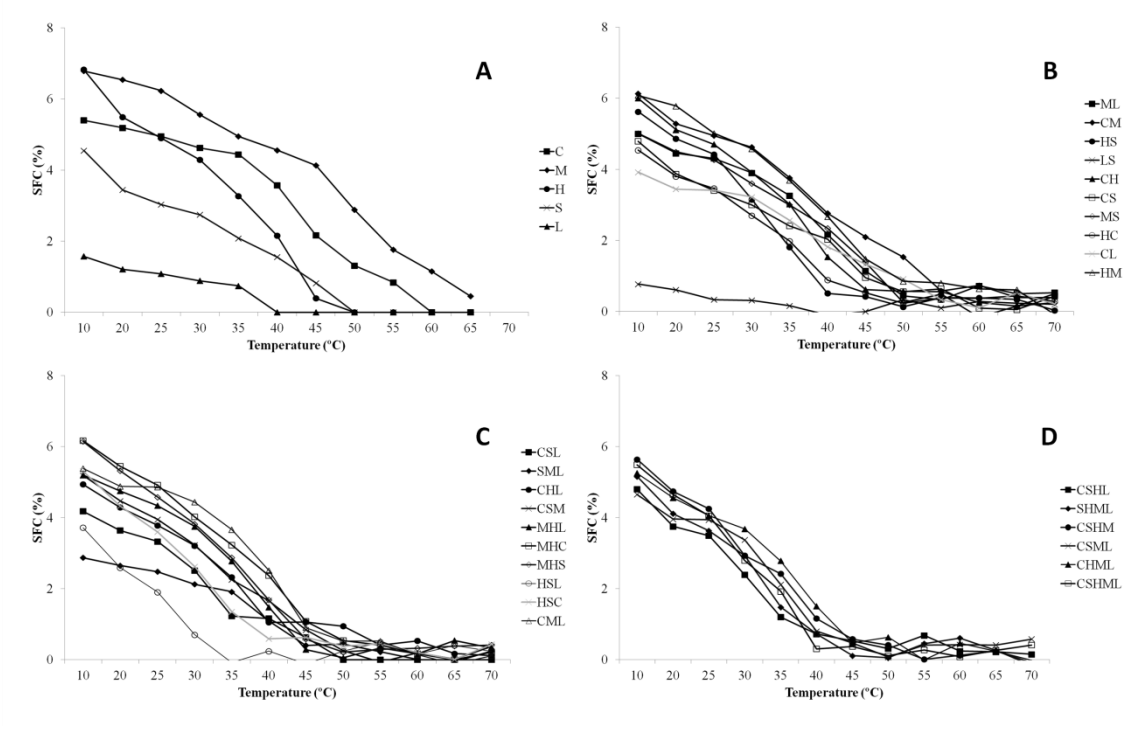
Simples	Crystalline elements	Mean density	Mean D crystals (μm)	Agglomerated crystals (%)
C	1751.00	2.36	1.38	0.63
H	109.00	56.03	5.59	31.96
L	37.00	128.71	9.16	75.51
M	21246.00	14.54	3.15	32.73
S	9614.00	72.38	7.10	76.87
CH	3005.00	2.40	1.49	1.37
CL	2542.00	9.13	1.61	3.30
CM	4787.00	24.91	3.57	31.29

CS	525.00	32.00	4.21	35.69
HL	11820.00	26.33	4.58	53.07
HM	5147.00	30.46	5.28	41.00
HS	232.00	35.78	6.42	64.62
ML	9435.00	32.33	3.36	40.62
LS	141.00	59.53	4.41	50.18
MS	4966.00	36.81	3.98	53.44
CHL	4794.00	70.71	7.36	74.20
CHM	10458.00	15.88	2.69	33.03
CHS	8786.00	41.32	1.57	20.16
CLM	4199.00	85.60	10.95	84.25
CLS	11165.00	26.28	2.21	26.21
CMS	3376.00	38.14	6.47	58.92
HLM	5158.00	28.05	5.74	57.59
HLS	960.00	64.57	6.10	62.95
HMS	5503.00	76.18	3.96	65.52
LMS	1644.00	51.05	6.70	55.47
CHLM	2983.00	94.77	8.90	80.38
CHLS	1257.00	67.52	6.42	80.42
CHMS	501.00	3.25	1.87	0.37
CLMS	14006.00	45.24	5.07	78.08
HLMS	2524.00	112.36	12.66	80.31
CHLMS	897.00	42.23	3.61	37.72

555 **C:** candelilla wax; **H:** palm oil hardfat; **L:** soybean lecithin, **M:** Grindsted Crystallizer 100
556 monoacylglycerols and **S:** sorbitan monostearate.

557 **FIGURE CAPTIONS**

558



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560

561 **Figure 1**. Solids profile of the organogels.

562 **SFC:** Solid fat content; **C:** candelilla wax; **H:** palm oil hardfat; **L:** soybean lecithin, **M:** Grindsted

563 Crystallizer 100 monoacylglycerols and **S:** sorbitan monostearate.

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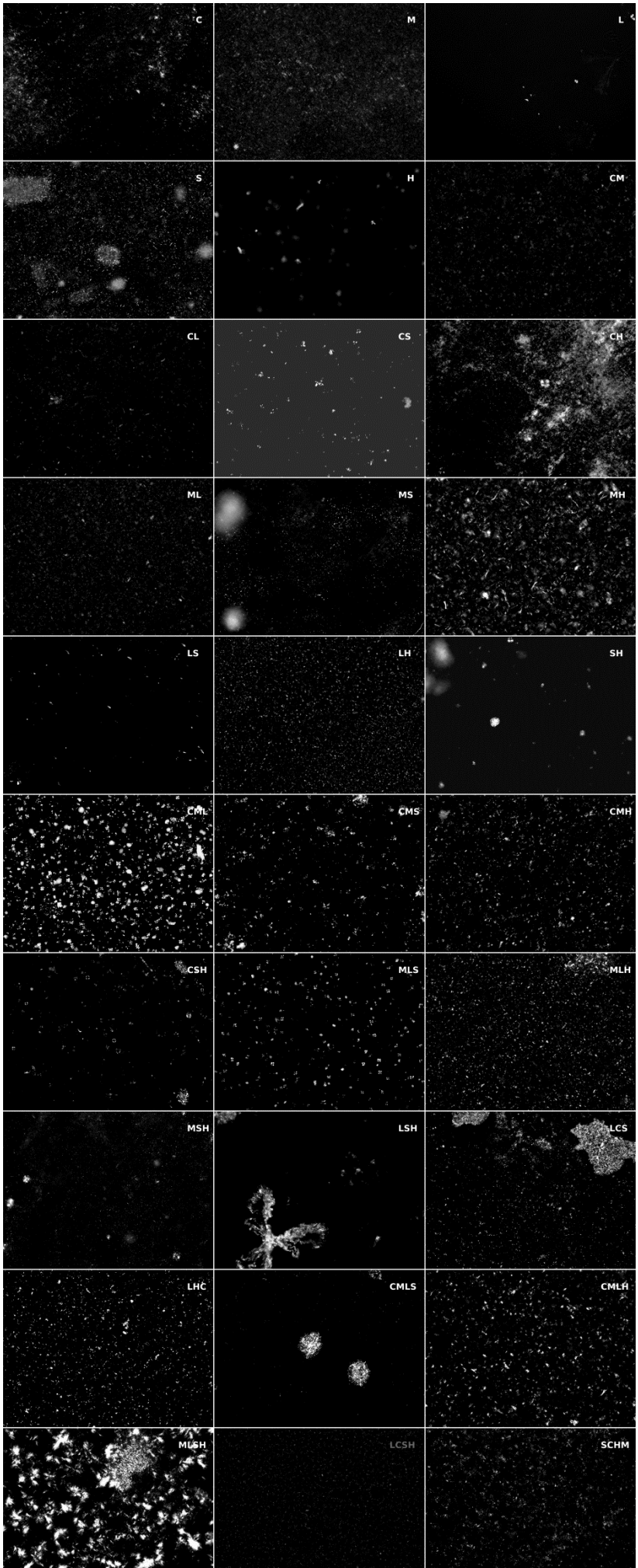
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565 **Figure 2**. Microstructure of the organogels at a magnification of 20x, after stabilization at 25°C for 24
566 hours.
567 **C:** candelilla wax; **H:** palm oil hardfat; **L:** soybean lecithin, **M:** Grindsted Crystallizer 100
568 monoacylglycerols and **S:** sorbitan monostearate.
569

CAPÍTULO 6

FOOD GRADE HYBRID ORGANOGELATOR SYSTEMS FOR STRUCTURING OF HIGH OLEIC SUNFLOWER OIL

Artigo Submetido

Grasas y Aceites

Food grade hybrid organogelator systems for structuring of high oleic sunflower oil

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SUMMARY: Organogels are formed by low-molecular-weight structuring agents and some organic solvents. The aim of this study was to evaluate and characterize high oleic sunflower oil organogels, structured with candelilla wax (C), sorbitan monostearate (S) and monoglycerides (M) for physicochemical properties and crystallization behavior of lipid systems. The solid fat content (SFC) of the organogels ranged from 4.72 to 6.84% at 10ºC; all organogels showed full melting at 65ºC. The hardness values ranged from 0.13 (6% S) to 32.00 (6% C) N. All organogels showed short spacings of 4.6 Å, typical of the β polymorph; and had similar and frequency- and temperature-independent rheological properties, with G' higher than G'', indicating solid material behavior. The results from thermal analysis of organogels by differential scanning calorimetry (DSC) showed behavior matching thermal properties. The best results of thermal and mechanical stability were obtained when using a concentration of 2% of each structuring agent (C, S and M).

KEYWORDS*: Candelilla wax; High oleic sunflower oil; Monoglycerides; Organogel; Sorbitan monostearate.*

RESUMEN: *Los organogeles están formados por agentes estructurantes de bajo peso molecular y algunos solventes orgánicos. El objetivo de este estudio fue evaluar y caracterizar los organogeles de aceite de girasol con alto contenido de ácido oleico, estructurados con cera de candelilla (C), monoestearato de sorbitán (S) y monoglicéridos (M) para determinar las propiedades fisicoquímicas y el comportamiento de cristalización de los sistemas lipídicos. El contenido sólido de los organogeles varió de 4.72 a 6.84% a 10ºC; Todos los organogeles mostraron fusión total a 65ºC. Los valores de dureza variaron de 0.13 (6% S) a 32.00 (6% C) N. Todos los organogeles mostraron espaciamientos cortos de 4.6 Å, típicos del polimorfo β; y tenía propiedades reológicas similares e independientes de la frecuencia y la temperatura, con G 'más alto que G' ', lo que indica el comportamiento del material sólido. Los resultados del análisis térmico de organogeles por calorimetría diferencial de barrido mostraron un comportamiento que combina las propiedades térmicas. Los mejores resultados de estabilidad térmica y mecánica se obtuvieron al usar una concentración del 2% de cada agente estructurante (C, S y M).*

PALABRAS CLAVE*: Aceite de girasol alto oleico; Cera de candelilla; Monoestearato de sorbitan; Monoglicéridos; Organogel.*

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1. INTRODUCTION

Vegetable oils lack the physical properties necessary for direct application in lipid-based foods that require consistency, limiting their application in their unchanged forms due to their particular fatty acid and triacylglycerol composition. In conventional lipid modification processes, the basic structure of oils and fats can be redesigned depending on the desired plasticity profile and on the intrinsic characteristics of the raw materials using techniques such as hydrogenation, interesterification or fractionation (O'Brien, 2008).

However, all these technological alternatives, when used together or alone, are unable to reduce the SFA content of lipid formulations for industrial applications. Most often, obtaining crystallization properties, thermal resistance and consistency characteristic of technical fats even requires significantly increasing the proportion of SFA in lipid compositions, which has been a major hurdle for the area of oils and fats (Menaa, 2013).

The lipid crystallization behavior has important implications, especially for the industrial processing of products whose physical characteristics strongly depend on fat crystals such as chocolates, margarines and lipid-based products in general. Crystal formation and growth rates and polymorphic transformations are key determinants of the functionality and application of oils and fats (Sato, 2001).

New raw materials and production processes are currently being researched as potential alternatives for structuring lipid-based materials towards reducing the content of SFA and *trans* fats (Chaves *et al.*, 2018; Garcia *et al.*, 2013; Godoi *et al.*, 2019; Marangoni and Garti, 2011).

Within the framework of unconventional structuring of organic phases, lipid systems composed of liquid vegetable oils can be structured as gels. These structures form

continuous lattices of low-molecular-weight aggregates, which are organized as liquid crystals, micelles or fibrillar lattices and are termed organogels. These materials, classified as soft-materials, are characterized by various structural arrangements described in the literature (Chaves *et al.*, 2019; Okuro *et al.*, 2018; Pernetti *et al.*, 2007; Siraj *et al.*, 2015). However, further studies must be performed to evaluate molecular interactions between structuring agents using, for example, phase diagrams and X-ray diffraction patterns (Silva *et al.*, 2018).

High oleic sunflower oil (HOSO) contains from 75 to 85% oleic acid, representing an interesting alternative for the development of organogels thanks to its different composition from and higher oxidative stability than other vegetable oils resulting from their monounsaturated fatty acid content. However, most edible vegetable oils, such as HOSO, contain a high proportion of unsaturated fatty acids, which hinders their direct use at processing and storage temperatures and thus requires using structuring agents (Cardenia *et al.*, 2011; O'Brien, 2009).

The addition of structuring agents to lipid matrices composed of liquid oils makes it possible to produce structured lipid systems with functional and technological characteristics similar to plastic fats, thereby generating formulations with specific thermal and consistency properties for product-driven applications (Chaves *et al.*, 2018; Co and Marangoni, 2012; Rogers, 2009).

Waxes are composed of varied fractions of n-alkanes, fatty alcohols and fatty acids, in a specific, origin-dependent composition. The proportions of these constituents are of great importance for gelling vegetable oils. Candelilla wax (C), considered food-grade, is of interest as a food-grade structuring agent for use in organogels (Toro-Vazquez *et al.*, 2007).

Sorbitan esters are emulsifiers commonly used in foods. Sorbitan Monostearate (S) has a tasteless and odorless characteristic and is capable of forming semi-solid and thermoreversible organogels. Its organization in the oil phase occurs through the self-assembly mechanism, forming a tubular molecular structure that contributes to the formation of the three-dimensional lattice, immobilizing the organic fluid represented by vegetable oil (Rogers 2009; Smith *et al.*, 2011). Studies on S effects suggest its use as a structuring agent of vegetable oils, through a positive interaction with triglycerides, especially with high contents of fatty acids with 18 carbon atoms (Cerqueira *et al.*, 2017; Oliveira *et al.*, 2015; Sonwai *et al.*, 2017).

Saturated monoglycerides (M) have high potential for food applications and can be used to gel unsaturated vegetable oils with plastic fat characteristics and with high SFA content, thus enabling the formulation of products with low SFA and *trans* fat content (Ojijo *et al.*, 2004; Pieve *et al.*, 2010). The effects of different M on the thermal properties, microstructure and consistency of organogel-emulsions developed with C and safflower oil was investigated by Toro-Vazquez *et al.*, (2013).

The combination of self-assembly structuring agents with crystalline particles in vegetable oils results in hybrid systems with high potential for the formation of stable organogels. Studies show that the combined use of specific structuring agents may result in a satisfactory synergistic effect that can overcome the limitations of organogels consisting of only one type of structuring agent (Chaves *et al.*, 2018).

This study aimed to evaluate and characterize HOSO organogels, structured with C, S and M, using mixture systems represented by a ternary diagram with respect to the physicochemical properties and crystallization behavior of lipid systems with low SFA content, high oleic acid proportion and technological functionality for food applications.

2. MATERIALS AND METHODS

2.1. Material

The organic phase used was high oleic sunflower oil purchased from Cargill Agrícola S.A., Brazil; the following structuring agents were used: candelilla wax (Light Special Candelilla REAL®), purchased from the company Multiceras S.A., Mexico; sorbitan monostearate, purchased from Sigma Aldrich, USA and monoglycerides Grindsted Crystallizer 100, purchased from DuPont do Brasil S.A., Brazil.

2.2. Methods

2.2.1. Organogel formulation

Organogels were prepared by mixing HOSO and the structurants, the oil was heated at a temperature above the melting point of the structurants (80°C), the structurants were added and the samples stirred for 3 minutes for complete homogenization (visually assessed), samples were cooled at the storage temperature (5°C) as previously described at literature (Rocha *et al.*, 2013; Stahl *et al.*, L). The organogels were stabilized for 24 hours at 25°C before the analyses.

2.2.2. Organogel evaluation

Solid fat content (SFC). SFC was determined using a Nuclear Magnetic Resonance (NMR) Spectrometer Bruker pc120 Minispec and high-precision dry baths (0 - 70°C) Tcon 2000 (Duratech, USA). AOCS Cd 16b- 93 method: direct method, serial sample reading, tempering for non-stabilized fat (AOCS, 2009).

Crystallization kinetics. Crystallization kinetics were assessed by initial sample tempering (100ºC for 15 min, 1 h at 70ºC), and the SFC was monitored at 25ºC (± 0.5ºC) on a NMR Spectrometer Mq20 NMR AnalyzerBruker (Wassell and Young, 2007). Data were automatically acquired, measuring every minute, for 1h. Crystallization kinetics were characterized according to the induction period (τSFC) - regarding the beginning of crystal formation, maximum solid fat content (SFCmax) (Campos, 2005; Stahl *et al.*, 2018).

Thermal behavior. The thermal behavior of the samples was analyzed on a differential scanning calorimeter (DSC) TA Q2000, coupled to a RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing system used was Universal V4.7A (TA Instruments, Waters LLC, New Castle). The following conditions of analysis were used: sample mass: ~ 10 mg; modified AOCS Cj 1-94 (AOCS, 2009) method: temperature ranging from -60 to 100°C, with a 5°C/min ramp (crystallization and melting). The following parameters were used to evaluate the results: onset crystallization and melting temperatures (T_{oc} and T_{om}), peak crystallization and melting temperatures (T_{pc} and T_{pm}), crystallization and melting enthalpies (ΔH_c and ΔH_m) and final crystallization and melting temperatures (T_{fc} and T_{fm}) (Barbosa *et al.*, 2018; Campos, 2005).

Polymorphism. Determined by x-ray diffraction, according to procedures by Sawalha and colleagues (Sawalha *et al.*, 2012). Analyses were performed using a Philips (PW 1710) diffractometer and Bragg-Bretano geometry (θ:2θ) with Cu-Ka radiation (l = 1.54056Å, 40-KV voltage and 30-mA current). Measurements were taken with 0.02° steps in 2θ and 2-second acquisition time, with 15-to-30° scans (2θ scale) at 25ºC. Crystalline forms were identified from the characteristic short spacings (SS) of crystals (AOCS, 2009).

Microstructure. The microstructure of the organogels was evaluated under polarized light microscopy. Using a capillary tube, a drop of sample was placed on a glass slide, which was covered with a coverslip, and kept at 5ºC for 24 hours and subsequently at 25ºC for 24 hours. Organogels were analyzed at 25°C using 20x magnification. The microstructure of the crystals was evaluated using a polarized light microscope (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). Images were acquired using the image analysis software Image Pro-Plus 7.0 (Media Cybernetics, United States) in four different visual fields of each slide for each sample, and the mean particle diameter result was expressed as the mean and standard deviation of these values (Cindio and Cacace, 1995; Toro-Vazquez *et al.*, 2013).

Hardness. Hardness was determined using a microcomputer-controlled texturometer (TA-XTi2, Stable Microsystems, United Kingdom). For analysis, 30 mL of organogels were placed in 50-mL beakers and incubated in a Bio-Oxygen Demand (BOD) incubator, at 5ºC, for 24 h. A compression/extrusion test was performed using a 25-mm-wide and 35-mm-long acrylic cylindrical probe, at a rate of 1.0 mm/s, and at a fixed probe-penetration distance of 15 mm. The value considered was the maximum force obtained (Rocha *et al.*, 2013).

Rheological properties. Rheological properties were assessed according to the method proposed by Rocha *et al.*, (2013) using a shear stress-controlled rheometer (Physica MCR 301, Anton Paar, Germany), with parallel plate geometry of stainless steel and with a rough surface (50mm wide and with a 200μm gap). Temperature was controlled using a Peltier system. Temperature scans at a 5°C/min rate were performed from 5°C to 100°C, subsequently cooled down from 100 to 5°C and reheated from 5 to 100ºC. In this analysis, 1Hz frequency (f) and 1% deformation were used, within the linear viscoelasticity range. The organogels were analyzed by shear stress and frequency

scanning to evaluate their mechanical resistance and behavior at different observation times, respectively. Shear stress scans were performed from 0.1 to 10 Pa (f = 1Hz) at 25ºC. Frequency scans were performed from 0.01 to 10 Hz, within the linear viscoelasticity range, and at the same temperature as that used in shear stress scans. The following parameters were determined: elastic modulus (G'), viscous modulus (G"), complex modulus (G*), phase angle (δ) and complex viscosity (η*).

2.3.Statistical analysis

Interactions between structuring agents were studied by varying the concentrations of C, S and M using mixing systems represented by ternary diagrams. At a total concentration of 6% structuring agents, HOSO organogels were prepared by varying the content of each structuring agent, using a simplex centroid design of seven experiments (1 to 7 and 11) and three additional experiments (8 to 10) for model validation, as outlined in Table 1. The solid fat content (SFC), crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness and rheological properties of the resulting organogels were characterized.

For statistical analysis of the results, differences were determined by analysis of variance (ANOVA), followed by the Tukey test at $p<0.05$. For measurable experimental results, regression models were applied as a function of the proportion of each structuring agent (x1, x2, x3): $\hat{y}i = \beta1x1 + \beta2x2 + \beta3x3 + \beta12x1x2 + \beta13x1x3 + \beta23x2x3 + \beta123x1x2x3$, wherein $\hat{y}i$ = estimated response, βi = coefficients estimated using the least squares method, and xi = dependent variables. The quality of the models was evaluated by ANOVA and using the adjusted coefficient of determination (Barros Neto *et al.*, 2001;

211 Cornell, 2002). All statistical tests were performed using the software Statistica 8.0 (Stat
212 Soft, 2007).

213

214 ## 3. RESULTS AND DISCUSSION

215 ### *Solid fat content (SFC)*

216 Figure 1 shows the solid profiles determined for the organogels and from 10 to 70ºC.
217 The SFC of the organogels ranged from 4.72 to 6.84% at 10ºC. All organogels showed
218 fully melted at 65ºC. Organogel 3 (6% M) had the highest thermal resistance at all
219 temperatures. Organogels 3 (6% M), 5 (3% C and 3% M), 6 (3% S and 3% M) and 10
220 (1% C 1% S and 4% M) had SFC higher than 6% at 10ºC (6.84, 6.02, 6.01 and 6.14%
221 solids, respectively), which is higher than the total content of structuring agents. These
222 formulations contain a higher concentration of M (6, 3, 3 and 4%, respectively), most
223 likely due to the different compositions of fatty acids of M and to the presence of stearic
224 (47.82%) and behenic (41.30%) acids at high concentrations (Silva *et al.*, 2018), which
225 may induce crystallization of the most saturated triacylglycerol fraction of HOSO.
226 Organogels 2 (6% S), 4 (3% C and 3% S) and 9 (1% C, 4% S and 3% M) showed reduced
227 SFC at 10ºC (4.72, 4.86 and 5.15%, respectively), with values lower than 6% structuring
228 agents added in total. These formulations contain an increased concentration of S (6, 3
229 and 4%, respectively), the structuring agent with the lowest SFC, either alone or
230 combined with C and M.

231 The SFC at different temperatures describes the melting profiles of organogels,
232 thereby qualifying organogels for possible industrial applications. This analysis made it
233 possible to determine the solid fraction of each organogel during melting. At low
234 temperatures (4 to 10°C), the values of SFC typify the spreadability of cooled organogels.

At room temperature (20 to 22°C), a minimum of 10% solid fat is essential to ensure the resistance to exudation of the oil and product stability when referring to conventional fat. The SFC of organogels ranged from 3.79 (organogel with 6% S) to 5.88% (organogel with 6% M) at 20ºC because they are unconventional lipid bases. At temperatures between 30 and 35°C, general purpose fats, for example, palm oil, stand out for their melting, with concomitant flavor release, and the SFC provides an estimate of taste sensory attributes (Wassell and Young, 2007). Body temperature (37.5ºC) is critical for the sensory quality of lipid-based products. In this range, the SFC should be lower than 5% to minimize possible waxy sensation (Masuchi *et al.*, 2014; Oliveira *et al.*, 2014). All organogels had SFC lower than 4.29% at 37.5ºC.

The SFC values of the organogels at 25ºC are outlined in Table 1. The values ranged from 2.94 to 5.60%; the minimum was assessed in organogel 9 (1% C, 4% S and 1% M) and the maximum in organogel 3 (6% M). Thus, C and M contributed more to the increase in SFC than S.

The structuring agents C, S and M had a significant ($p<0.05$) effect on SFC at 25ºC. The R^2 value (88.42%) indicates that this model (SFC at 25ºC = 4.71*C + 2.74*S + 5.54*M) is suitable to evaluate the behavior of the SFC of organogels formed with C, S and M. From these data, triangular diagrams were generated (Figure 2A).

Crystallization kinetics

Table 1 outlines the induction time (IT) and SFCmax at 25ºC of the organogels. The IT ranged from 3 to 7 minutes and SFCmax from 2.66 to 5.44%. Organogel (1% C, 4% S and 1% M) showed slower crystallization, with an IT of 7 minutes, followed by organogels 2 (6% S), 4 (3% C and 3% S) and 11 (2% C, 2% S and 2% M) (6 minutes), 7 (2% C, 2% S and 2% M) and 8 (4% C, 1% S and 1% M) (5 minutes), 1 (6% C) and 5 (3%

C and 3% M) (4 minutes) and 3 (6% M), 6 (3% S and 3% M) and 10 (1% C, 1% S and 4% M) (3 minutes). These results match the SFC of the organogels.

The SFCmax of organogel 3 (6% M) was the highest, most likely due to the different compositions of fatty acids of M, in line with the SFC profile.

The IT and SFCmax values of the organogels are outlined in Table 1. The IT values ranged from 3 to 7 minutes, with minima in organogels 3 (6% M), 6 (3% S and 3% M) and 10 (1% C, 1% S and 4% M) and a maximum in organogel 9 (1% C, 4% S and 1% M). Thus, C and S contributed more to the increase in IT than M. The SFCmax values ranged from 2.66 to 5.44%, with a minimum in organogel 2 (6% S) and maximum in organogel 3 (6% M). Thus, C and M contributed more to the increase in SFCmax than S. These results are in line with the SFC at 25ºC.

The structuring agents C, M and S had a significant ($p<0.05$) effect on SFCmax. The R^2 values of SFCmax (83.76%) indicate that this model (SFCmax = 4.72*C + 2.28*S + 5.23*M) is suitable to evaluate the variation in SFCmax of organogels. From these data, triangular diagrams were generated (Figure 2B).

Thermal behavior

The thermal behavior of the organogels is shown in Table 2. The melting and crystallization curves can be subdivided into different regions, reflecting different triacylglycerol classes present in organogels.

In terms of thermal behavior in crystallization, organogel 9 (1% C, 4% S and 1% M) had the lowest onset crystallization temperature (-32.12ºC) among the study organogels because organogel 9 did not show peak 1, whereas the T_{oc} of the other organogels ranged from 24.39 to 56.48ºC, for organogels 6 (3% S and 3% M) and 3 (6% M), respectively. Organogels with the highest concentration of S, together with another structuring agent,

had the lowest $T_{oc,}$ indicating crystallization induction, as observed, for example, in organogels 4 (3% C and 3% S) and 6 (3% S and 3% M), which had T_{oc} of 37.23 and 24.39ºC, respectively.

The crystallization curves showed two broad peaks (representing fractions with higher melting points), which are associated with the presence of these different triacylglycerol classes of structuring agents and HOSO (Manzocco *et al.*, 2014; Saberi *et al.*, 2011).

All structuring agents showed co-crystallization with each other and with HOSO, except S. The thermal behavior parameters of the organogels indicated a first melting peak, concerning the most unsaturated triglycerides, most likely of HOSO, and a second melting peak referring to the structuring agent used and to the saturated fraction of HOSO also, although the melting peak was not identified in organogel 9 (1% C, 4% S and 1% M), in contrast to the others, thus indicating higher content of triacylglycerol components with a lower melting point, in line with lower SFC at 25ºC and higher IT values in crystallization kinetics (Table 1).

T_{oc} values of the organogels are outlined in Table 2. The T_{oc} values ranged from -32.12 to 56.48ºC, with a minimum in organogel 9 (1% C, 4% S and 1% M) and a maximum in organogel 3 (6% M). Thus, the structuring agents C and M contributed more to the increase in T_{oc} than S. These structuring agents may be used as crystallization initiators or inducers, and these results are in line with SFC at 25ºC and with IT.

The structuring agents C and M had a significant ($p<0.05$) effect on T_{oc}. The R^2 value (75.07%) indicates that this model ($T_{oc} = 56.03*C + 63.06*M$) is suitable to evaluate the behavior of the T_{oc} of the organogels. From these data, triangular diagrams were generated (Figure 2D).

Polymorphism

Figure 3 shows the X-ray diffractograms of the organogels. The crystalline forms are characterized by specific SS. Typical SS correspond to 4.15 Å for the α form; 3.8 and 4.2 Å for β' and 4.6 Å for β, and they are used to determine the relative proportion and type of polymorphisms present in lipid bases (Stahl *et al.*, 2017).

Organogel 2 (6% S) was liquid at room temperature, which precluded the analysis. The other organogels had SS of 4.6 Å, typical of β polymorphism. Organogels 1, 3, 4, 5, 7, 8 and 11 had SS of 3.8 and 4.2 Å (Table 3), typical of β' polymorphism, which favors the spreadability and creaminess of the products in which lipid bases can be applied (Wassell and Young, 2007).

Organogels 1, 4, 5 and 8 showed more defined peaks, due to their higher C content, composed of hydrocarbons and wax esters, which have high melting points (Doan *et al.*, 2017). These results are in line with the crystallization kinetics parameters because these organogels also have a higher equilibrium SFC (Table 1).

Microstructure

The lipid composition and crystallization conditions affected the crystalline microstructure, and different polymorphic forms and crystalline morphologies are possible. Crystals aggregate into larger structures forming a lattice, which characterizes the microstructural level of fat. The microstructure concept includes information on the state, quantity, shape, size, spatial relationship and interaction between all components of the crystal lattice and has a strong effect on the macroscopic properties of fats (Oliveira *et al.*, 2015; Ribeiro *et al.*, 2009; Shi *et al.*, 2005).

Triacylglycerols generally crystallize as spherulites, which correspond to the aggregation of crystalline lamellae. They grow radially from the same central nuclei and may develop ramifications during ripening (Rousset, 2002). Eventually, depending on the

cooling conditions or even on the melting profile of each fat, triglycerides are associated with other morphologies, such as needles and discs (Oliveira *et al.*, 2015).

Organogels can form a 3D fibrous lattice in which the solvent is trapped in the structuring agent matrix, avoiding solvent mobility. The lattice is stabilized by weak interactions between the chains, such as hydrogen bonding, van der Waals forces and π *staking* (Huang *et al.*, 2014; Lupi *et al.*, 2016; Pirner *et al.*, 2016; Simsolo *et al.*, 2018). Although organogels are known to form through weak intermolecular interactions between molecules of structuring agents, resulting in three-dimensional lattices (Steed, 2011), our fundamental understanding of the types of interactions remains limited (Nikiforidis *et al.*, 2015). The association between the self-assembly and structuring of crystalline particles in vegetable oils is therefore a hybrid system with high potential for organogel formation.

Table 4 outlines the number of crystalline elements, mean density, mean D of crystals (μm), crystal clusters (%) and mean D of individual crystals (μm) of the organogels, after static isothermal stabilization at 25ºC, for 24 hours. Figure 4 shows images of the microstructure of the organogels with stabilization at 25ºC and 20x magnification.

Organogels 1 (6% C), 2 (6% S), 5 (3% C and 3% M), 6 (3% S and 3% M) and 7 (2% C, 2% S and 2% M) had a higher number of crystalline elements (Table 4), highlighting the effect of C and S on the formation of a microstructure typical of conventional crystallization. The mean crystal diameter ranged from 1.08 (organogel with 4% C, 1% S and 1% M) to 1.76 (organogel with 6% S) μm, thus indicating that this parameter remained unchanged at higher C and S concentrations.

C organogels showed decreased lattice density and crystal size, in contrast to S organogels, which showed high mean density and mean crystal Ds. In general, crystal

size is related to the hardness resulting from lipid bases, and small crystals are more hardness than large crystals (Hwang *et al.*, 2012).

Organogels 2 (6% S), 7 (2% C, 2% S and 2% M), 9 (1% C, 4% S and 1% M), 10 (1% C, 1% S and 4% M) and 11 (2% C, 2% S and 2% M) showed more visually defined crystals (Table 4 and Figure 4), most likely due to the high mean density.

The mean diameter of the organogels was smaller than 30 μm, minimizing the possible negative sensory impact of grittiness (Beckett, 2008).

Hardness

The hardness values of the organogels are outlined in Table 1. The values ranged from 0.13 to 32.00 N, with a minimum in organogel 2 (6% S) and maximum in organogel 1 (6% C); therefore, the structuring agent C contributed the most to the increase in hardness.

The R^2 value (87.87%) indicates that this model (Hardness = 29.34*C – 56.46*C*M) is suitable to evaluate the hardness behavior of the organogels. From these data, triangular diagrams were generated (Figure 2C).

Organogels 1 (6% C), 4 (3% C and 3% S), 7 (2% C, 2% S and 2% M) and 11 (2% C, 2% S and 2% M) showed increased hardness, compatible with high SFC at 25ºC. These organogels had a higher number of crystalline elements and a smaller crystal diameter (Table 4), which are characteristics typical of crystal lattices with higher density and consistency (Campos, 2005).

Crystal size can be partly associated with organogel hardness, sand small crystals are more hardness than large crystals (Hwang *et al.*, 2012).

Organogels 1 (6% C), 2 (6% S) and 3 (6% M) showed 32.00, 0.13 and 1.52 N hardness, respectively, which are extreme values. However, organogels 7 and 11, which

contain 2% C, 2% S and 2% M, had intermediate hardness suitable for food applications as lipid bases. This result contradicts a study which reported the need for at least 3% C to obtain a semi-solid organogel in which soft organogels were prepared at concentrations lower than 2% C, even when combined with M and a fully hydrogenated vegetable oil at high concentrations (4 to 5%) (Silva *et al.*, 2018). These characteristics may be favorable in relation to lipid applications in which hardness and texture are important and may be beneficial replacing fats which require increased flowability for products such as spreads (Manzocco *et al.*, 2014). In general, organogel hardness parameters can be associated with the formation of small crystals of fat dispersed in high proportion of liquid oil, promoting the formation of less cohesive crystal lattices.

Frequency scanning

The results from frequency scanning analysis of the organogel deformation behavior within the linear viscoelasticity region are shown in Figure 5. All organogels showed similar behavior and frequency-independent properties, and G' was higher than G'', thus indicating solid material behavior (Steffe, 1996).

Frequency scanning analysis showed that all organogels had intermediate G' and G'' values, except organogels 1 and 2 although the concentration of all organogels was 6%. Organogel 1 (6% C) had the highest G' and G'' values, and these results are in line with hardness (Table 1). Organogel 2 (6% S) had the lowest values of these parameters, which explains the low SFCmax at 25ºC and the low hardness values.

Organogels 1 (6% C) and 7 (2% C, 2% S and 2% M) had the highest number of crystalline elements (Table 4), which is directly related to high G' and G'' values and to high hardness values as well.

Temperature scanning

The results from the temperature scanning analysis of the organogels are shown in Figure 6, characterized by similar behavior, determined by apparent viscosity, with an increase in elastic modulus (G') and viscous modulus (G") within the same temperature range, and G' was higher than G''.

The results from the temperature scanning analysis of the organogels showed that the behavior was the same as that observed by DSC with respect to thermal properties, with similar onset and final crystallization and melting temperatures (Table 2), as well as similar frequency scanning behavior (Figure 5).

Temperature scanning shows that organogels can be used as fat substitutes in industrial processes involving shear stress, even if the process temperature is higher than the formation temperature of the organogel because they are thermoreversible materials, as reported by other researchers (Alvarez-Mitre *et al.*, 2012; Dassanayake *et al.*, 2011; Pernetti *et al.*, 2007; Smith *et al.*, 2011).

4. CONCLUSIONS

The organogels analyzed in this study were stable, except for the organogel formulated with 6% S. This result may be associated with other organogel properties such as hardness, thermal and mechanical stability and polymorphism. In addition, β' and β polymorphs, together with appropriate hardness values, G' higher than G'' and thermal resistance provide organogels applicable as technical fat substitutes in industrial processes, thereby addressing the lack of *trans* fatty acids and the reduction of saturated fatty acids in formulations with higher stability.

Although the structuring agent S showed negative results alone, when combined with the structuring agents C (3% C and 3% S) and with C and M (2% C, 2% S and 2% M), S shows satisfactory results in preparing organogels for application in lipid-based products requiring lower consistency. The most positive thermal and mechanical stability results were assessed when using

a concentration of 2% of each structuring agent (C, S and M; organogels 7 and 11), these organogels can be used to various food applications based on results showed.

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583 **TABLES**

584

585 **Table 1.** Induction time, maximum solid content, solid content at 25ºC and hardness

Oleogels	C (%)	S (%)	M (%)	IT (minutes)	SFCmax (%)	SFC a 25ºC (%)	Hardness (N)
1	6	0	0	4	5.09	4.96	32.00
2	0	6	0	6	2.66	3.04	0.13
3	0	0	6	3	5.44	5.60	1.52
4	3	3	0	6	3.82	3.69	6.22
5	3	0	3	4	5.07	5.20	1.59
6	0	3	3	3	3.62	4.56	0.82
7	2	2	2	5	3.46	4.41	4.54
8	4	1	1	5	4.07	4.37	1.91
9	1	4	1	7	3.03	2.94	0.66
10	1	1	4	3	5.09	4.71	1.96
11	2	2	2	6	3.53	4.13	4.17

586 C - Candelilla Wax; S – Sorbitan monostearate; M – Monoglycerides Grindsted Crystallizer
587 100; IT – Induction time; SFC – solid fat content.

588 **1:** 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S,
589 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S,
590 2% M.

591

592 **Table 2.** Thermal behavior of oleogels

Crystallization

Oleogels	Peak 1				Peak 2			
	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)
1	45.38	37.09	15.44	5.82	-32.99	-38.04	-49.51	37.96
2	47.95	18.30	3.11	1.98	-10.34	-36.47	-50.48	50.39
3	56.48	48.07	7.12	7.61	-26.22	-33.43	-47.96	49.33
4	37.23	29.74	11.97	2.95	-32.66	-37.79	-49.85	41.92
5	48.08	31.38	11.29	4.41	-28.51	-39.00	-51.33	38.70
6	24.39	18.37	2.67	1.29	-31.84	-36.11	-50.74	59.91
7	47.03	16.30	-5.25	5.55	-31.41	-37.96	-50.84	41.73
8	40.37	30.62	5.26	4.92	-34.22	-38.29	-51.16	43.18
9	n.d.	n.d.	n.d.	n.d.	-32.12	-36.37	-49.38	45.97
10	52.00	20.30	8.12	3.86	-31.61	-38.94	-50.79	39.09
11	46.22	26.23	7.80	2.85	-32.33	-37.93	-49.92	40.36

Melting

Oleogels	Peak 1				Peak 2			
	T_{om} (ºC)	T_{pm} (ºC)	T_{fm} (ºC)	ΔH_m (J/g)	T_{om} (ºC)	T_{pm} (ºC)	T_{fm} (ºC)	ΔH_m (J/g)
1	-14.88	-5.14	9.32	52.67	36.19	47.07	57.43	1.78
2	-15.99	-3.38	11.34	49.94	26.26	45.26	58.08	5.81
3	-15.99	-3.54	11.54	57.73	35.96	53.40	70.15	7.78
4	-14.88	-5.03	7.34	57.03	24.52	35.84	60.69	1.23

5	-15.82	-4.76	11.93	54.31	24.82	47.55	67.28	6.44
6	-14.80	-4.76	8.14	54.67	21.72	28.10	36.86	0.66
7	-14.21	-4.57	8.66	56.55	6.78	26.40	58.77	2.90
8	-14.56	-5.12	5.10	55.88	33.78	45.35	58.91	1.52
9	-15.01	-4.67	6.56	58.82	n.d.	n.d.	n.d.	n.d.
10	-14.40	-3.88	31.32	56.55	36.10	49.79	66.65	3.89
11	-14.24	-4.24	7.23	52.25	18.18	26.37	59.73	2.49

593 **T_{oc} and T_{om}**: onset crystallization and melting temperature; **T_{pc} and T_{pm}** peak crystallization
594 and melting temperature; **T_{fc} and T_{fm}:** final crystallization and melting temperature; **ΔH_c, ΔH_m**
595 **and ΔH_{total}:** crystallization, melting and total enthalpies; **n.d.:** not detected.

596 **1:** 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S,
597 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S,
598 2% M.

599

600 **Table 3.** Lipid phase diffractograms of oleogels

Oleogels	**Short spacings**			**Polymorphic form**
	4.6	**4.2**	**3.8**	
1	4.47	4.11	3.71	β' + β
2	liquid	liquid	liquid	-
3	4.57	4.18	3.88	β' + β
4	4.55	4.14	3.73	β' + β
5	4.57	4.14	3.73	β' + β

6	4.60	n.d.	n.d.	β
7	4.55	4.16	3.71	β' + β
8	4.57	4.15	3.72	β' + β
9	4.55	n.d.	n.d.	β
10	4.57	n.d.	n.d.	β
11	4.48	4.14	3.72	β' + β

n.d.: not detected

1: 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S, 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S, 2% M.

Table 4. Microstructure of oleogels.

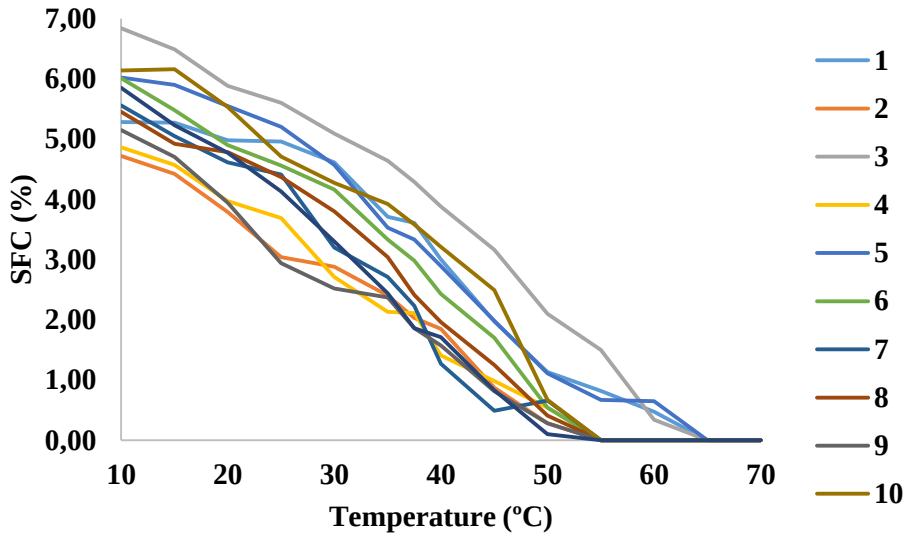
Oleogels	Crystalline elements	Mean density (µm)	Mean D crystals (µm)	Agglomerated crystals (%)
1	49238.00	9.90±7.20	1.33±1.49	14.26
2	13256.00	68.24±16.35	1.76±1.62	32.30
3	97.00	72.35±14.49	1.71±2.47	16.49
4	1825.00	37.06±9.07	1.45±1.19	24.16
5	22100.00	16.65±2.75	1.36±1.34	16.40
6	48810.00	10.00±7.20	1.19±1.04	11.93
7	43845.00	13.95±8.28	1.30±2.42	12.38

8	3062.00	29.48±3.89	1.08±1.21	8.62
9	3190.00	54.03±11.41	1.52±1.51	23.10
10	10240.00	32.12±3.71	1.56±2.69	20.04
11	526.00	38.86±6.95	1.66±2.11	20.15

607 **1:** 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S,
608 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S,
609 2% M.

610

611 **FIGURE CAPTIONS**



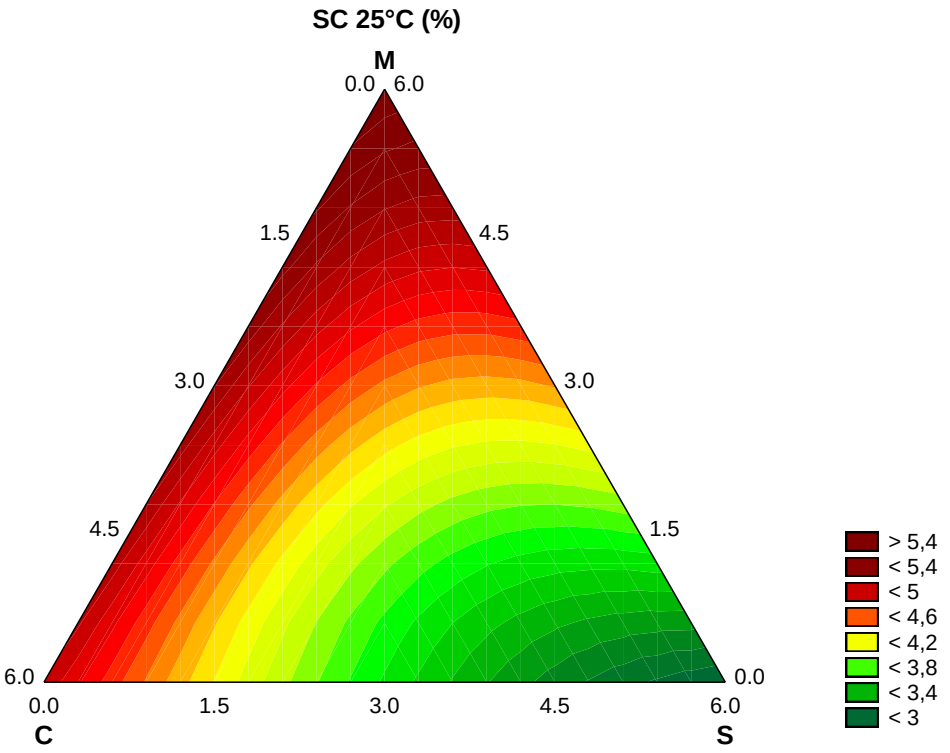
612

613 **Figure 1.** Fat solids profile of oleogels.

614 **1:** 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S,

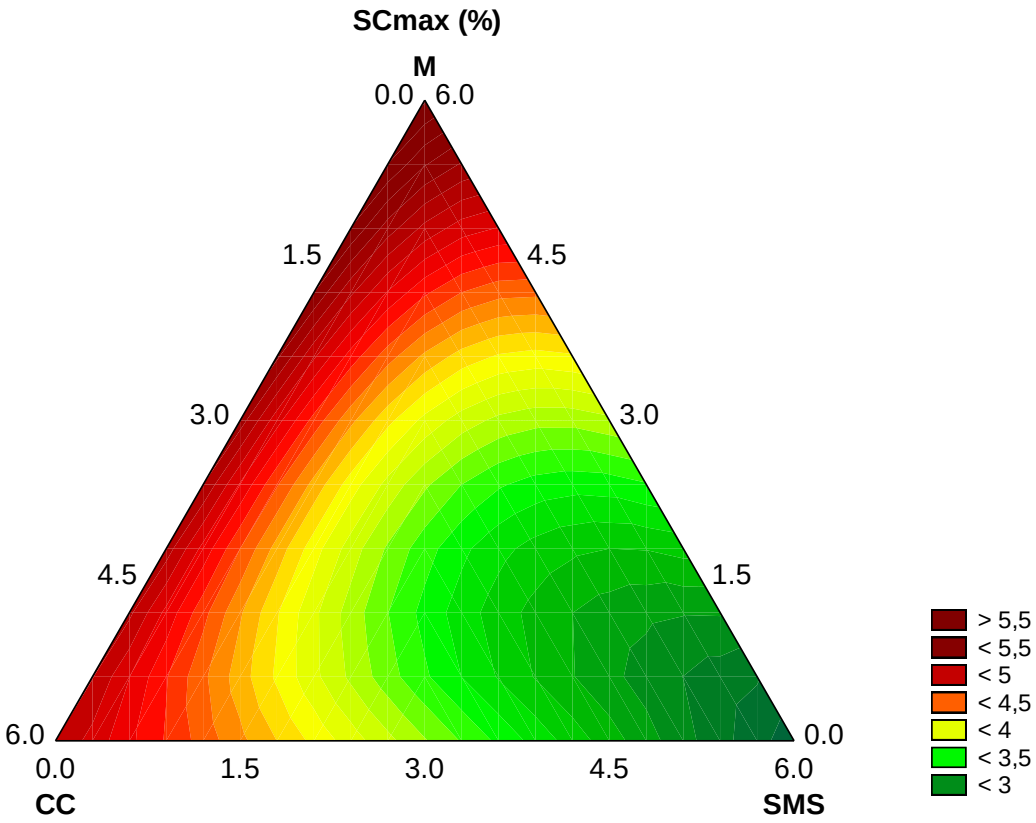
615 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S,

616 2% M.



617

618 **(A)**



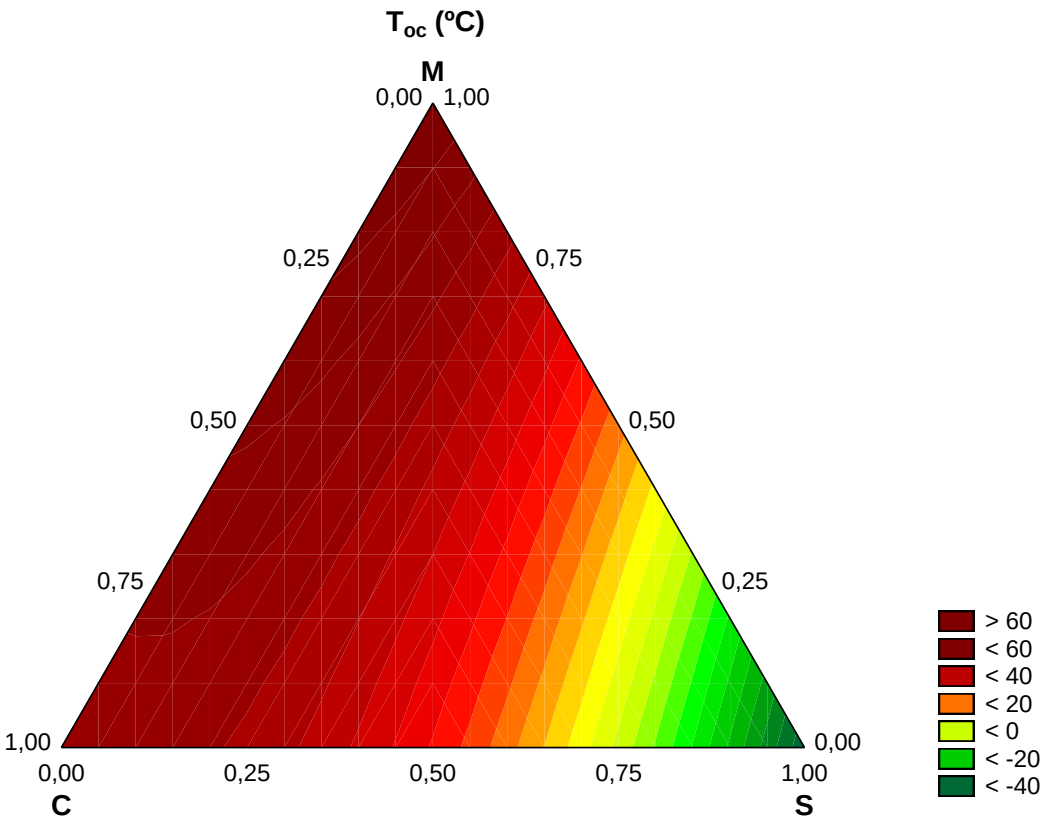
619

620 **(B)**


Hardness (N)
M
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
CC
SMS
> 25
< 25
< 20
< 15
< 10
< 5
< 0

621

622 **(C)**



(D)

Figure 2. Triangular diagrams for solid content at 25ºC (A), maximum solid fat content (SFCmax) (%) (B), hardness (N) (C) and onset crystallization temperature (Toc) (ºC) (D) as a function of candelilla wax (C), sorbitan monostearate (S), monoglycerides (M).

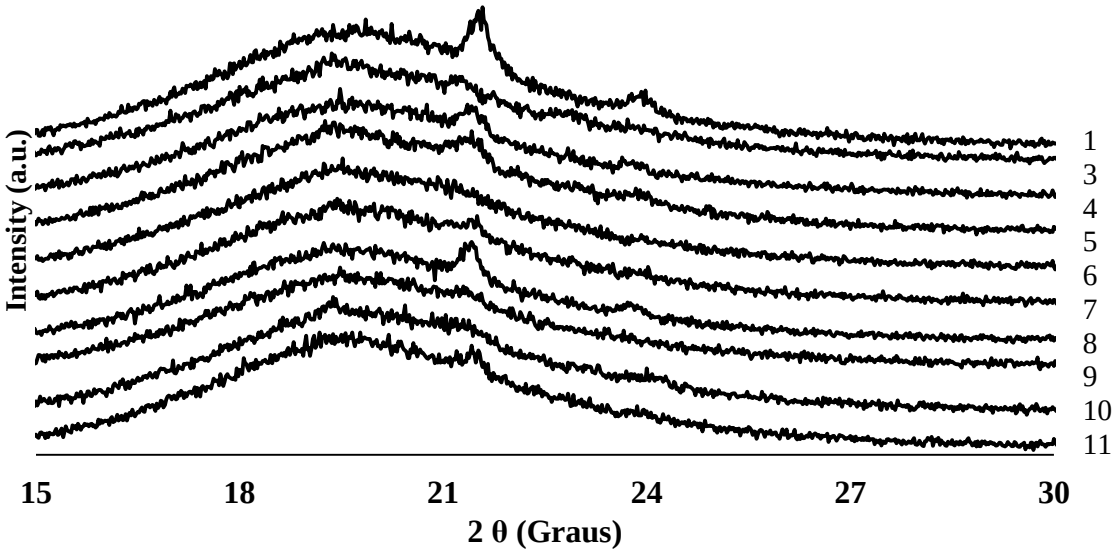
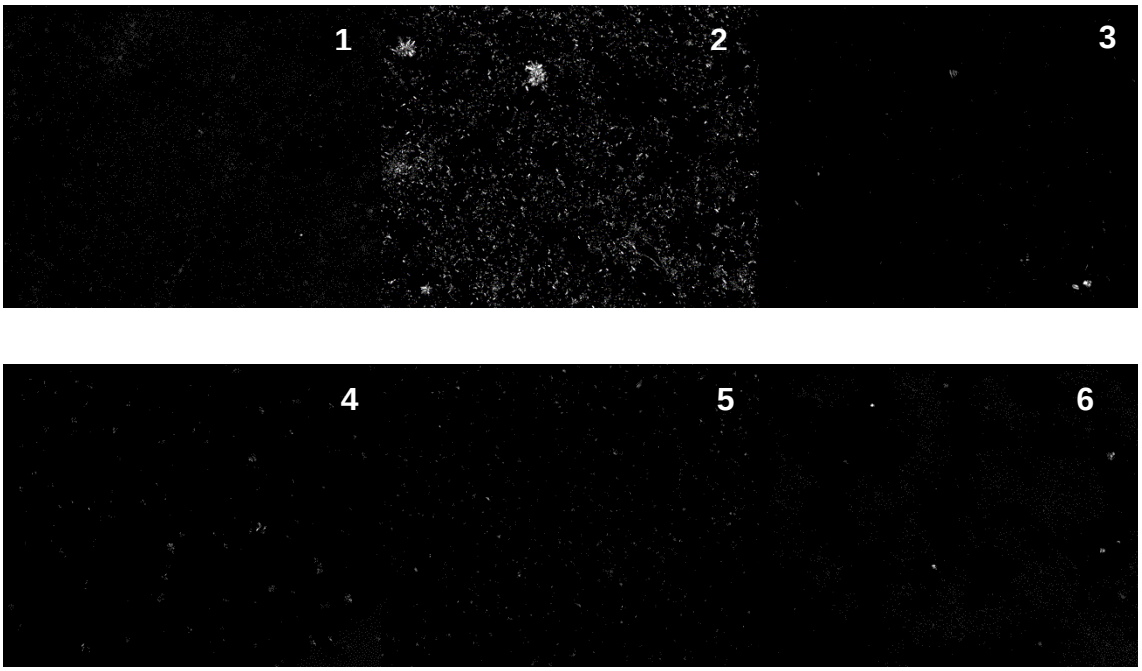


Figure 3. Lipid phase diffractograms of oleogels.

1: 6% C; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S, 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S, 2% M.



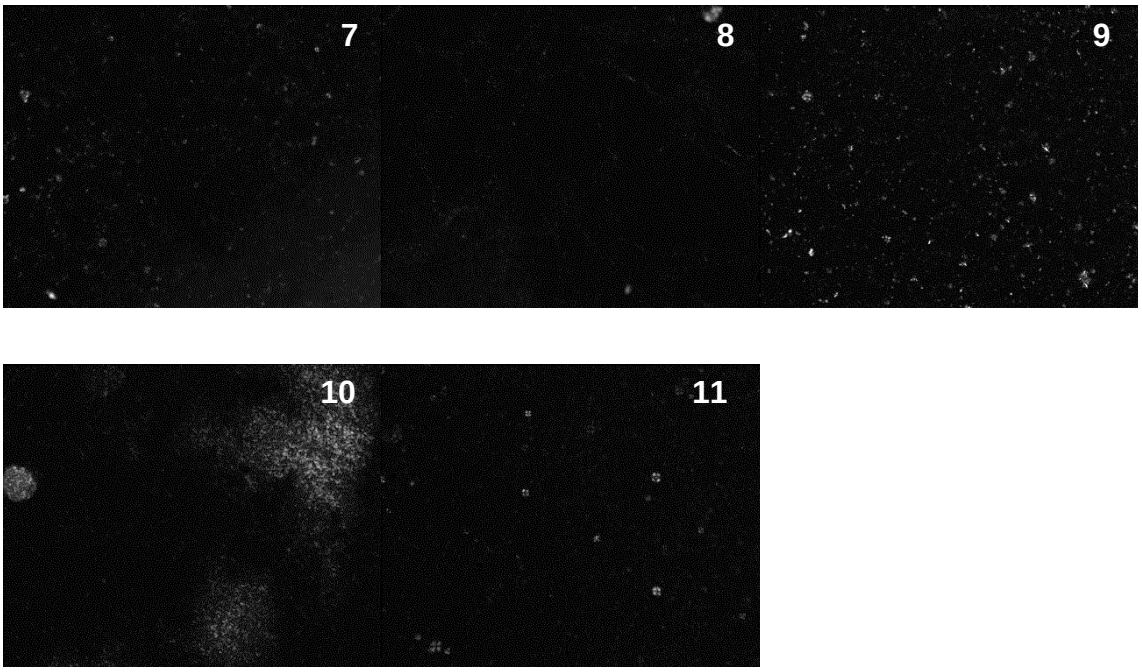
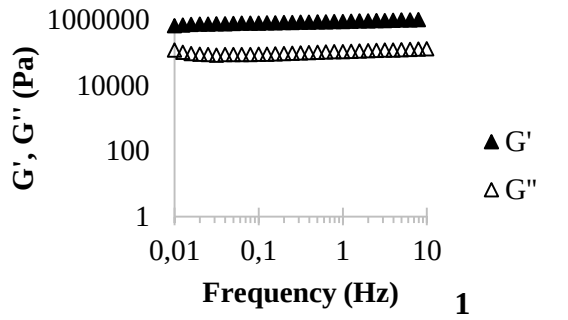
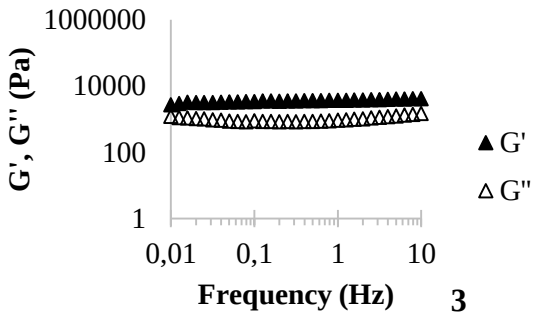
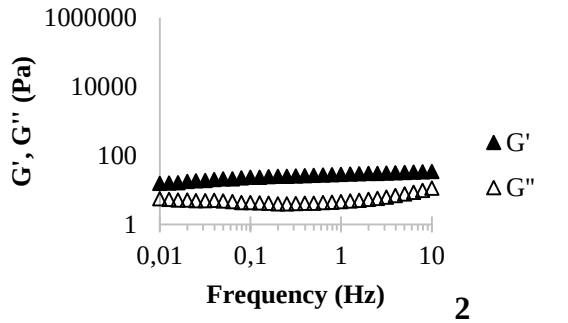


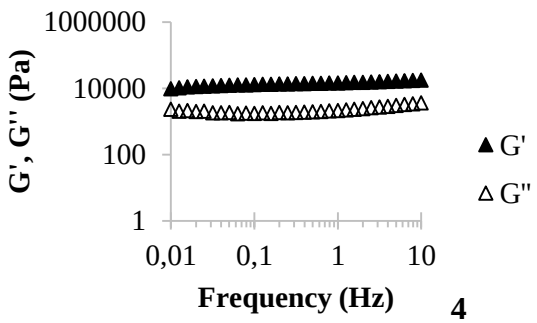
Figure 4. Microstructure of oleogels at 20x magnification and 25°C.

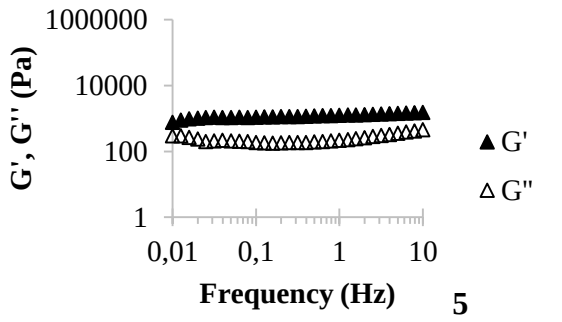
1: 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S, 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S, 2% M.







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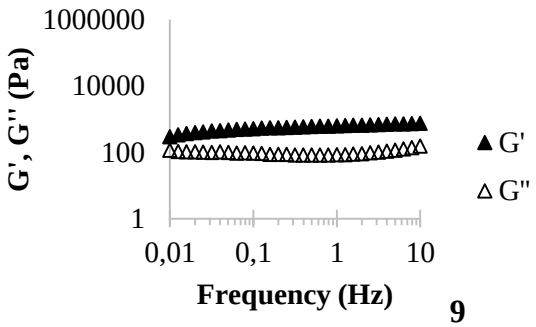
1
10
G', G'' (Pa)
Frequency (Hz)
G'
G''

5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G', G'' (Pa)
Frequency (Hz)
G'
G''

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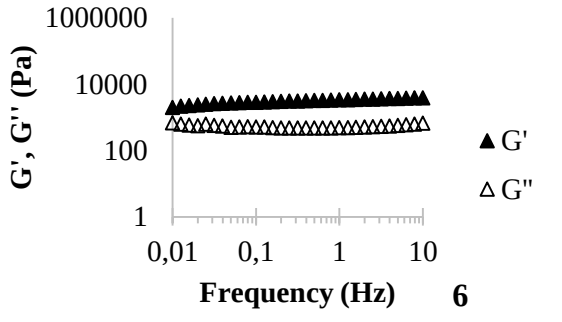
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G', G'' (Pa)
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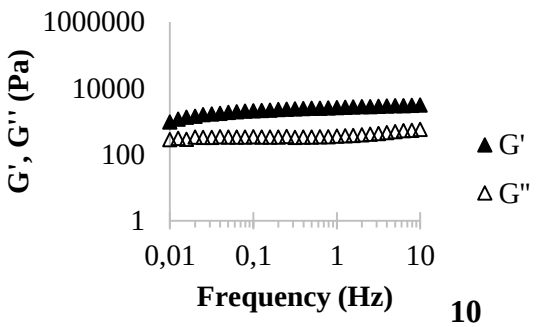
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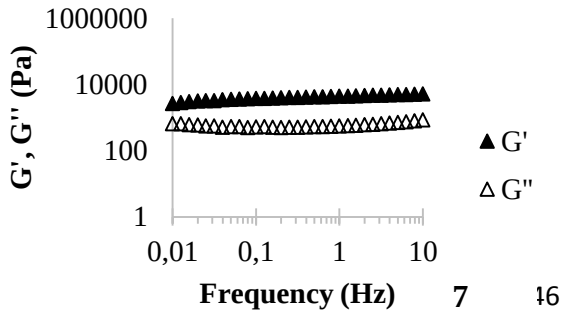
G', G'' (Pa)
Frequency (Hz)
G'
G''

10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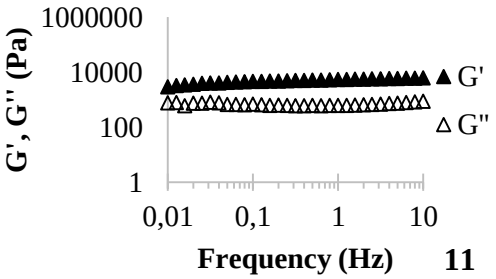
G', G'' (Pa)
Frequency (Hz)
G'
G''

7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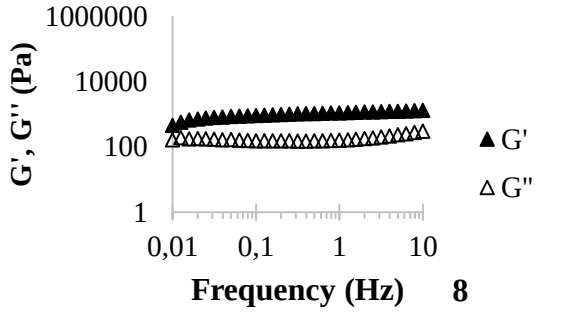
G', G'' (Pa)
Frequency (Hz)
G'
G''

11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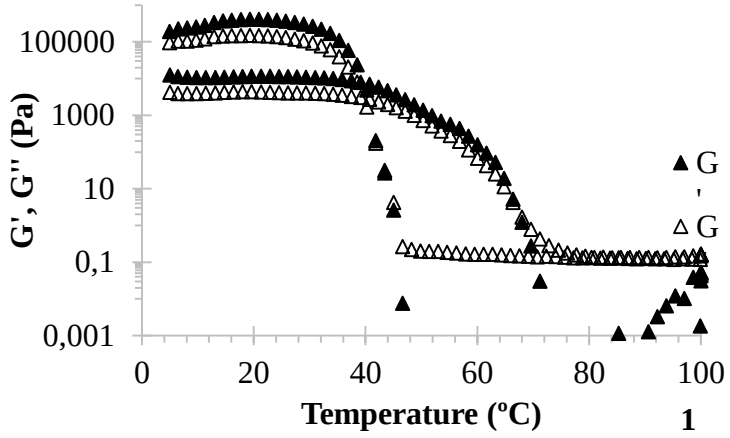
G', G'' (Pa)
Frequency (Hz)
G'
G''

8

Figure 5. G’ (a) and G’’ (b) of the frequency scanning of oleogels.

1: 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S, 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S, 2% M.



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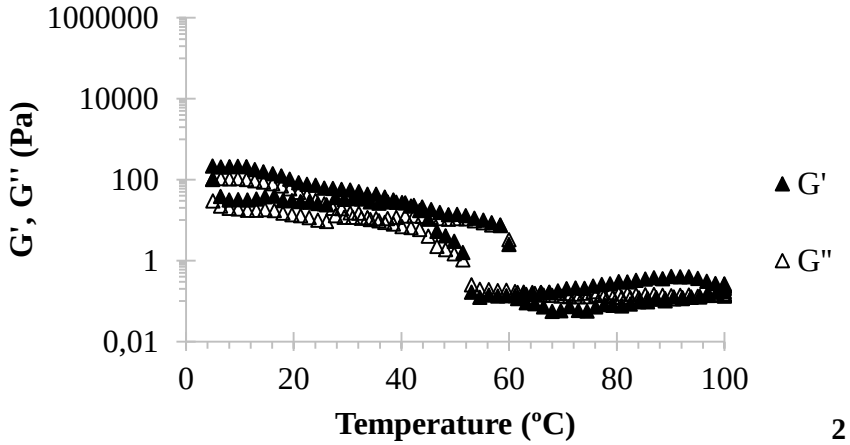

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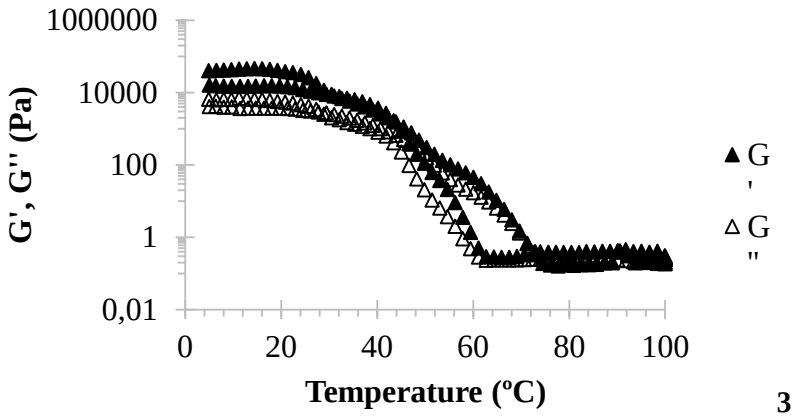

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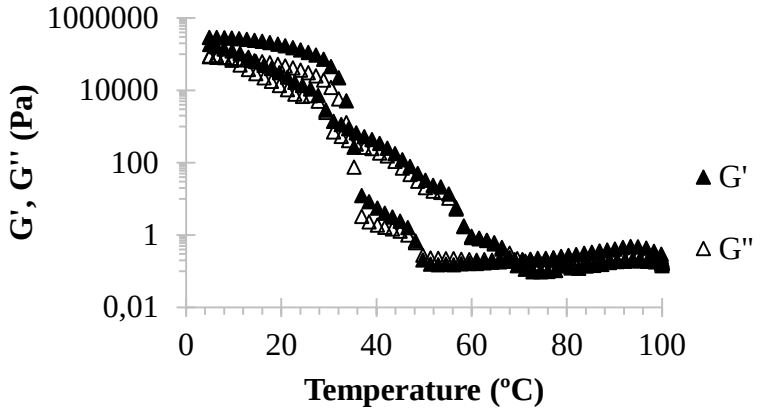
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G', G'' (Pa)
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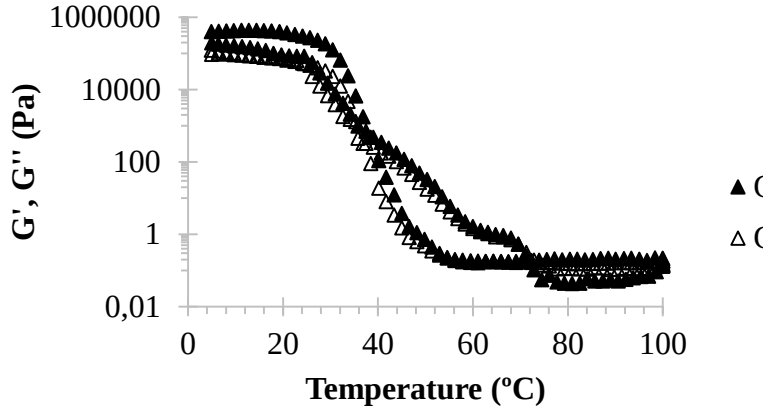
20
40
60
80
100
Temperature (ºC)
▲ G'
△ G''

4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G', G'' (Pa)
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40
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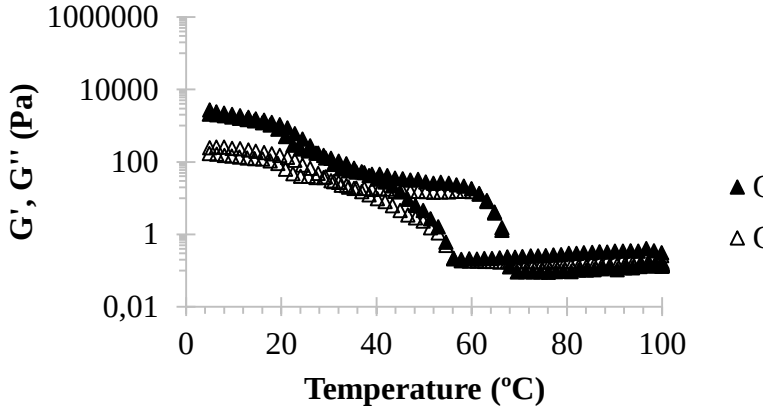
Temperature (ºC)
▲ G'
△ G''

5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G', G'' (Pa)
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Temperature (ºC)
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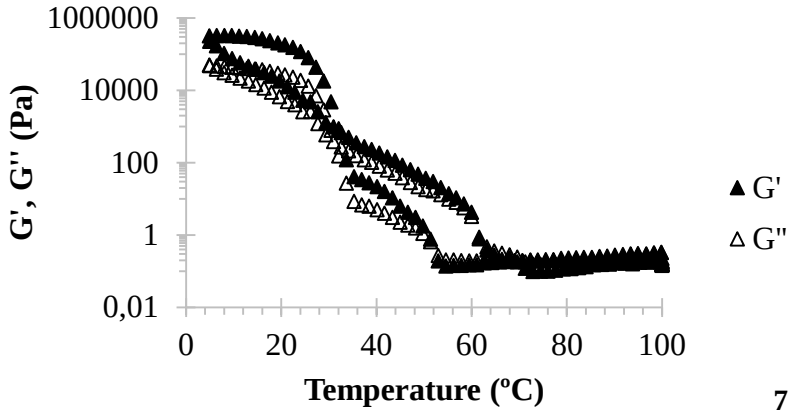
6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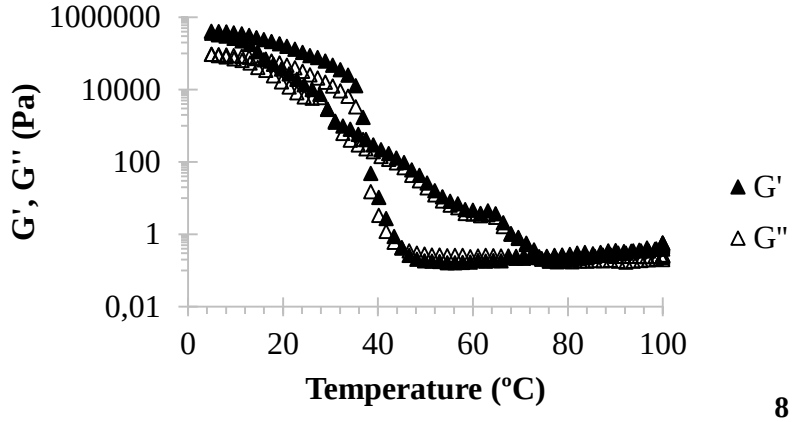
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G', G'' (Pa)
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Temperature (ºC)
G'
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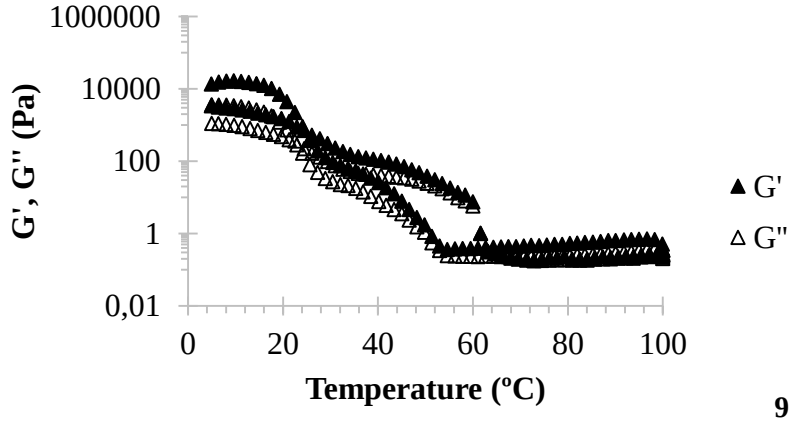
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G', G'' (Pa)
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Temperature (ºC)
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G''

9

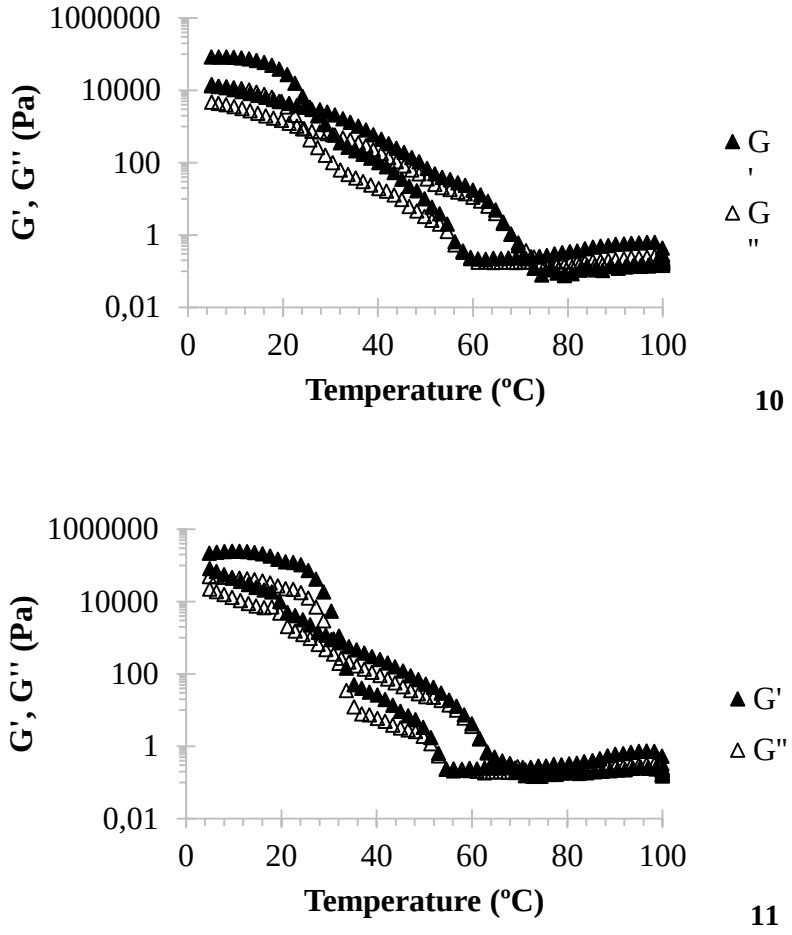

Figure 6. G’ (a) and G’’ (b) of the temperature scanning of oleogels.

1: 6% C; **2:** 6% S; **3:** 6% M; **4:** 3% C, 3% S; **5:** 3% C, 3% M; **6:** 3% S, 3% M; **7:** 2% C, 2% S, 2% M; **8:** 4% C, 1% S, 1% M; **9:** 1% C, 4% S, 1% M; **10:** 1% C, 1% S, 4% M; **11:** 2% C, 2% S, 2% M.

CAPÍTULO 7

FORMULATION OF LIPID BASES USING A TERNARY SYSTEM

Artigo Submetido

Journal of the American Oil Chemists' Society

Wiley

Formulation of lipid bases using a ternary system

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Abstract

Lipid systems composed of liquid vegetable oils can be structured as gels, structures that form continuous networks of small molecules that congregate in liquid crystals, micelles or fibrillar networks and are called organogels. The aim of this study was to evaluate and characterize high oleic sunflower oil (HOSO) organogels structured with candelilla wax (C), sorbitan monostearate (S) and palm oil hardfat (H). The organogels were evaluated for their physicochemical properties and their crystallization behavior, aiming at obtaining lipid bases. All organogels showed complete fusion at 55ºC; C and H were determinant for SC increase at 25ºC. H was the most effective structuring factor for the increase of IT. The thermal behavior organogels parameters indicated two melting peaks, relative to HOSO unsaturated triacylglycerols and to the structuring used, respectively. The presence of C as structuring induces the formation of smaller crystals. Structurer C was more effective to increase hardness, both for isolated structurers and for their combinations. Structurer S alone promoted lower hardness (6% 0.13N), but when combined with structurer H, which also resulted in low hardness organogel (6% 0.56N), it had a synergistic action for this parameter. All organogels showed similar rheological behavior and frequency and temperature independent properties, with G' superior to G", characteristic of solid material. Temperature scan analysis showed that the organogels properties corresponded to thermal behavior parameters. Organogels have been characterized as potential lipid bases for use as fat substitutes in industrial processes, with effective reduction of saturated fatty acids.

Keywords: high oleic sunflower oil; candelilla wax; sorbitan monostearate; palm *hardfat*; organogel.

1. INTRODUCTION

Organogels are a class of materials that can hold large volumes of organic liquids in self-assembled networks, with many practical applications in the pharmaceutical, chemical and food industries (Nikiforidis et al., 2015). Organogels are formed by low molecular weight structurers and some immobilized organic solvents in a three-dimensional network. The biodegradable characteristic of organogels allows them to be used in drugs such as protein carriers, medicines and vaccines, for example. (Mandal et al., 2015; Uzan et al., 2016; Zhao et al., 2017).

Research on food grade organogels has recently received widespread academic attention due to the versatility of applications (Barbut et al., 2016a, 2016b; Bemer et al., 2016; Chaves et al., 2018; Hwang et al., 2016, 2013; Jang et al., 2015; Manzocco et al., 2014a; Mert and Demirkesen, 2016; Moschakis et al., 2016; Patel et al., 2014; Silva et al., 2018; Tanti et al., 2016; Yilmaz and Ogutcu, 2015; Zevenbergen et al., 2009; Zulim Botega et al., 2013a, 2013b).

The most commonly used structuring agents are fatty acids, fatty alcohols, fatty acids and fatty alcohols mixtures, phytosterols/oryzanols mixtures, sorbitan monostearate, lecithin mixtures, sorbitan tristearate and waxes (Rogers et al., 2009). Mixing these compounds can have a synergistic effect on the structuring potential of oils compared to the use of pure materials (Pernetti et al., 2007).

Waxes are composed of varied fractions of n-alkanes, fatty alcohols and fatty acids, depending on their origin. The proportions of these constituents are of great importance in the gelation of organic phases, without negative effects on health. Candelilla wax (C) is of interest as a food grade structuring potential for use in organogels (Toro-Vazquez et al., 2007). C is derived from leaves of a small shrub of the family *Euphorbiaceae* found in northern Mexico and in the southwestern United States. (Kuznesof, 2005).

Sorbitan esters are emulsifiers commonly used in foods. Studies have shown this group of compounds as potential structuring agents of vegetable oils, also modulating the crystallization process. Specifically, sorbitan monostearate (S) is tasteless and odorless, capable of forming semisolid and thermo-reversible organogels. The organization of S in the oil phase occurs through a self-assembly mechanism, from a tubular molecular structure that contributes to the formation of a three-dimensional network, immobilizing the organic fluid represented by vegetable oil (Rogers, 2009; Sith, Bhaggan,

Talbot and van Malssen, 2011). Recent studies on the structuring effects of S suggest its positive interaction with high melting triglycerides (TAG) (Oliveira, Stahl, Ribeiro et al., 2015; Cerqueira et al., 2017; Sonwai, Podchong, & Rousseau, 2017).

In this context, fully hydrogenated vegetable oils, technically referred to as *hardfats*, have been effective in modifying crystalline properties of lipid bases, acting as structuring elements of crystallization process acceleration in low and medium melting TAG composite systems. They come from the total catalytic hydrogenation of natural oils and are characterized by a homogeneous fatty acids and high melting TAG composition, being considered low cost products from the industrial point of view (Oliveira, Ribeiro, & Kieckbusch, 2015; Ribeiro, Basso, & Kieckbusch, 2013).

The combination of *self-assembly* structuring agents and crystalline particles in vegetable oils constitute hybrid systems with high potential for stable organogels formation. Studies show that the combined use of specific structuring agents can result in a satisfactory synergistic effect that may overcome possible disadvantages of organogels consisting of only one type of structuring agent (Chaves et al., 2018).

The objective of this study was to evaluate and characterize high oleic sunflower oil (HOSO) organogels, structured with C, S and palm oil *hardfat* (H), using ternary diagram mixtures, with regard to physical-chemicals properties and crystallization behavior of lipid systems with low levels of saturated fatty acids, high proportion of oleic acid and technological functionality for food application.

2. MATERIAL AND METHODS

Material

The organic phase used was HOSO provided by Cargill Agrícola S.A., Brazil; As structuring agents were used lentil-shaped candelilla wax (C) (Light Special Candelilla REAL®), supplied by Multiceras S.A., Mexico; sorbitan monostearate (S) supplied by Sigma Aldrich, USA and fully hydrogenated palm oil (H) (*hardfats*) supplied by SGS Agricultura e Indústria Ltda, Brazil.

Methods

Formulation of organogels

The organogels were prepared by mixing HOSO and the structuring agents under heating above the melting point of the structuring agents (80° C), stirring for 3 minutes for complete homogenization and cooling at 5° C storage temperature (Rocha et al., 2013; Stahl et al., 2018a).

Structural interactions were studied by varying C, S and H concentrations. This evaluation was performed using a mixture system represented by a ternary diagram. For a total concentration of 6% structuring agents, HOSO organogels were obtained, varying the contents of each structuring agent, through simplex centroid experimental design of seven experiments and 3 additional experiments to validate the model, according to Table 1. The obtained organogels were characterized according to solids content, crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness, oil exudate content and rheological properties.

In the statistical analysis results, differences were determined by analysis of variance (ANOVA), followed by Tukey test at $p < 0.05$. For the measurable experimental results, regression models were applied as a function of the proportion of each structuring agent. (x1, x2, x3): $\hat{y}i = \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 + \beta 123x1x2x3$, where $\hat{y}i$ =estimated response, βi = estimated coefficients by the method of minimum squares, and xi =dependent variables. The models quality was evaluated by ANOVA and adjusted determination coefficient (BARROS NETO et al., 2001; Cornell, 2002). The statistical analyses were conducted using Statistic 8.0 (STAT SOFT, 2007).

Organogel Evaluation

Solids content (SC). It was determined by Nuclear Magnetic Resonance Spectrometer (NMR) Bruker pc120 Minispec, with dry baths of high precision (0 - 70°C) Tcon 2000 (Duratech, USA). Method AOCS Cd 16b- 93: direct method, series sample reading, tempering for non-stabilized fats (AOCS, 2009).

Crystallization kinetics. It was obtained through the initial tempering of samples (100ºC for 15 min, 1 h at 70ºC) and the solids content was monitored at 25ºC (± 0.5ºC) in the Nuclear Magnetic Resonance Spectrometer Mq20 NMR AnalyzerBruker (Wassell and Young, 2007). Data were acquired automatically, measured every minute for 1h. The characterization of crystallization kinetics was performed according to the induction time (IT) - relative to the beginning of crystal formation, maximum solids content (SCmax) (Campos, 2005; Stahl et al., 2018b).

Thermal behavior. The samples' thermal analysis was performed using a differential scanning calorimeter (DSC) TA Q2000, coupled to RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing system used was the Universal V4.7A (TA Instruments, Waters LLC, New Castle). The analysis conditions were: sample mass: ~ 10 mg; method AOCS Cj 1-94 (AOCS, 2009) modified: temperature between -60 and 100°C, with ramp of 5° C/min (crystallization and melting). The following parameters were used to evaluate the results: crystallization and melting onset temperature (T_{oc} and T_{om}), peak crystallization and melting temperatures (Tpc and Tpm), crystallization and melting enthalpies (ΔH_c and ΔH_m) and crystallization and melting completion temperature (T_{cc} and T_{mc}) (Barbosa et al., 2018; Campos, 2005).

Polymorphism. Determined by x-ray diffraction, according to procedures by Sawalha et al. (Sawalha et al., 2012). Analyses were conducted on a Philips diffractometer (PW 1710), using Bragg-Bretano (θ:2θ) geometry with Cu-ka (l = 1.54056Å, 40 KV tension and 30 mA current) radiation. Measurements were obtained with steps of 0.02° at 2q and 2 seconds acquisition time, with *scan*s of 15 to 30° (scale 2θ) at 25ºC. The identification of crystalline forms was performed with *short spacings* (SS) characteristics of crystals (AOCS, 2009).

Microstructure. The organogels microstructure was evaluated by microscopy under polarized light. With the aid of a capillary tube, a drop of sample was placed on a glass slide, which was covered with a coverslip and kept at 5ºC for 24 hours and then at 25ºC for 24 hours. Organogels were analyzed at 25° C using 20x magnification. The microstructure of crystals was evaluated using a polarized light microscope (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). Images were captured by Image Pro-Plus 7.0 (Media Cybernetics, United States) in four different visual fields on each slide for each sample and the mean particle diameter result was expressed as mean and standard deviation of these values (Cindio and Cacace, 1995; Toro-Vazquez et al., 2013).

Toughness. Determined using a texturometer (TA-XTi2, Stable Microsystems, England) controlled by microcomputer. For the analyses, 30 mL of organogels were placed in 50 mL beakers and placed in a B.O.D. oven at 5ºC for 24 h. A compression/extrusion test was performed using a 25 mm

diameter and 35 mm long cylindrical acrylic probe with a velocity of 1.0 mm/s and a 15 mm fixed distance for probe penetration. The value considered was the maximum force obtained (Rocha et al., 2013).

Oil exudate content. It was evaluated on organogels to test samples stability which were analyzed 24 hours after obtaining organogels at 25° C. All samples were monitored for stability with phase separation or liquid oil exudation observation (Rocha et al., 2013).

Rheological properties. The rheological analyses were performed according to methodology proposed by (Rocha et al., 2013) using a tension-controlled rheometer (Physica MCR 301, Anton Paar, Germany). The geometry of parallel plates of rough surface stainless steel (50mm diameter and 200μm gap) was used. The temperature was controlled using a Peltier system. Temperature scans at rate of 5° C/min were done from 5° C to 100° C, then cooled from 100 to 5° C and reheated from 5 to 100° C. Frequency (f) of 1Hz and 1% deformation were used within the linear viscoelasticity range. The organogels were analyzed by voltage and frequency scanning to evaluate their mechanical resistance and behavior against different observation times, respectively. Voltage scans were performed from 0.1 to 10 Pa (f = 1Hz) at 25ºC. Frequency scans were obtained between 0.01 and 10 Hz, within the linear viscoelastic range, and within the same range as voltage scans. The following parameters were determined: elastic modulus (G'), viscous module (G"), complex module (G*), phase angle (δ) and complex viscosity (η*).

3. RESULTS AND DISCUSSION

Solids content

Figure 1 shows solids profiles determined for organogels at 10 to 70° C. Organogel 3 (6% H) (6.57%) showed higher thermal resistance, followed by organogels 10 (1% C 1% S and 4% H) (6.31%), 5 (3% C 3% H) (6.17 %) and 6 (3% S 3% H) (5.81%). All of them have 6% of structurers in its composition, and H is present in higher concentration, 6, 4, 3 and 3%, respectively. Organogels 2 (6% S) and 4 (3% C, 3% S) showed SC of 4.72 and 4.86% and had in their composition 6 and 3% of S, respectively. Organogels 1 (6% C), 7 (2% C 2% S 2% H), 8 (4% C 1% S 1% H), 9 (1% C 4% S 1% H)

and 11 (2% C 2% S 2% H) exhibited similar SC (5.28, 5.24, 5.27, 5.23 and 5.20%, respectively), mainly at 10° C and all showed complete fusion at 55° C. During lipid phase crystallization, materials with similar chemical composition and molecular structure are more prone to co-crystallization (Marangoni, 2005).

The SC at different temperatures describes the organogels' melting profiles, thus directing possible industrial applications. The SC as a function of temperature provides an indication of basic physicochemical attributes and technological performance of lipid bases (Braipson-Danthine and Deroanne, 2006). At low temperatures (4 to 10 ° C), SC values typify spreadability. As the SC organogels was from 4.72 (6% S organogel) to 6.57% (6% H organogel), all samples are suitable for spreadable products. At room temperature (20 to 22° C), a minimum of 10% solid fat is essential to ensure oil exudate resistance and stability when it comes to conventional lipid bases, however organogels at 20° C showed SC from 3.97 (with 3% C and 3% S organogels) to 5.76% (6% H organogel) and were stable and without HOSO exudation, with the exception of organogel 2 (6% S) which did not form a structured three-dimensional network with isolated S only. SC values at temperatures below 25° C qualify hardness parameters, while SC values between 25 and 30° C refer to thermal resistance, since in this temperature range most lipid bases begin the melting process (Braipson-Danthine and Deroanne, 2006). At temperatures between 30 and 35° C, commonly used fats, such as palm oil, are distinguished by melting, with concomitant release of flavor; and SC provides an estimate of sensory attributes in tasting (Wassell and Young, 2007). The body temperature (37.5ºC) is critical for sensory quality lipid-based products for the food industry. In this range, SC should be less than 5%, to minimize possible waxy sensation (De Oliveira et al., 2014; Masuchi et al., 2014); organogels exhibited SC 0.82 (with 3% S and 3 % H organogel) to 3.61% (6% C organogel).

The experimental values obtained for organogels in relation to SC at 25ºC are presented in Table 1. The values are 3.04 to 5.02%, where the minimum value corresponded to organogel 2 and the maximum to organogel 5. Consequently, C and H were more relevant to the increase of SC at 25ºC.

Structurers C, S and H showed a statistically significant influence ($p < 0.05$) on SC at 25ºC. The value of R^2 (90.68%) indicates that this model (SC at 25ºC = 4.87* C + 3.01* S + 4.84 * H) is adequate

to evaluate SC behavior of organogels. From these data, it was possible to generate triangular diagrams (Figure 2A).

Crystallization kinetics

Table 1 shows the induction time (IT) and SCmax of organogels. The IT was from 4 to 18 minutes, where the minimum value corresponded to organogel 1 (6% C) and the maximum to organogel 10 (1% C 1% S 4% H), so H was the most relevant structuring for IT increase. The SCmax was from 1.66 to 5.70%, where the minimum value corresponded to organogel 6 (3% S 3% H) and the maximum to organogel 3 (6% H); these results were consistent with SC at 25ºC, where H was important for increasing solids levels, probably due to the presence of palmitic (43.09%) and stearic (54.11%) fatty acids (Oliveira et al., 2015). Organogels 3 (6% H) and 10 (1% C 1% S 4% H) showed slower crystallization, with IT equal to 15 and 18 minutes, respectively, followed by organogels 11 (2% C 2% S 2% H) (9 minutes), 6 (3% S 3% H) and 7 (2% C 2% S 2% H) (7 minutes), 2 (6% S), 4 (3% C 3% S) , 5 (3% C 3% H), and 9 (1% C 4% S 1% H) (6 minutes), 8 (4% C 1% S 1% H) (5 minutes) and 1 (6% C) (4 minutes), results consistent with SC of organogels (Table 1).

The organogel 3 SCmax value (6% H) was the highest, probably due to the different triacylglycerol class compositions of H, results consistent with the SC profile.

Organogels 1 (6% C), 4 (3% C 3% S), 5 (3% C 3% H) and 8 (4% C 1% S 1% H) showed the highest SCmax values, 5.09, 3.82, 4.77 and 3.38%, respectively, possibly due to the C high concentration used in organogels, and IT of these organogels were 4, 6, 6, and 5 minutes. Again, there is an effect of high levels of saturated fatty acids and alcohols and the alkanes present in C (Doan et al., 2017a). This effect is similar to that observed by Basso et al. (2010) in studies on structuring with behenic acid, as they are long chain saturated components with high melting point.

Organogels 2 (6% S), 6 (3% S 3% H), 7 (2% C 2% S 2% H), 9 (1% C 4% S 1% H) and 11 (2% C 2% S 2% H) showed SCmax values below 2.59%, indicating that possibly SCmax is associated with S molecular dissimilarity compared to other structurers, as the presence of esterified sorbitol to the molecule makes molecular packaging difficult (Marangoni, 2005).

The C, S and H showed statistically significant influence ($p < 0.05$) on the IT and SCmax. The R^2 value of IT (71.24%) and SCmax (92.62%) indicates that these models (IT = 16.87* H) (SCmax = 4.90* C + 2.76* S + 5.92* H - 9.43* S* H) to evaluate the IT organogels behavior. From these data, it was possible to generate triangular diagrams (Figures 2B and 2C).

Thermal behavior

The thermal behavior of organogels is shown in Table 2 and Figure 3. The melting and crystallization curves can be subdivided into different regions, reflecting the different triglyceride classes present in organogels.

Regarding the thermal behavior on crystallization, organogels 3 (6% H), 6 (3% S 3% H) and 10 (1% C 1% S 4% H) showed lower crystallization *onset* temperature (T_{oc}) (26.06, 24.61 and 26.56ºC, respectively), probably due to TAG high concentration with H higher melting point, structuring present in higher concentration in these organogels. The *hardfats* play an interesting role in TAG structuring due to their insolubility, or limited solubility, in polyunsaturated vegetable oils, and the ability to form a solid crystal network. The different types of *hardfats* have similar melting characteristics, high melting point, high enthalpy compared to conventional fats and the ability to form a matrix that will crystallize at high temperatures, forming a fine dispersion of stable crystals (Alander and Lidefelt, 2007; Norberg, 2006; Talbot, 1989).

The other organogels showed T_{oc} from 34.04 to 47.95ºC for organogels 9 (1% C 4% S 1% H) and 2 (6% S), respectively. The crystallization curves showed two wide peaks (representative of the lowest and highest melting point TAG fractions, respectively) and overlapping, which are associated with the presence of these different triglyceride classes (Manzocco et al., 2014b; Saberi et al., 2011).

All organogels showed co-crystallization with each other and with HOSO, except for S. The thermal behavior parameters indicated that organogels have a first melting peak, relative to the most unsaturated TAGs, probably from HOSO, and the second melting peak is relative to the structuring used and also HOSO saturated fraction.

Polymorphism

Figure 4 shows the X-ray diffractograms of organogels. Crystalline forms are characterized by *short spacings* (SS) or interplanar distances between fatty acid chains, which can be determined by X-ray diffraction. The characteristic SS correspond to 4.15 Å to form α, 3,8 and 4,2 Å for β' and 4,6 Å for β, and are used to determine the relative proportion and type of polymorphs present in organogels (AOCS, 2009; Stahl et al., 2017).

The organogel with 6% S was liquid at room temperature which made the analysis impossible. For the other organogels SS were verified equal to 4.6 Å, characterizing the β polymorphic habit (Table 3).

The more heterogeneous the structuring composition, the greater tendency to stabilize the β'-polymorphic habit, due to the difficulty of crystalline packaging for the most stable crystalline form (Campos, 2005; Marangoni, 2005). Organogels 1, 3, 4, 5, 7, 8, and 11 showed SS equal to 3.8 and 4.2 Å, characterizing the β' polymorphic habit, which favors the spreadability and creaminess of products to which lipid bases can be applied (Wassell and Young, 2007). According to different literature studies that characterize palm oil as β', Stahl et al. (2017) and Basso et al. (2010) reported that the presence of stable crystals in the form β' in palm oil, due to its diverse fatty acid composition and, specially, the greater amount of palmitic acid.

The organogels 1 (6% C), 4 (3% C 3% S), 5 (3% C 3% H) and 8 (4% C 1% S 1% H), showed more defined peaks due to higher C contents, due to hydrocarbons and wax esters in their composition (Doan et al., 2017b). These results agree with crystallization kinetics parameters, as these organogels also have high solids in equilibrium (Table 1).

Microstructure

Lipid composition and crystallization conditions affects crystal shape, and different polymorphic forms and crystalline morphologies are possible. The crystals are aggregated into larger structures forming a network, which characterizes the fat microstructural level. The microstructure concept includes information about state, quantity, shape, size, spatial relationship, and interaction between all

components of the crystal network, and has a huge influence on fat macroscopic properties (Marangoni and Hartel, 1998; Oliveira et al., 2015; Ribeiro et al., 2009; Shi et al., 2005).

TAGs generally crystallize as spherulites, which is an aggregation of crystalline lamellae, growing radially from the same central nuclei and may develop branches during ripening (Rousset, 2002). Eventually, depending on the cooling conditions or even the melting profile characteristic of each fat, TAGs may also crystallize into other characteristic morphologies such as needles and discs (Oliveira et al., 2015).

Organogels can structure into a 3D fibrous network, where the solvent is trapped in the structuring matrix, immobilizing the organic fluid. The network is stabilized by weak interactions between chains, such as hydrogen bonding, van der Waals and π staking (Huang, Chen, Huang, & Xu, 2014; Lupi et al., 2016; Pirner, Dulle, E. J. Mauer, & Förster, 2016; Simsolo, Eroglu, Tanriverdi, & Ozer, 2018). Although it is known that organogels are formed through weak intermolecular interactions between structuring molecules, which generates three-dimensional networks (Steed, 2011). There is still a fundamental lack of understanding considering the type of interactions that are needed. (Nikiforidis, Gilbert, & Scholten, 2015). The association between self-assembly and structuring of crystalline particles in vegetable oils constitute hybrid systems with high potential for organogels formation.

Table 4 shows the number of crystalline elements, mean density, mean D crystals (μm), agglomerated crystals (%) and mean D of individual crystals (μm) of organogels after static isothermal crystallization at 25° C for 24 h. The images in Figure 5 are shown at 20x magnification.

Organogels 1 (6% C), 5 (3% C and 3% H), 7 (2% C, 2% S and 2% H), 8 (4% C, 1% S and 1% H), 10 (1% C, 1% S and 4% H) and 11 (2% C, 2% S and 2% H) showed higher number of crystalline elements and lower mean density, which is directly related to higher SCmax at 25ºC (Table 1). The mean diameter of organogel crystals was less than 30 μm from 1.33 (6% C organogel) to 2.25 μm (6% H organogel), indicating that the presence of C induces the formation of smaller crystal. Hence, it should not present sandy mouth perception, resulting from the lipid phase (Beckett, 2008). Generally, crystal size is related to hardness (Table 1) of organogels or other lipid materials, with smaller crystals being more resistant than larger crystals (Hwang et al., 2012).

Hardness

Hardness is the resistance of a material against permanent deformation, and its magnitude measures the structure mechanical strength as a result of interaction forces between the materials'components (Walstra, 2003). The hardness values of organogels are presented in Table 1. The values ranged from 0.13 to 32.00 N, where the minimum value corresponded to 6% S organogel and the maximum value, to 6% C organogel. Thus, C was more relevant for increased hardness.

The value of R^2 (87.98%) indicates that this model (Hardness = 29.58* C) is suitable for assessing organogels' hardness behavior. From these data, it was possible to generate triangular diagrams (Figure 2D).

Organogels with 6% C and 3% C and 3% H showed higher hardness, compatible with high levels of SC at 25ºC. These organogels, together with organogels 4 (3% C 3% S), 7 (2% C 2% S 2% H), 8 (4% C 1% S 1% H), 10 (1% C 1% S 4% H) and 11 (2% C 2% S 2% H) showed higher number of crystalline elements and smaller crystal diameter (Table 4), according to typical characteristics of more cohesive and harder crystalline grids (Campos, 2005). The size of crystals is related to organogels' hardness, and smaller crystals are more resistant than larger crystals (Hwang et al., 2012).

C-containing organogels produced gels with higher hardness for both isolated structurers and for the combinations. This information is consistent with that observed in the literature for this structurer (Doan et al., 2015; Rocha et al., 2013; Toro-Vazquez et al., 2009).

Organogels with 6% C, S and H showed 32.00, 0.13 and 0.56 N hardness, respectively, which are extreme and discrepant values, which makes the use of C, S and H structurers at 6% concentration impossible in the isolated form, justifying the studies for hybrid systems. Consequently, organogels 7 (4.25N) and 11 (4.91N), containing 2% C, 2% S and 2% H respectively, showed intermediate hardness value, ideal for application in food as lipid base. These characteristics may be favorable in relation to lipid applications where hardness and texture are important and may be beneficial in technological applications requiring increased fluidity for products such as *spreads* (Manzocco et al., 2014b). In organogels 2 (6% S), 3 (6% H) and 9 (1% C 4% S 1% H), lower hardness values (0.13, 0.56 and 1.06N, respectively) were observed, in contrast to SC at 25ºC (Table 1), which indicates that despite SC

presence, they are not able to form a three-dimensional network to trap HOSO. This feature is negative in lipid applications where hardness is important for product structuring.

Structurer S in isolated form caused low hardness value for organogels (6% 0.13N), but when combined with structurer H, which also resulted in low hardness value (6% 0.56N) they showed synergistic action, possibly resulting in an increase in hardness, with a value of 4.55N with 3% S and 3% H, but lower than the values obtained for organogels 1, with 6% C isolated (32.00N) and 5, with 3% C and 3% H. This effect may be due to intermolecular interactions between TAGs of H and long chain fatty acids present in structurer S, followed by formation of crystal network. The fatty acid chains may have been adsorbed or physically incorporated into the surface of the crystal network, creating an occluded structure, trapping HOSO, as in the *pickering* effect for emulsions (Dickinson, 2012).

Organogels with high S concentrations provided lower hardness, 0.13 and 1.06 N at 6 and 4% S, respectively, probably due to the hydrophilic groups of emulsifiers, which exert repulsive forces due to chemical composition and number of hydrophilic portions (Sangwal, 2007), forming a less cohesive structure. In general, hardness parameters of organogels can be associated with small fat crystals formation dispersed in high proportion of liquid oil, promoting the formation of less cohesive crystal networks.

Frequency Scan

The frequency scan analyses to investigate the deformation behavior of organogels within the linear viscoelastic region are presented in Figure 6. All organogels showed similar rheological behavior, with an observed value of elastic modulus (G') higher than the viscous modulus (G"), indicating solid material behavior (Steffe, 1996).

Organogels 1 and 5 showed the highest values for G' and G" and organogel 2 showed the lowest value for this parameter, results consistent with SCmax values at 25ºC and hardness (Table 1). The other samples showed intermediate values of G' and G" in the frequency scan analysis.

Temperature scan

The temperature scanning analyses of organogels are presented in Figure 7, showing a similar behavior for the different organogels, through apparent viscosity, with an increase of elastic modulus

(G') and viscous modulus (G") within the same range of temperature, with an observed value of G' higher than G", according to the frequency scan analysis (Figure 6).

The organogels' temperature scanning analysis results showed that the rheological behavior was similar to that observed for the thermal behavior, with initial and final crystallization and fusion (Table 2), indicating that the first observed peak for thermal analysis is related to the organogel network breakdown, results consistent with other work that evaluated the rheological behavior for organogels (Rocha et al., 2013).

The values of G' and G" decreased with increasing temperature, but increased during cooling of organogels, probably by recrystallization and reorganization of the three-dimensional organogel network. The temperature scan shows that organogels can be used as fat substitutes in industrial processes using shear, even if the process temperature is higher than the organogel formation temperature as these materials are thermo-reversible, as reported by other researchers (Alvarez-Mitre et al., 2012; Dassanayake et al., 2011; Pernetti et al., 2007; Smith et al., 2011).

Crystallization temperatures were also higher than those determined for melting (Figure 3), and G'-G" crossover can be observed, which can be used as a simple criterion for gel point (Rocha et al., 2013). Gelation temperature refers to crystallization phenomena and grouping of crystals in clusters (Lupi et al., 2012). When the molten system is cooled, fat crystals in α polymorphism are obtained; and its size and number increase with decreasing temperature. In addition, during the cooling process, potential transitions (polymorphic transformation $\alpha \rightarrow \beta'$) and aggregations occur forming a three-dimensional crystal network (Campos, 2005; Marangoni, 2005).

4. CONCLUSION

The analyzed organogels in this study were stable with the exception of 6% S organogel. The different organogels characterization parameters were associated in terms of characteristics such as SCmax at 25ºC, hardness, β' polymorphism, mean crystal density and behavior of solid material, since they presented G' greater than G". Structurer S presents negative results in isolation, but when associated with structurers C and H, as in organogels 4 (3% C, 3% S), 7 (2% C, 2% S, 2% H), 9 (1% C, 4% S, 1% H), and 11 (2% C, 2% S, 2% H) showed satisfactory results, justifying the importance of studies on

hybrid systems for organogels. The characteristic hardness of organogels has been shown to be suitable for spreadable products, except for organogel 1, which is indicated for application to higher consistency products, such as chocolate. The set of properties evaluated characterize the analyzed organogels as lipid bases with potential for use as fat substitutes in industrial processes, to meet an important demand for reduction of saturated fatty acids and healthier products.

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7. TABLES AND FIGURES

Table 1. Induction time, maximum solids, solid content at 25ºC and hardness

Oleogels	C (%)	S (%)	H (%)	IT (minutes)	SCmax (%)	SC a 25ºC (%)	Hardness (N)
1	6	0	0	4	5.09	4.96	32.00±0.15
2	0	6	0	6	2.66	3.04	0.13±0.01
3	0	0	6	15	5.70	4.87	0.56±0.10
4	3	3	0	6	3.82	3.69	6.22±0.21
5	3	0	3	6	4.77	5.02	11.94±0.81
6	0	3	3	7	1.66	4.33	4.55±0.09
7	2	2	2	7	2.31	4.05	4.25±0.28
8	4	1	1	5	3.38	3.79	3.28±0.18
9	1	4	1	6	2.59	3.13	1.06±0.12
10	1	1	4	18	4.36	4.18	2.80±0.39
11	2	2	2	9	2.27	3.87	4.91±0.24

C - candelilla wax; S - sorbitan monostearate; H - palm oil *hardfat*; IT – Induction time; SC - solid content.

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

Table 2. Thermal behavior of oleogels

Crystallization								
Oleogels	**Peak 1**				**Peak 2**			
	T_{oc} (ºC)	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**T_{oc} (ºC)**	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**
1	45.38	37.09	15.44	5.82	-32.99	-38.04	-49.51	37.96
2	47.95	18.30	3.11	1.98	-10.34	-36.47	-50.48	50.39
3	26.06	22.15	0.27	6.31	-26.06	-33.06	-48.76	49.95
4	37.23	29.74	11.97	2.95	-32.66	-37.79	-49.85	41.92
5	40.07	16.99	4.21	4.36	-30.83	-36.77	-49.82	44.97
6	24.61	16.95	5.89	1.72	-28.86	-32.92	-46.24	47.54
7	36.10	14.45	1.97	3.49	-31.82	-38.42	-49.56	39.86
8	42.87	33.06	-5.74	5.46	-32.64	-38.31	-49.87	40.67
9	34.04	13.12	-8.04	2.92	-32.08	-36.71	-49.85	50.16
10	26.56	19.39	5.65	3.12	-28.90	-37.00	-50.42	44.51
11	36.79	15.14	3.05	3.47	-31.70	-38.30	-50.60	42.81
Melting								
Oleogels	**Peak 1**				**Peak 2**			
	T_{oc} (ºC)	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**T_{oc} (ºC)**	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**
1	-14.88	-5.14	9.32	52.67	36.19	47.07	57.43	1.78
2	-15.99	-3.38	11.34	49.94	26.26	45.26	58.08	5.81
3	-15.48	-3.38	17.59	51.07	25.26	45.29	59.20	6.08
4	-14.88	-5.03	7.34	57.03	24.52	35.84	60.69	1.23
5	-14.71	-4.19	21.87	56.80	28.65	41.05	54.72	3.80
6	-14.40	-3.47	9.51	50.61	16.46	36.75	48.29	2.35
7	-14.90	-4.19	8.14	53.16	26.98	33.82	48.60	1.40
8	-14.68	-4.10	7.34	54.19	25.63	45.25	56.54	2.21
9	-14.92	-4.90	12.61	53.22	17.44	27.59	39.04	1.00

10	-14.88	-4.17	24.29	53.19	27.48	40.00	52.96	3.93
11	-15.51	-4.95	11.45	53.69	26.31	33.71	46.56	1.49

T_{oc} and T_{om}: onset crystallization and melting temperature; **T_{pc} and T_{pm}** peak crystallization and melting temperature; **T_{fc} and T_{fm}:** final crystallization and melting temperature; **ΔH_c, ΔH_m and ΔH_{total}:** crystallization, melting and total enthalpies.

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

Table 3. Lipid phase diffractograms of oleogels

Oleogels	**Short spacings**			**Polymorphic form**
	4.6	**4.2**	**3.8**	
1	4.47	4.11	3.71	β' + β
2	liquid	liquid	liquid	-
3	4.55	4.14	3.73	β' + β
4	4.43	4.14	3.75	β' + β
5	4.53	4.14	3.75	β' + β
6	4.59	n.d.	n.d.	β
7	4.48	4.14	3.72	β' + β
8	4.53	4.11	3.72	β' + β
9	4.47	n.d.	n.d.	β
10	n.d.	n.d.	3.77	β
11	4.51	4.16	3.71	β' + β

n.d.: not detected

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

Table 4. Microstructure of oleogels

Oleogels	Crystalline elements	Mean density (µm)	Mean D crystals (µm)	Agglomerated crystals (%)
1	49238.00	9.90	1.33	14.26
2	13256.00	68.24	1.76	32.30
3	611.00	69.45	2.24	29.79
4	1825.00	37.06	1.45	24.16
5	38229.00	17.07	1.34	13.28
6	210.00	52.10	1.94	25.24
7	47367.00	11.76	1.93	23.64
8	47353.00	10.62	1.34	12.76
9	732.00	53.05	1.79	31.15
10	50562.00	8.85	2.25	36.19
11	51990.00	7.81	2.14	32.19

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

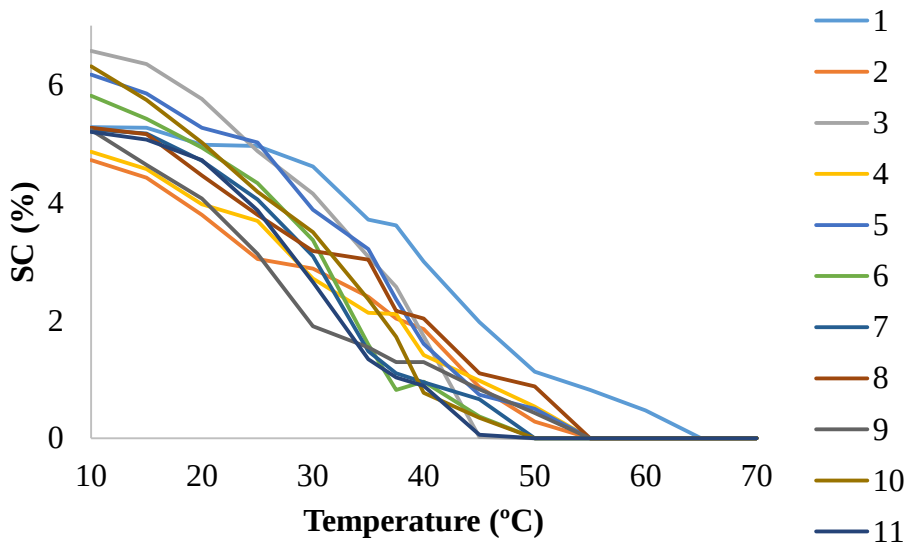
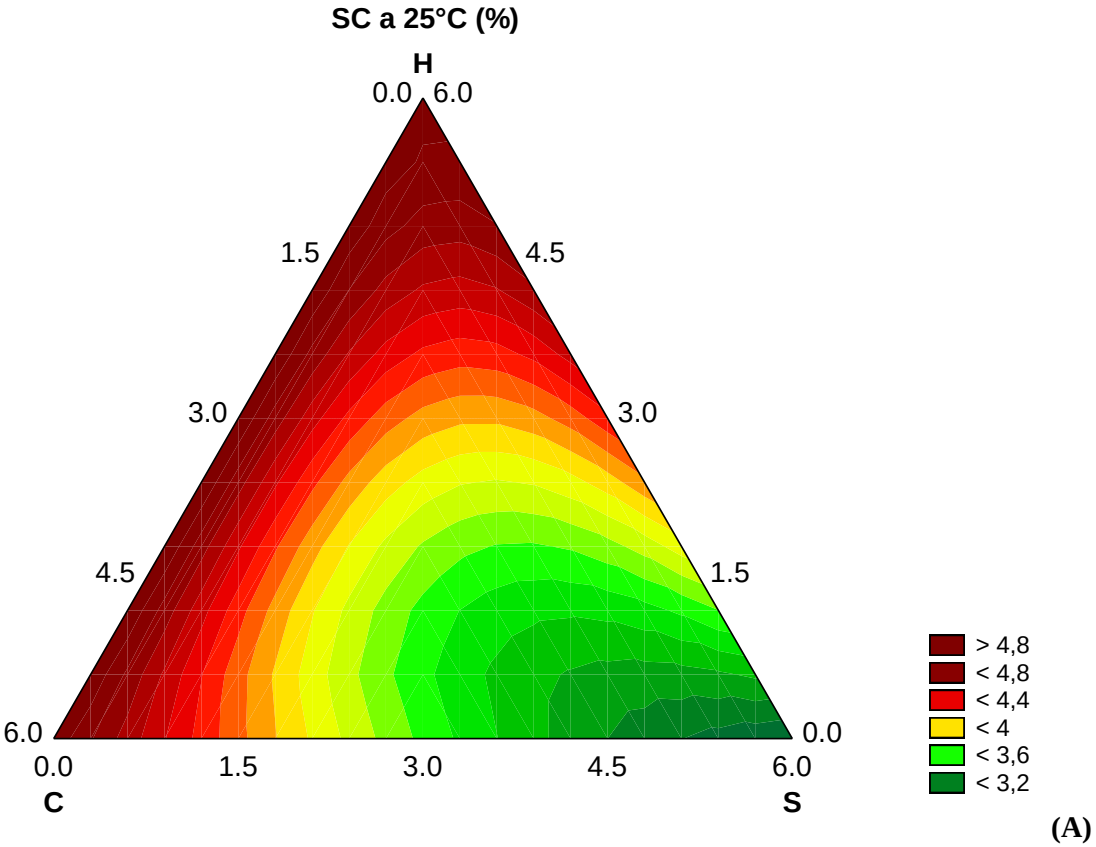
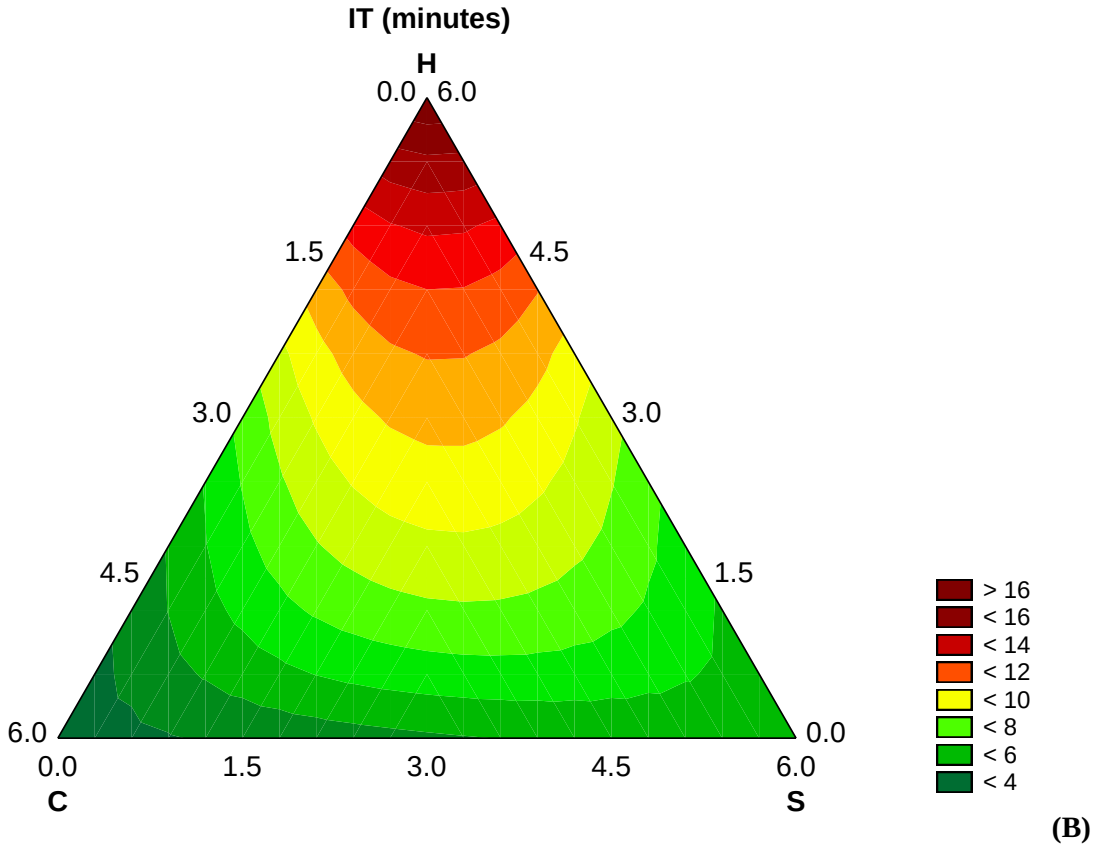


Fig. 1 Fat solids profile of oleogels.

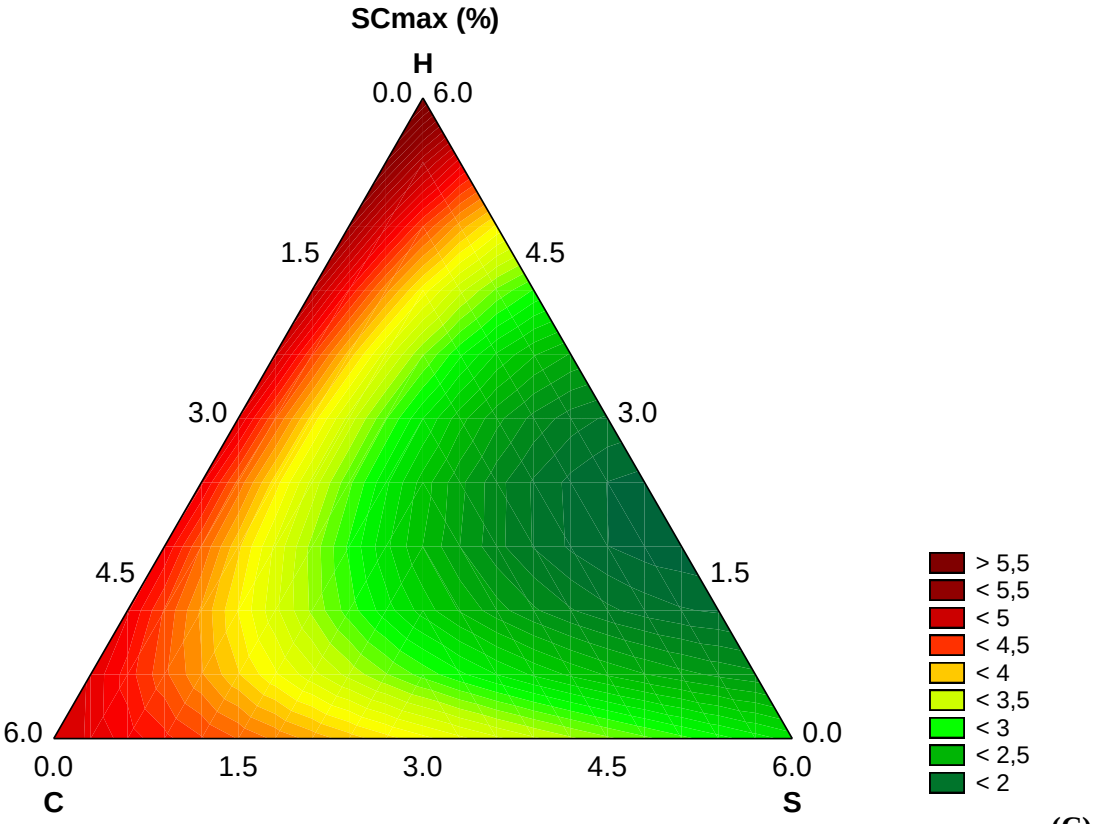
1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.


SC a 25°C (%)
H
0.0 6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0 1.5 3.0 4.5 6.0
C
S
> 4,8
< 4,8
< 4,4
< 4
< 3,6
< 3,2

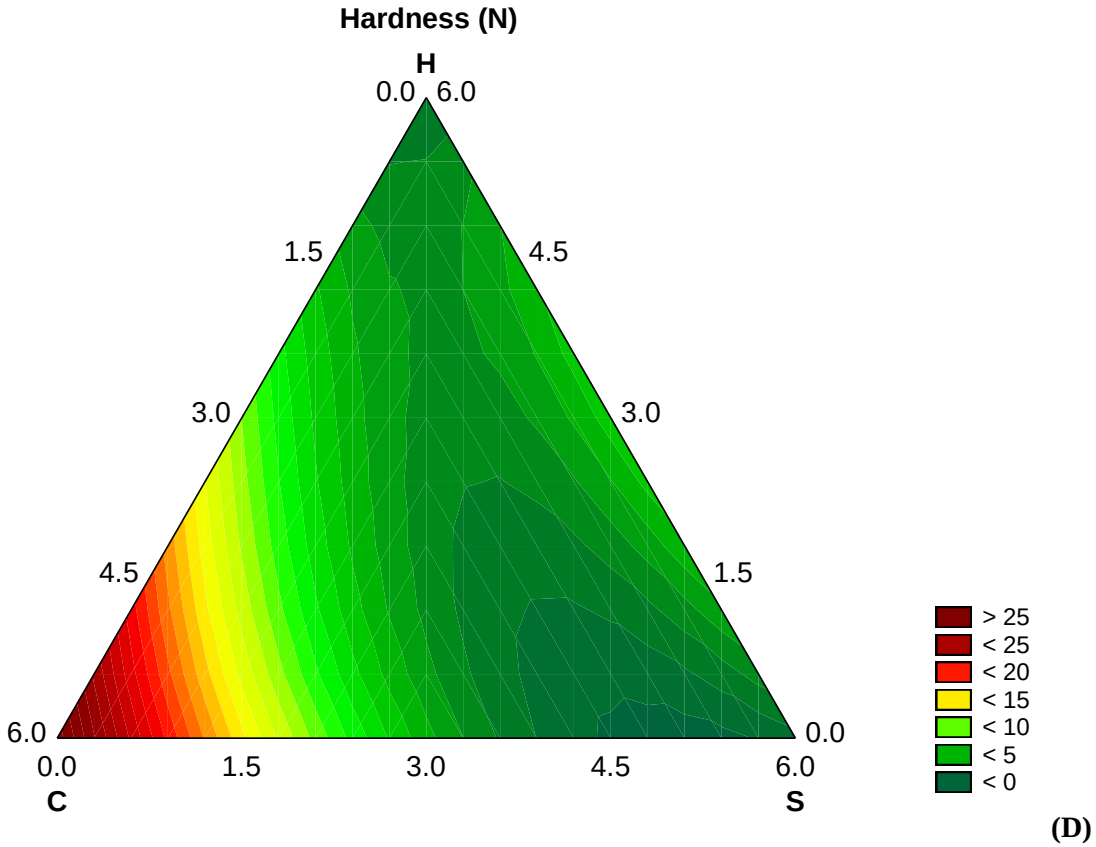
(A)


IT (minutes)
H
0.0 6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0 1.5 3.0 4.5 6.0
C
S
> 16
< 16
< 14
< 12
< 10
< 8
< 6
< 4

(B)

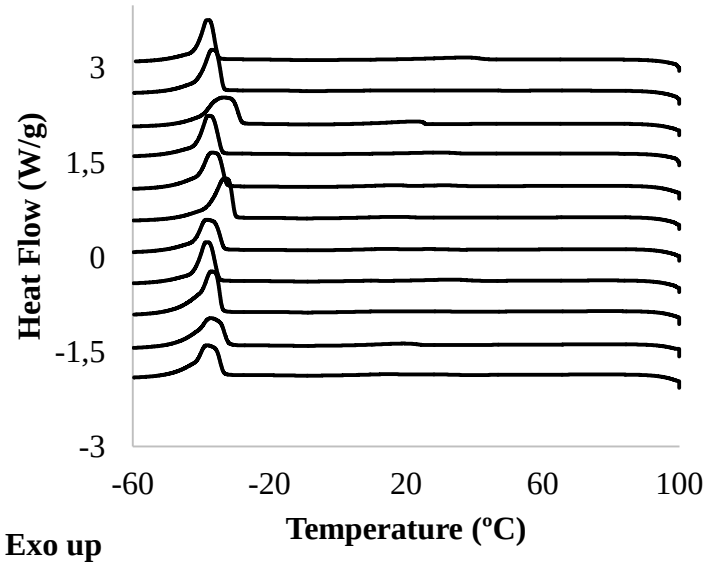

SCmax (%)
H
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
C
S
> 5,5
< 5,5
< 5
< 4,5
< 4
< 3,5
< 3
< 2,5
< 2

(C)


Hardness (N)
H
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
C
S
> 25
< 25
< 20
< 15
< 10
< 5
< 0

(D)

Fig. 2 Triangular diagrams for solid content at 25ºC (A), induction time (IT) (minutes) (B), maximum solid content (SCmax) (%) (C) and hardness (N) (D) as a function of candelilla wax (C), sorbitan monostearate (S), palm oil *hardfat* (H).



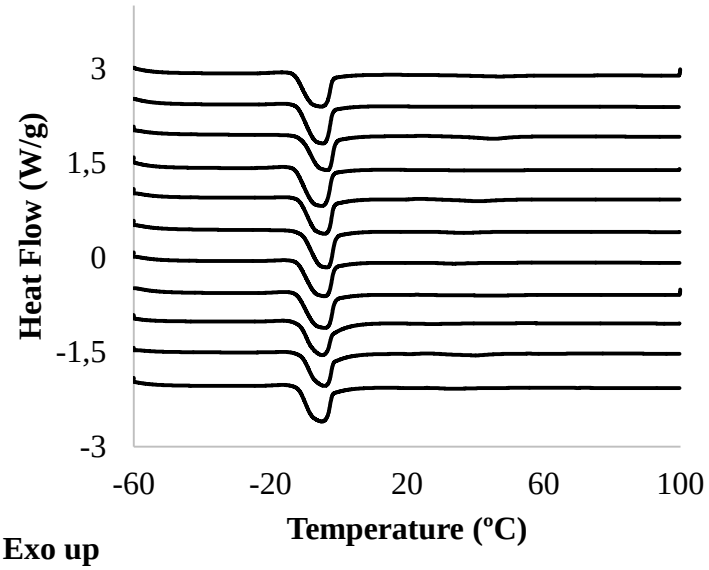


Fig. 3 Melting and crystallization curves of organogels.

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

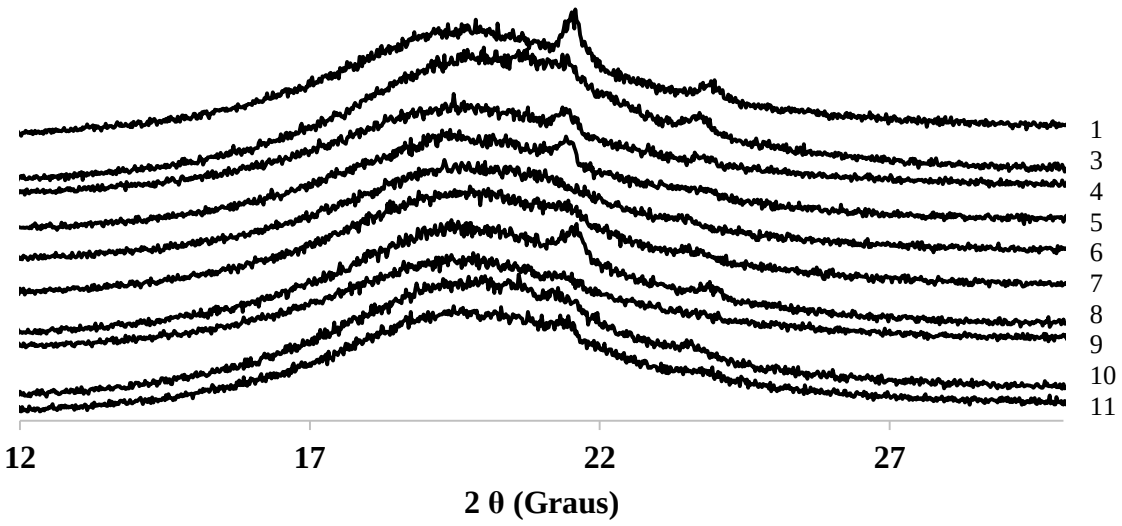
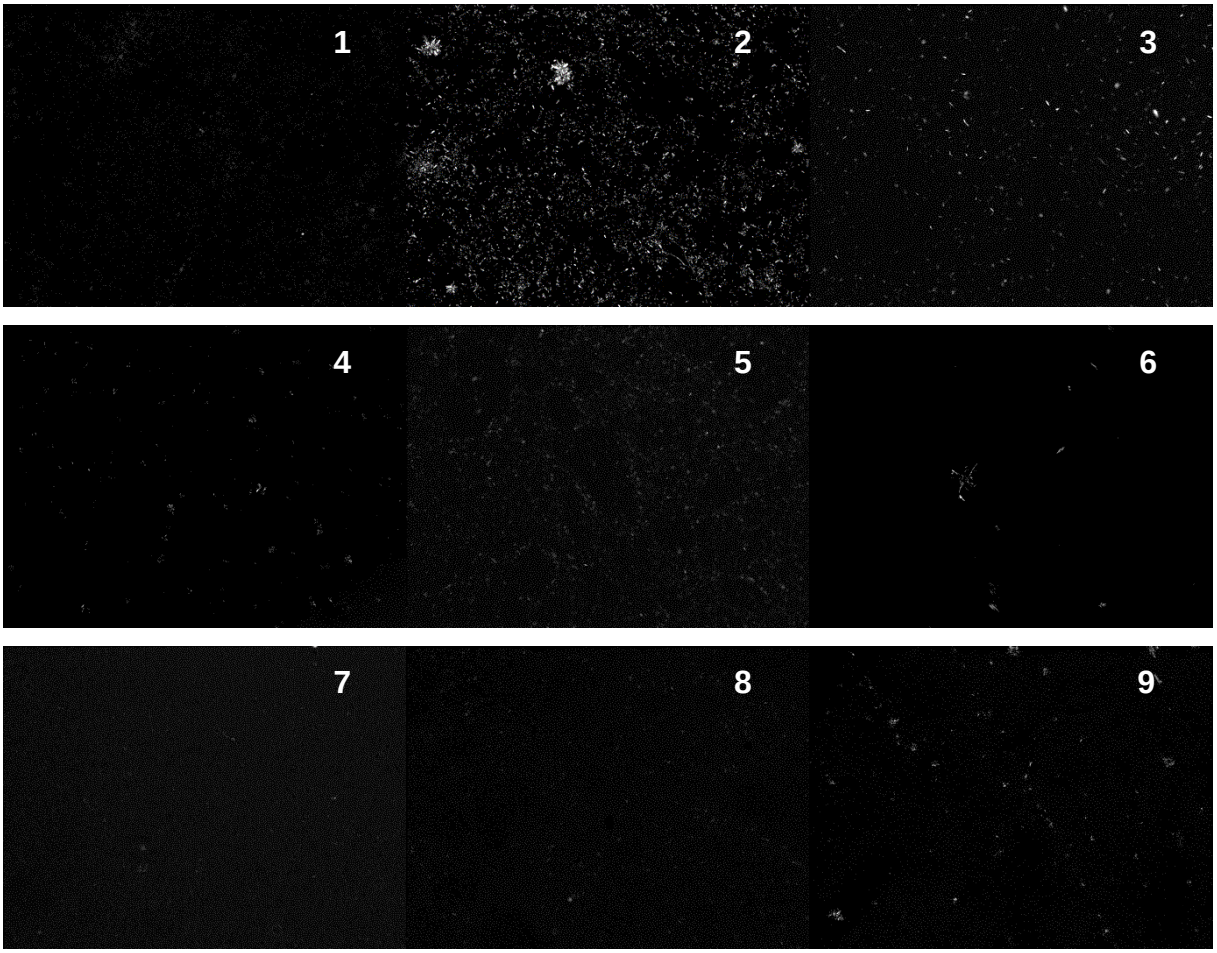


Fig. 4 Lipid phase diffractograms of oleogels.

1: 6% C; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.



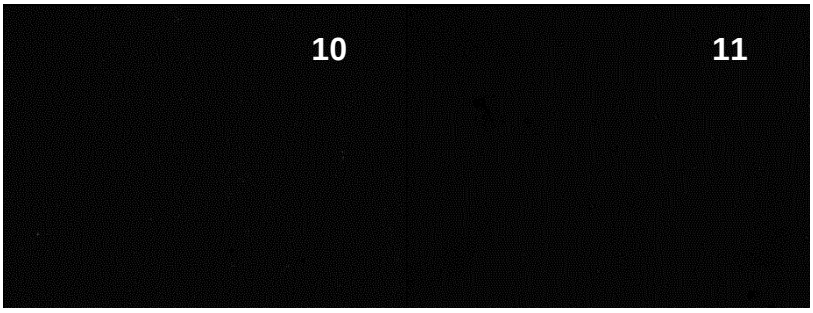


Fig. 5 Microstructure of oleogels at 20x magnification and 25°C.

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

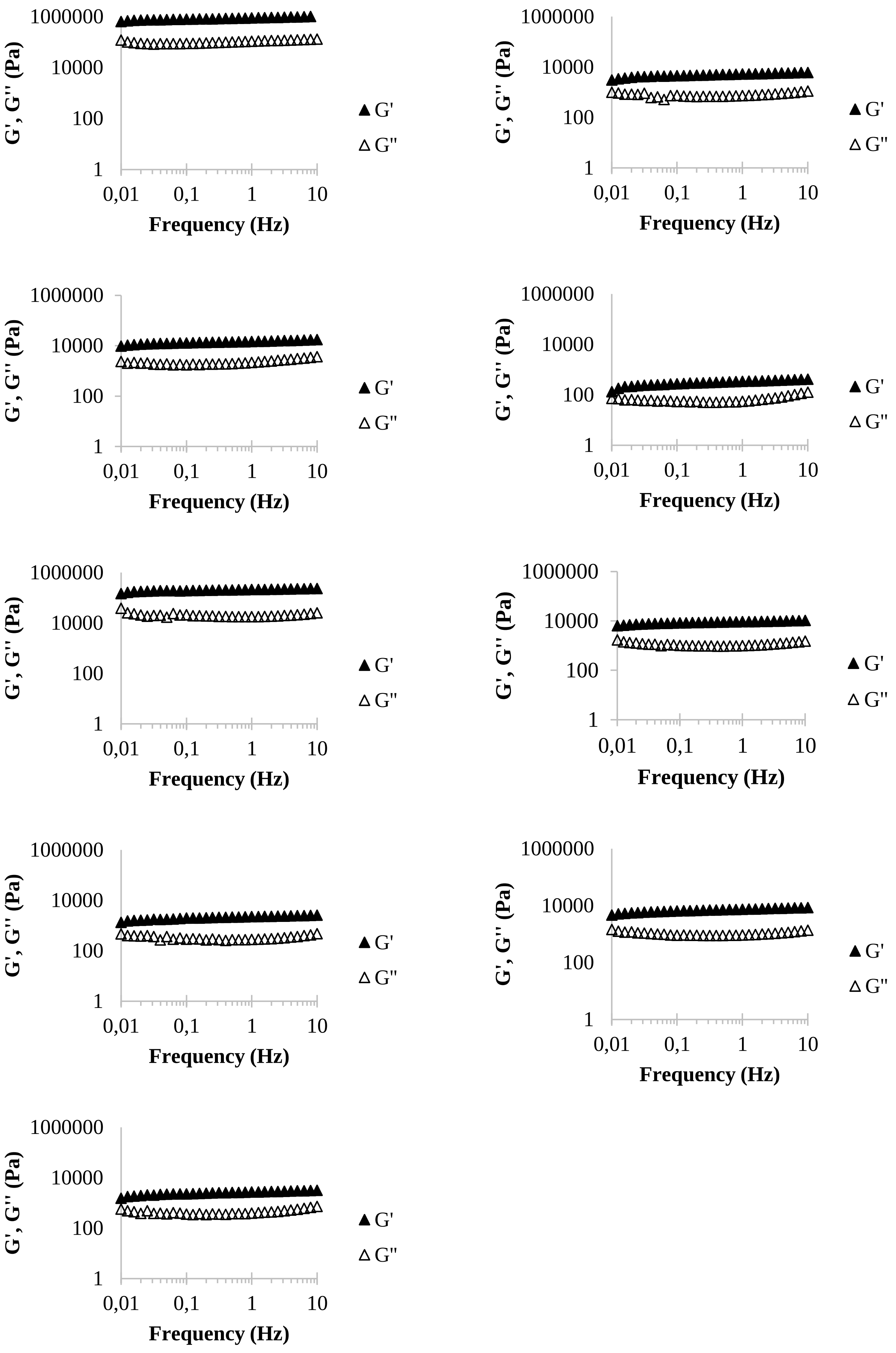
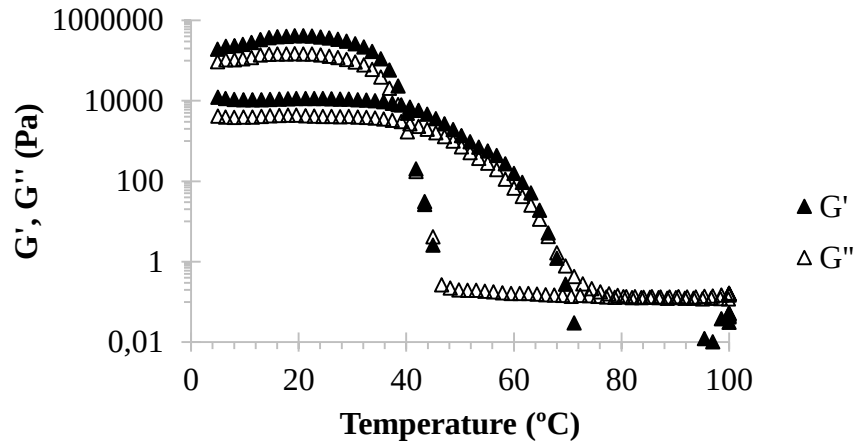
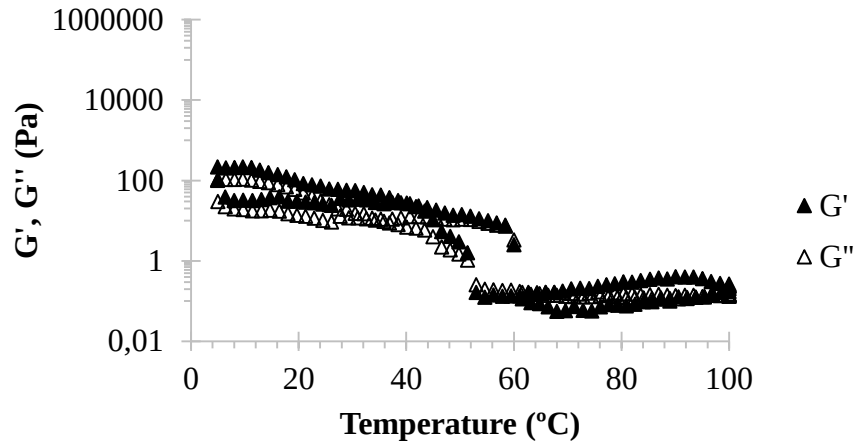
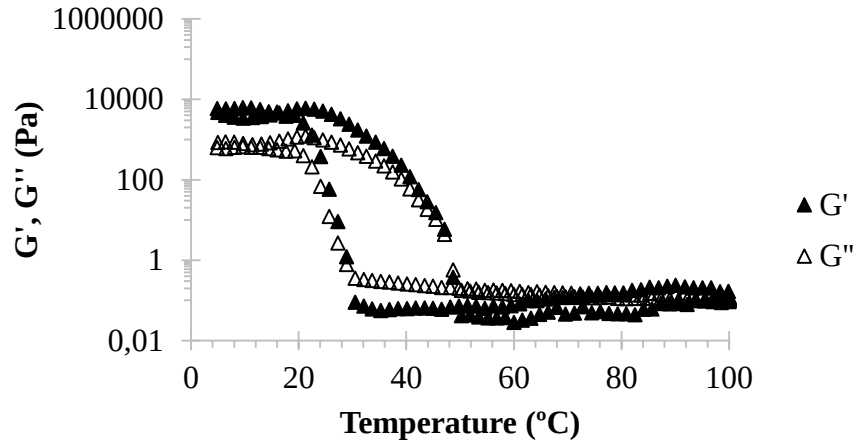

1000000
10000
100
1
0,01
0,1
1
10
G', G'' (Pa)
Frequency (Hz)
G'
G''

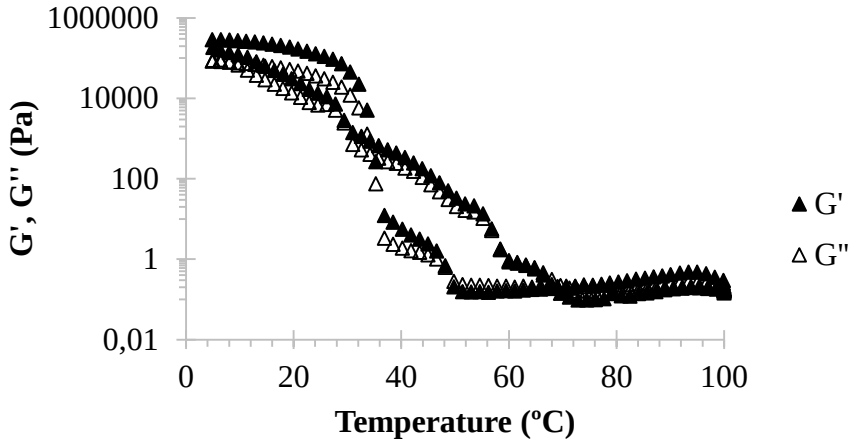
Fig. 6 G’ (a) and G’’ (b) of the frequency scanning of oleogels.

1: 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H; **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S, 2% H.

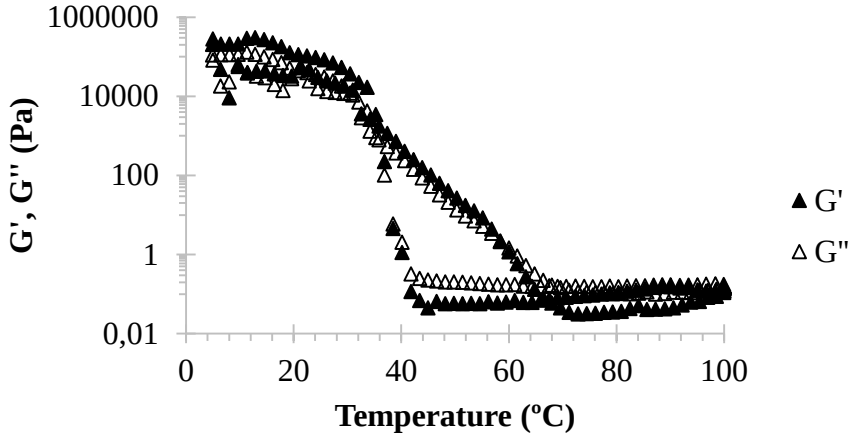




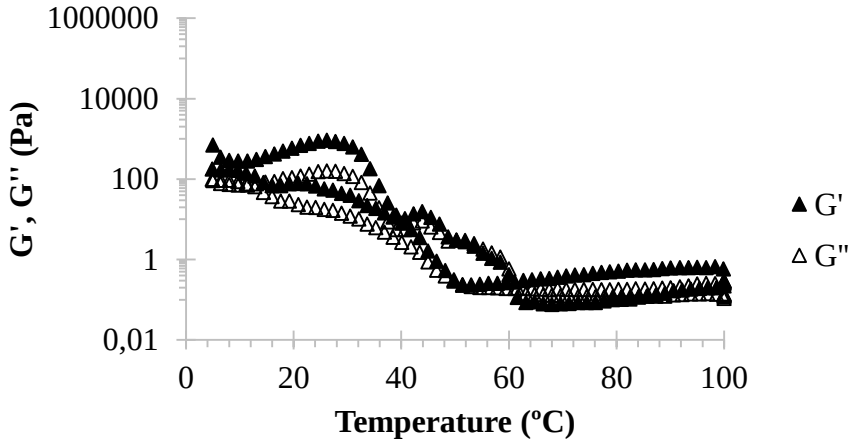



1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
G'
G''

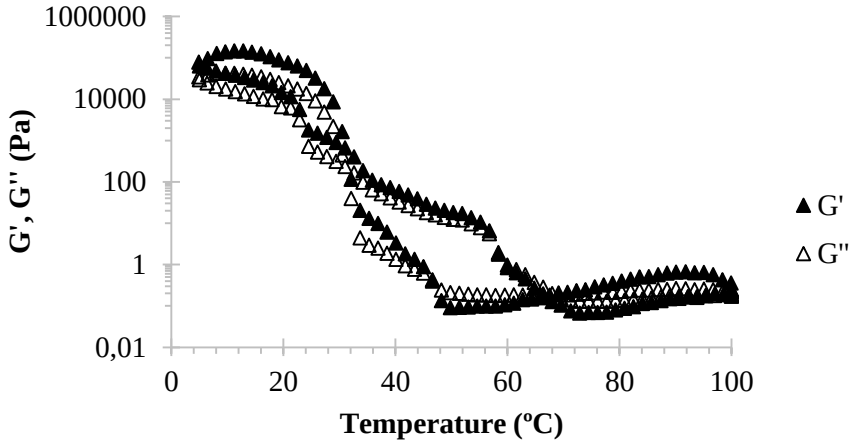
1


1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
G'
G''

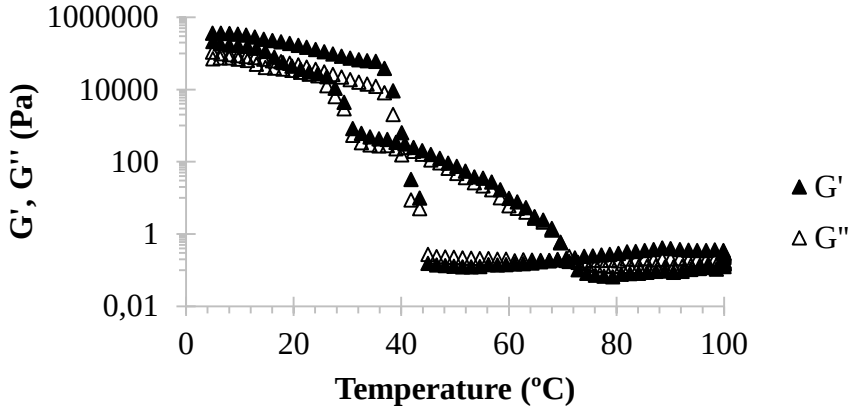
2


1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
G'
G''

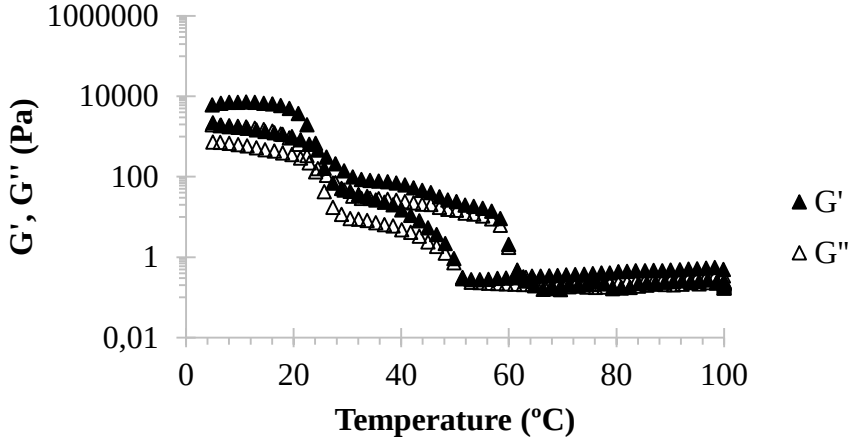
3


1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
▲ G'
△ G''

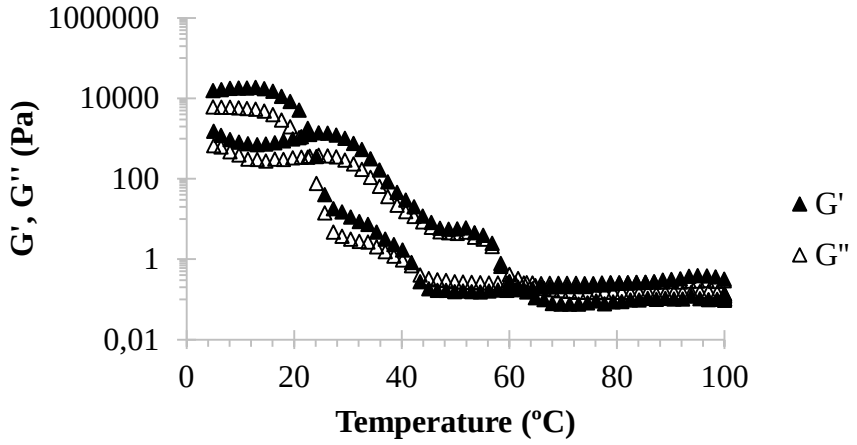
1


1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
▲ G'
△ G''

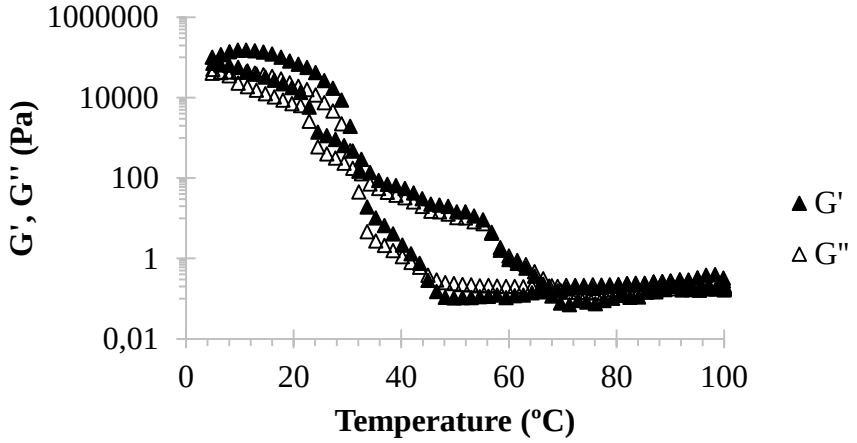
2


1000000
10000
100
1
0,01
G', G'' (Pa)
0
20
40
60
80
100
Temperature (ºC)
▲ G'
△ G''

3



1



2

3 **Fig. 7** G' (a) and G'' (b) of the temperature scanning of oleogels.

4 **1:** 6% C; **2:** 6% S; **3:** 6% H; **4:** 3% C, 3% S; **5:** 3% C, 3% H; **6:** 3% S, 3% H; **7:** 2% C, 2% S, 2% H;
5 **8:** 4% C, 1% S, 1% H; **9:** 1% C, 4% S, 1% H; **10:** 1% C, 1% S, 4% H; **11:** 2% C, 2% S,

CAPÍTULO 8

OBTAINING ZERO TRANS/LOW SAT LIPID BASES WITH NON-CONVENTIONAL STRUCTURING

Artigo Submetido

Food Science and Technology - LWT

Obtaining zero trans/low sat lipid bases with non-conventional structuring

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ABSTRACT

The objective of this study was to evaluate and characterize high oleic sunflower oil organogels structured with sorbitan monostearate (S), hardfat palm oil (H) and monoacylglycerols (M). The organogels were evaluated for their physicochemical properties and crystallization behavior for food application. All organogels melted completely at 55ºC, except the organogel containing 6% M. H was the structuring agent promoting the greatest effect in IT increase; H and M were more effective in increasing the maximum SC, the highest value for the 6% H organogel, probably due to the different compositions in H triacylglycerol classes, results that are consistent with SC profile at 25ºC. The structuring agent S, isolated, resulted in lower hardness (0.13N at 6%). However, when combined with H, which also led to low hardness organogel (0.56N at 6%), disclosed a synergistic action for this parameter. All organogels had similar rheological behavior and properties independent on frequency and temperature, with G' (elastic modulus) above G'' (viscous modulus), typical of solid materials. Temperature scanning analysis showed that organogel properties corresponded to the thermal behavior parameters. The organogels were characterized as potential lipid bases to be used as fat substitutes in industrial processes, offering an effective reduction in saturated fatty acids.

Keywords: high oleic sunflower oil; sorbitan monostearate; palm oil hardfat; monoacylglycerols; organogel.

1. INTRODUCTION

Organogels are viscoelastic materials composed by structuring agents and a non-polar liquid phase, which distinguish them from other gels, made out of hydrosoluble compounds, also known as hydrogels. They are semi-solid systems, where an oil phase is immobilized by a self-sustaining three-dimensional network of the structuring agent (Dassanayake, Kodali, Ueno, & Sato, 2009; Rogers, Smith, Wright, & Marangoni, 2007; Rogers, Wrightb, & Marangoni, 2009).

The organogels production methods are various, as well as their physicochemical properties. According to Co & Marangoni (2012), the possibilities of formation of these compounds occur through crystalline particles, crystalline fibers, and polymeric tapes, among others. These methods vary according to the structuring agent used and the process conditions to which the raw materials are exposed (Chaves, Silva, Domingues, Barrera-Arellano, & Ribeiro, 2019).

Organogels can structure themselves into a 3D fibrous network, where the solvent is trapped in the structuring matrix, preventing solvent flow. The network is stabilized by weak interactions between chains, such as hydrogen bonding, van der Waals forces and π staking (Huang, Chen, Huang, & Xu, 2014; Lupi et al., 2016; Pirner, Dulle, Mauer, & Förster, 2016; Simsolo, Eroglu, Tanriverdi, & Ozer, 2018).

The organic fluid used in structuring, in the development of organogels for food application, is oil or fat (Pernetti, Vanmalssen, Kalnin, & Floter, 2007). High oleic sunflower oil, considered as a premium raw material, is often used in food applications requiring the use of liquid oil with exceptional oxidative stability. It has neutral taste and aroma, and, therefore, high application potential in foods, cosmetics and pharmaceutical products (Gunstone, 2005). The typical composition of the high oleic sunflower oil is 3-5% palmitic acid, 2-6% stearic acid, 75-88% oleic acid and less than 1% of linolenic acid, which guarantees oxidative stability 10 times higher than regular soybean, canola and sunflower oils. Additionally, the regiospecific distribution of the high oleic sunflower oil is distinctive, with high linolenic acid content in sn-2, which also explains its high oxidation stability (Grompone, 2005). Such attributes make the high oleic sunflower oil a high quality liquid lipid source for producing organogels for food applications.

Sorbitan esters are emulsifiers regularly used in foods. Recent studies disclosed this group of compounds as potential structuring agents for vegetable oils, modulating the fat crystallization process. Sorbitan monostearate (S), particularly, is tasteless, odorless and has the ability to form semi-solid and thermoreversible organogels. The organization of S in the oil phase occurs through the self-assembly mechanism, creating a tubular molecular structure that contributes to the formation of the three-dimensional network, immobilizing the organic fluid

represented by the vegetable oil (Co & Marangoni, 2012; Rogers, 2009; Smith, Bhaggan, Talbot, & Van Malssen, 2011). Sorbitan monostearate is a hydrophobic, non-ionic surfactant, often used combined with polysorbates in cakes, creamy fillings and toppings, improving volume and softness, as well as showing a high potential for modifying the crystallization properties in lipid systems (Alejandro G. Marangoni & Narine, 2002). Recent studies on the structuring effect of S suggest its use as a potential structuring element for vegetable oils, by means of a positive interaction with triacylglycerols (Cerqueira et al., 2017; Oliveira et al., 2015; Sonwai, Podchong, & Rousseau, 2017).

Fully hydrogenated vegetable oils, technically designated *hardfats*, have been shown as potentially effective in modifying the crystalline properties of lipid bases, acting as structuring elements to accelerate the crystallization process in low and medium melting point triacylglycerol systems. Fatty acids are characterized by a homogeneous composition of fatty acids and high melting point triacylglycerols, arising from total catalytic hydrogenation of natural oils, and are considered low cost materials from the industry viewpoint (Oliveira, Ribeiro, & Kieckbusch, 2015; Ribeiro, Basso, & Kieckbusch, 2013). Palm *hardfat* (H), especially, is characterized by polymorphic habit β', crystalline property that indicates this oil to different applications in lipid based food (Ribeiro et al., 2013). In addition to the crystallization primary agent function, *hardfats* modify the physical properties of continuous lipid systems, allowing several adaptations related to the development of organogels, a property that justified many studies on TAGs (triacylglycerol) conventional structuring (Smith et al., 2011; Wassell et al., 2010).

Saturated monoacylglycerols (M) present high potential for wide application in foods and can be used in the gelation of unsaturated vegetable oils in order to reach characteristics of high SFA content plastic fats. Thus, it is possible to develop low SFA and *trans* content products (Da Pieve et al., 2010; Ojijo et al., 2004). The structuring of vegetable oils through M occurs through self-assembly, micelle or inverse lamellar phases formation when cooling the formed system, i.e., M molecules can be structured as oil-in-water emulsions, trapping the oil phase (Lopez-Martínez et al., 2015; Valoppi et al., 2016; Wang et al., 2016).

The combination of *self-assembly* and crystalline particle structuring in vegetable oils, constitute hybrid systems with high potential for the production of stable organogels. Researches show that the combined utilization of specific structuring agents can result in a satisfactory synergistic effect, which may overcome the disadvantages of organogels consisting of one single type of structuring agent (Chaves et al., 2018).

The purpose of this study was to evaluate and characterize HOSO organogels, S, H and M structured, utilizing mixture systems represented by ternary diagram, regarding the physicochemical properties and the crystallization behavior of lipid systems with low SFA content, high oleic acid proportion and technological feature for food application.

2. MATERIAL AND METHODS

2.1. Material

The utilized organic phase was HOSO supplied by Cargill Agrícola S.A., Brazil; the structuring agents used were S, supplied by Sigma Aldrich, USA, H, from SGS Agricultura e Indústria Ltda, Brazil and M Grindsted Crystallizer 100, supplied by DuPont do Brasil S.A., Brazil.

2.2. Methods

2.2.1. Organogels formulation

Organogels were prepared by mixing HOSO with the structuring agents, heated at a temperature above the structuring agents melting point (80ºC), stirred for 3 minutes for complete homogenization and cooled at storage temperature, 5ºC, in accordance with (Rocha et al., 2013; Stahl et al., 2018a).

Structuring agents interactions were studied by varying the concentrations of S, H and M. This evaluation utilized mixtures systems, represented by ternary diagrams. For a total 6% structuring agents concentration, HOSO organogels were obtained, varying the contents of each structuring agent, through simplex centroid experimental planning of seven experiments and 3 additional experiments to validate the model, according to Table 1. The obtained organogels were characterized by solids content, crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness, oil exudate content and rheological properties.

The statistical analysis of the results relied on differences determined by analysis of variance (ANOVA), followed by Tukey test at $p < 0.05$. For the measurable experimental results, regression models were applied as a function of the proportion of each structuring agent (x1, x2, x3): $\hat{y}i = \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 + \beta 123x1x2x3$, where $\hat{y}i$ = estimate response, βi = coefficients estimated by the least square method and xi = dependent variables. Models quality was evaluated by ANOVA and the determination coefficient was adjusted (BARROS NETO et al., 2001; Cornell, 2002). The statistical analysis were performed by the Statistica 9.0 *software*.

2.2.2. Organogels evaluation

Solids content (SC). Determined by Bruker pc120 Minispec Nuclear Magnetic Resonance Spectrometer (NMR), with the aid of high precision dry baths (0 - 70°C) Tcon 2000 (Duratech, USA). Method AOCS Cd 16b- 93: direct method, serial samples reading, tempering for unstabilized fats (AOCS, 2009).

Crystallization kinetics. Obtained through initial samples tempering (100ºC for 15 min, 1 h at 70ºC) and solids content was monitored at 25ºC (± 0.5ºC) in Mq20 NMR Analyzer Bruker Nuclear Magnetic Resonance Spectrometer (NMR) (Wassell and Young, 2007). Data were automatically acquired, measured each minute, during 1h. The crystallization kinetics characterization was carried out in accordance with the induction time (IT) - related to the beginning of crystal formation, maximum solids content (SCmax) (Campos, 2005; Stahl et al., 2018b).

Thermal behavior. Samples thermal analysis was conducted in a differential scanning calorimeter (DSC) TA Q2000, coupled to RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The utilized data processing system was the Universal V4.7A (TA Instruments, Waters LLC, New Castle). The analysis conditions were: sample mass: ~ 10mg; AOCS Cj 1-94 modified method (AOCS, 2009): temperature between -60 and 100°C, with 5°C/min slope (crystallization and melting). The following parameters were used to evaluate the results: crystallization and melting (T_{oc} and T_{of}) *onset* temperatures, crystallization and melting peak temperatures (T_{pc} and T_{pf}), crystallization and melting enthalpy (ΔH_c and ΔH_f) and crystallization and melting end temperatures (T_{fc} and T_{ff}) (Barbosa et al., 2018; Campos, 2005).

Polymorphism. Determined by x-ray diffraction, according to Sawalha et al. (Sawalha et al., 2012) procedures. Analyses were performed on a Philips diffractometer (PW 1710) using Bragg-Bretano geometry (θ:2θ) with Cu-ka radiation (l = 1.54056Å, 40 KV voltage and 30 mA current). Measurements were obtained with 0.02° steps in 2θ and 2 seconds acquisition time, 15 to 30° *scans* (2θ scale) at 25ºC. The identification of the crystalline forms was made from the characteristic crystals' *short spacings* (SS) (AOCS, 2009).

Microstructure. Organogels microstructure was evaluated by microscopy under polarized light. With the aid of a capillary tube, a sample droplet was placed on a glass slide, covered with a coverslip and kept at 5ºC for 24 hours and then at 25ºC for 24 hours. Organogels were analyzed at 25 ° C using 20x magnification. The crystals microstructure was evaluated using a polarized light microscope (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). Images were captured by the Image

Pro-Plus 7.0 application (Media Cybernetics, United States) in four different visual fields on each slide for each sample and the mean particle diameter result was expressed as the mean and standard deviation of these values (Cindio and Cacace, 1995; Toro-Vazquez et al., 2013).

Hardness. Determined using a microcomputer-controlled texturometer (TA-XTi2, Stable Microsystems, England). For the analysis, 30 mL of organogels were placed in 50 mL beakers and placed in a B.O.D. (Bacteriological Oxygen Demand) oven at 5ºC for 24 h. A compression / extrusion test was conducted using a 25 mm diameter and 35 mm long cylindrical acrylic probe with 1.0 mm/s speed and 15 mm fixed probe penetration distance. The value considered was the maximum obtained force (Rocha et al., 2013).

Oil exudation content. Determined in organogels in order to test the stability of samples analyzed 24 hours after obtaining the organogels at 25°C. All samples were monitored with respect to stability, observing phases separation or exudation of liquid oil (Rocha et al., 2013).

Rheological properties: Rheological analysis were carried out according to the methodology proposed by (Rocha et al., 2013), utilizing a controlled stress rheometer (Physica MCR 301, Anton Paar, Germany). The geometry of parallel stainless-steel plates with rough surface (50mm diameter and 200μm gap) was used. The temperature was controlled using a Peltier system. Temperature scans at 5° C/min rate were taken, from 5°C to 100°C, then cooled from 100 to 5°C and reheated from 5 to 100°C. 1 Hz frequency (f) and 1% deformation were used, within the linear viscoelasticity range. The organogels were analyzed by stress and frequency scanning, in order to evaluate their mechanical strength and behavior against different observation times, respectively. Stress scans were performed from 0.1 to 10 Pa (f = 1Hz) at 25ºC. Frequency scans were obtained between 0.01 and 10 Hz, within the linear viscoelasticity range, and at the same stress scans. The following parameters were determined: elastic modulus (G'), viscous modulus (G”), complex modulus (G*), phase angle (δ) and complex viscosity (η*).

3. RESULTS AND DISCUSSION

Solids content

Figure 1 shows the solids profile determined for organogels between 10 and 70ºC. Organogel containing 6% M (6.84%) had better thermal resistance, followed by organogel 6 (3% H, 3% M) (6.75%), 2 (6% H) (6.57%), 11 (2% S, 2% H, 2% M) (6.40%), 9 (1% S, 4% H, 1% M) (6.33%), 10 (1% S, 1% H, 4% M) (6.22%), 5 (3% S, 3% M) (6.01%) and 7 (2% S, 2% H, 2% M) (6.00%). All of them have 6% structuring agents in their composition. The others (1 (6% S), 4 (3% S, 3% H) and 8 (4% S, 1% H, 1% M)) have 4.72, 5.81 and 5.32% SC,

respectively, at 10ºC and present, in their composition, 6, 3 and 4% S, respectively, demonstrating that high S concentrations, along with H and M, leads to a lower SC. All melted completely at 55ºC, the exception was organogel 3, containing 6% M.

SC at different temperatures describes the melting profiles of organogels, and, as a consequence, guide possible industrial applications. SC as a function of temperature gives an indication of basic physic-chemical attributes and technological performance behavior of lipid bases (Braipson-Danthine and Deroanne, 2006). At low temperatures, (4 to 10°C), SC values are typical of spreadability. Since organogels SC oscillated from 4.72 (organogel containing 6% S) to 6.75% (organogel with 6% H), all samples are appropriate for spreadable products. At room temperature (20 to 22°C), a minimum 10% solid fat is required in order to assure the oil exudation resistance and the stability, for conventional lipid bases. However, organogels at 20ºC displayed SC ranging from 3.79 (6% S organogel) to 5.88% (organogel containing 6% M) being stable and with no HOSO exudation, the only exception being organogel 1, that did not build a structured tridimensional network with isolated S only. SC values at temperatures below 25°C qualify hardness parameters, while SC between 25 and 30° C refer to thermal resistance, as at this temperature interval, most lipid bases starts to melt (Braipson-Danthine and Deroanne, 2006). At temperatures between 30 and 35°C, the general purpose fats, such as palm oil, distinguish from each other by melting, with simultaneous taste release; and SC offers an estimate of degustation sensory attributes (Wassell and Young, 2007). Body temperature (37.5ºC) is critical for the sensory quality of lipid-based products for the food industry. In this range, SC must be lower than 5%, in order to minimize the eventual waxy sense (De Oliveira et al., 2014; Masuchi et al., 2014), organogels exhibited SC varying from 0.82 (organogel with 3% H and 3% M) to 3.61% (6% S organogel).

Experimental values obtained for organogels related to SC at 25ºC are in Table 1. These values were from 3.04 and 5.60%, the minimum value corresponding to 6% S organogel and the maximum value, to organogel containing 6% M. Therefore, H and M were more relevant to the increase in SC at 25ºC.

Structuring agents C, S and M disclosed statistically significant influence ($p<0.05$) on SC at 25ºC. R^2 value (98.59%) indicates that this model (SC at 25ºC = 2.54*S + 5.78*H + 5.47*M – 10.17*S*H + 40.81*S*H*M) is adequate for evaluating the behavior of organogels SC. From these data, we were able to build the triangular diagrams (Figure 2A).

Crystallization kinetics

Table 1 displays induction time (IT) and SCmax of organogels. IT oscillated between 3 and 15 minutes, the minimum values corresponding to organogels 3 (6% M), 5 (3% S, 3% M) and 10 (1% S, 1% H, 4% M) and the maximum to the 6% H organogel. Therefore, H was the most relevant structuring agent for IT increase and SCmax ranged from 1.66 to 5.70%, the minimum value corresponding to organogel (3% S, 3% H) and the maximum to organogel containing 6% H. Organogels with 6% H and (1% S, 4% H, 1% M) presented slower crystallization and 15 and 12 minutes IT, respectively, followed by organogels 4 (3% S, 3% H) (7 minutes), 1 (6% S) and 6 (3% H, 3% M) (6 minutes), 8 (4% S, 1% H, 1% M) (5 minutes), 7 (2% S, 2% H, 2% M) and 11 (2% S, 2% H, 2% M) (4 minutes) and 3 (6% M), 5 (3% S, 3% M) and 10 (1% S, 1% H, 4% M) (3 minutes), results in accordance with organogels SC (Table 1).

Maximum SCmax value of 6% H organogel was the highest, probably due to the different compositions of triacylglycerol classes in H, results consistent with the SC profile.

Organogels 2 (6% H), 3 (6% M), 6 (3% H, 3% M), 9 (1% S, 4% H, 1% M) and 10 (1% S, 1% H, 4% M) disclosed high SCmax values: 5.70, 5.44, 5.38, 4.94 and 5.34%, respectively, possibly due to similar compositions of H and M in the organogel. This is because during lipid phase crystallization, materials of similar chemical composition and molecular structure are more likely to co-crystallization (Marangoni, 2005). IT of theses organogels were 15, 3, 6, 12 and 3 minutes, indicating a slower crystallization in H containing organogels and faster crystallization in M compounded organogels. In the case of H and M mixture, the SCmax value is intermediate. This M effect is similar to what was observed by Basso et al. (2010), who stated that behenic acid monoacylglycerols are probably more efficient as crystallization promoters, due to the occurrence of a longer fatty acid chain than those found in high proportions in fully hydrogenated palm oil monoacylglycerols, promoting greater crystallization stability.

Organogels 1 (6% S), 4 (3% S, 3% H) and 8 (4% S, 1% H, 1% M) had SCmax values below 2.60, a possible indication that SCmax is associated with S molecule dissimilarity in relation to other structuring agents, as the occurrence of sorbitol esterified to the molecule hampers the molecular packing (Marangoni, 2005).

H has statistically significant influence ($p<0.05$) on IT and both H and M influenced SCmax. R^2 value of IT (92.82%) and SCmax (93.82%) indicate that these models (IT = 5.96*S + 15.78H) (SCmax = 2.93*S + 4.85*H + 5.68*M) are appropriate for evaluating organogels IT behavior. These data allowed the creation of the triangular diagrams (Figures 2B and 2C).

Thermal behavior

The thermal behavior of organogels is shown in Table 2 and Figure 3. The melting and crystallization curves are subdivided into different regions, reflecting the different triacylglycerol classes in the organogels.

Regarding their thermal behavior in crystallization, organogels 2 (6% H), 4 (3% S, 3% H), 5 (3% S, 3% M), 7 (2% S, 2% H, 2% M), 8 (4% S, 1% H, 1% M), 9 (1% S, 4% H, 1% M) and 11 (2% S, 2% H, 2% M) displayed lower *onset* crystallization temperature (T_{oc}) (26.06, 24.61, 24.39, 26.14, 21.69, 27.79 and 25.53ºC, respectively, probably due to the high concentration of triacylglycerol with higher H melting point, the structuring agent occurring in higher concentration in these organogels. The *hardfats* play interesting role in triacylglycerols structuring, due to its insolubility, or limited solubility, in polyunsaturated vegetable oils and the ability to build a solid crystal network. Different *hardfat* types show similar melting characteristics, with high melting point, high melting enthalpy compared to conventional fats and the ability to build a matrix that will crystallize under high temperatures, forming a fine dispersion of stable crystals (Alander and Lidefelt, 2007; Norberg, 2006; Talbot, 1989).

The presence of *hardfats* as additives modify the crystalline habit and change the crystallization behavior, reducing the crystallization induction period, acting as crystallization germs (OLIVEIRA, 2011). This behavior is linked to the physical properties and the fat acids composition of the oil from which the *hardfat* was obtained (Alander and Lidefelt, 2007).

Specific *hardfats*, from a given oil source, present unique and differentiated triacylglycerol profile, distinguishing these materials as inductors of particular polymorphic habits. After cooling a *hardfat* containing lipid mixture, its high melting point (65-75°C) tri-saturated triacylglycerols promote the development of crystallization nuclei for the ordering of highly structured crystalline network from the liquid system (Pernetti et al., 2007).

Other organogels had T_{oc} ranging from 47.95 to 56.38ºC for organogels with 6% S and 6% M, respectively. Crystallization curves displayed two wide (representative of lower and higher melting point TAG fractions, respectively) and overlapped peaks, associated with the presence of these different triacylglycerol classes (Manzocco et al., 2014; Saberi et al., 2011).

The T_{oc} experimental values obtained for the organogels are in Table 2. They oscillated between 24.61 and 56.48ºC, the minimum value corresponding to the organogel with 3% S and 3% H and the maximum to the 6% M organogel. Therefore, H and M were more relevant to the increase in T_{oc}.

The structuring agents H and M disclosed statistically significant influence ($p<0.05$) on T_{oc}. R^2 value (82.02%) indicates that this model (T_{oc} = -31.65*S + 35.59*H + 63.95*M) is

adequate for the evaluation of organogels T_{oc} behavior. Triangular diagrams were built from these data (Figure 2E).

All organogels co-crystallized with each other and with HOSO, except S. The thermal behavior parameters indicated that the organogels have a first melting peak relative to the most unsaturated triacylglycerols, probably of HOSO, and the second melting peak is relative to the structuring agent used and to the saturated fraction of HOSO.

Polymorphism

Figure 4 shows the organogels X-ray diffractogram. The crystalline forms are characterized by *short spacing* (SS) or interplanar spacing between the fat acid chains, which can be determined by X-ray diffraction. The characteristic SS correspond to 4.15 Å for α form, 3.8 and 4.2 Å for β' and 4.6 Å for β, and are used for determining the relative proportion and type of polymorphs in the organogels (AOCS, 2009; Stahl et al., 2017).

The 6% S organogel was liquid at room temperature, which made the analysis impossible. Other organogels displayed SS values equal to 4.6 Å, characterizing the β polymorphic habit.

The more heterogeneous the composition of the structuring agent, the greater the tendency to stabilize the polymorphic habit β', due to the difficulty of crystalline packing into the most stable crystalline form (Campos, 2005; Marangoni, 2005). Organogels 2, 6 and 9 showed SS equal to 3.8 and 4.2 Å (Table 3), characterizing the polymorphic habit β', which favors the spreadability and creaminess of products to which lipid bases can be applied (Wassell and Young, 2007). In accordance with different literature studies characterizing the palm oil as β', Stahl et al. (2017) and Basso et al. (2010) reported the occurrence of β' form stable crystals in oil palm, due to the diversified fat acids composition and, particularly, to the greater amount of palmitic acid. Ribeiro et al., (2013) identified that *hardfats* from palm oils (HP) are distinguished by the β polymorphic habit and such crystallinity properties shall address them to distinct applications in lipid-based foods.

Although structuring agent M has a high concentration of behenic acid (C22:0) (Silva et al., 2018), which would favor the polymorphic habit β, its molecular structure is heterogeneous due to the typical regiospecific distribution of monoacylglycerols. Tripalmitin and behenic acid monoacylglycerols act as accelerators of the palm oil crystallization process, very important in the chocolate industry where the β-type crystal is desired (Basso et al., 2010). However, in *spreads* and margarines lipid phase, the crystals should preferably be stabilized in the β' form in order to favor product spreadability (Wassell & Young, 2007).

Microstructure

Lipid composition and crystallization conditions influence crystal shape, and different polymorphic forms and crystalline morphologies are possible. The crystals are aggregated into larger structures forming a network, which characterizes the microstructural level of fat. The microstructure concept comprises information on the condition, quantity, shape, size, spatial relationship and interaction between all components of the crystal network, and has a huge influence on macroscopic properties of fats (Oliveira et al., 2015; Ribeiro et al., 2009; Shi, Liang, & Hartel, 2005).

Triacylglycerols generally crystallize as spherulites, which correspond to the aggregation of crystalline lamellae growing radially from the same central nuclei and may develop ramifications during maturation (Rousset, 2002). Eventually, depending on the cooling conditions or even on the characteristic melting profile of each fat, triacylglycerols may also crystallize into other characteristic morphologies such as needles and discs (Oliveira et al., 2015).

Organogels can structure themselves into a 3D fibrous network, where the solvent is trapped in the structuring matrix, preventing solvent flow. The network is stabilized by weak interactions between the chains, such as hydrogen bonding, van der Waals forces and π staking (Huang, Chen, Huang, & Xu, 2014; Lupi et al., 2016; Pirner, Dulle, Mauer, & Förster, 2016; Simsolo, Eroglu, Tanriverdi, & Ozer, 2018). Although it is known that organogels are formed through weak intermolecular interactions between the structuring agents molecules, which generates three-dimensional networks (Steed, 2011), there is still a fundamental lack of understanding considering the type of required interactions (Nikiforidis, Gilbert, & Scholten, 2015). The association between the *self-assembly* and the structuring of crystalline particles in vegetable oils constitute hybrid systems with high potential for the formation of organogels.

Table 4 presents the number of crystalline elements, mean density (D), mean D of crystals (μm), agglomerated crystals (%) and mean D of individual crystals (μm) in organogels, after isothermal static crystallization at 25ºC, for 24 h. Images in Figure 5 are shown with 20 times magnification.

Organogels 3 (6% M), 5 (3% S, 3% M), 7 (2% S, 2% H, 2% M), 10 (1% S, 1% H, 4% M) and 11 (2% S, 2% H, 2% M) had higher number of crystalline elements (38229, 48810, 31934, 28125 and 18940, respectively) and lower mean density (17.07, 10.00, 18.94, 12.43 and 18.28, respectively). This is directly related with the higher SCmax values at 25ºC (Table 1), evidencing that the presence of M, as structuring agent, induces the development of smaller

crystals, therefore they may not create a sandy perception in the mouth, resulting from the lipid phase (Beckett, 2008). The average diameter of organogel crystals was below 30 μm, ranging from 1.19 (organogel with 3% S and 3% M) to 2.24 μm (organogel with 6% H), indicating that this parameter did not change and the presence of structuring agents promote the development of smaller crystals.

Organogels 1 (6% S), 5 (3% S, 3% M), 6 (3% H, 3% M) and 7 (2% S, 2% H, 2% M) showed lower network density and smaller crystal size, indicating that crystal size is generally related to the firmness of the material applied to the lipid bases, and smaller crystals are stronger than larger crystals (Hwang, Kim, Singh, Winkler-Moser, & Liu, 2012).

Hardness

Hardness is the resistance of a material against permanent deformation, and its magnitude measures the mechanical strength of a structure because of the interaction forces between the materials components (Walstra, 2003). Organogels hardness values are in Table 1. The values ranged from 0.13 to 4.55 N, where the minimum value corresponded to organogel with 6% S and the maximum value to organogel with 3% S and 3% H. Therefore, the interaction of structuring agents was relevant for the hardness increase.

R^2 value (90.96%) indicates that this model (Harness = 16.45*S*H + 8.93*H*M) is adequate for evaluating the organogels harness behavior. These data enabled the construction of the triangular diagrams (Figure 2D).

Organogels 4 (3% S, 3% H), 6 (3% H, 3% M), 7 (2% S, 2% H, 2% M), 9 (1% S, 4% H, 1% M) and 11 (2% S, 2% H, 2% M) had higher hardness (4.55, 3.45, 3.56, 4.01 and 3.49 N, respectively), compatible with high SC contents at 25ºC. Hardness relates to organogels crystal size (Table 4), small crystals being more resistant than the big ones (Hwang et al., 2012).

Organogels 1, 2 and 3, which contain the S, H and M structuring agents, isolated at 6% concentration, presented low hardness values, but organogels 7 and 11, which contained the same structuring agents at 2% concentration each, presented hardness suitable for use as lipid bases and may be beneficial in technological applications requiring increased flowability for products such as *spreads* (Manzocco, Calligaris, Camerin, Pizzale, & Nicoli, 2014).

Lower hardness values (0.13, 0.56, 1.52 and 1.76N), appeared for organogels 1 (6% S), 2 (6% H), 5 (3% S, 3% M) and 10 (1% S, 1% H, 4% M), respectively, indicating that despite the high concentration of M, it induces the negative hardness characteristic for lipid applications, where hardness is important for product structuring.

Structuring agent S, in isolated form, led to low hardness value for organogels (0.13N at 6%), but when combined with structuring agent H, which also resulted in low hardness value (0.56N at 6%), showed synergistic action. This may result in hardness increase, 4.55N with 3% S and 3% H. However, this does not happen when S is combined with M (0.82N), with 3% S and 3% M. This effect may result from intermolecular interactions between TAGs of H and long chain fatty acids present in structuring agent S, followed by the formation of the crystal network. The fatty acid chains may have been adsorbed or physically incorporated onto the crystal network surface, creating an occluded structure, trapping HOSO, as occurs in the *pickering* effect for emulsions (Dickinson, 2012).

Organogels with high S concentrations provided lower hardness, 0.13, 0.82 and 1.76 N with 6, 3 and 4% S, respectively, probably due to the hydrophilic groups in the emulsifiers that exert repulsive forces as a result of chemical composition and number of hydrophilic portions. Sangwal (2007) forming a less cohesive structure. In general, the organogels hardness parameters can be associated with the formation of small fat crystals dispersed in high proportion of liquid oil, promoting the formation of less cohesive crystal networks.

Frequency scanning

The frequency scanning analysis for investigating the deformation behavior of organogels within the linear viscoelastic region are presented in Figure 6. All organogels displayed similar behavior, with an elastic modulus value (G') higher than the viscous modulus (G"), indicating solid material behavior (Steffe, 1996).

Organogel with 6% S had the lowest G’ and G’’ values while the others had intermediate values at the frequency scanning analysis, compatible with 25ºC SCmax and hardness values (Table 1).

Temperature scanning

Organogel temperature scanning analysis are in Figure 7, displaying a similar behavior for different organogels, though apparent viscosity, with an increase in elastic modulus (G') and viscous modulus (G”), within the same temperature range. A G' value above G” was observed, according to the frequency scanning analysis (Figure 6).

The results obtained from the temperature scanning analysis of organogels showed that the rheological behavior was similar to that observed in the differential scanning calorimeter (DSC), with close crystallization and melting start and end values (Table 2), indicating that the first peak observed for thermal analysis is related to the breakdown of the organogel network,

results consistent with other studies that evaluated the organogels rheological behavior (Rocha et al., 2013). The crystallization of fats into different polymorphic crystalline forms can be explained by changes in G* curves slope (Lupi, Gabriele, & de Cindio, 2012).

During heating, G' and G'' values decreased with temperature increase; however, these values increased during organogels cooling, probably due to recrystallization and reorganization of organogels tridimensional network. Temperature scanning shows that organogels can be used as fat substitutes in industrial processes utilizing shear, even at temperatures above the organogel formation temperature, since these materials are thermoreversible, as reported by other researchers (Alvarez-Mitre, Morales-Rueda, Dibildox-Alvarado, Charó-Alonso, & Toro-Vazquez, 2012; Dassanayake, Kodali, & Ueno, 2011; Pernetti et al., 2007; Smith et al., 2011).

Crystallization temperatures were also higher than those determined for melting (Figure 3), and G'- G " crossover can be observed, which can be used as a simple criterion for gel point (Rocha et al., 2013). Gelation temperature refers to crystallization and crystals aggregation phenomena in *clusters* (Lupi et al., 2012). When the molten system is cooled, fat crystals in α polymorphism are obtained and their size and number increase with decreasing temperature. Additionally, during the cooling process, potential transitions (α → β′ polymorphic transformation) and aggregations occur, creating a three-dimensional crystal network (Campos, 2005; Marangoni, 2005).

4. CONCLUSION

The organogels analyzed in this study showed characteristic stability, except for the 6% S organogel. The different organogel characterization parameters were associated in terms of characteristics such as SCmax at 25ºC, hardness, β polymorphism, average crystal density and solid material behavior, since they presented G 'higher than G' '. Structuring agent S presents negative results in isolation, but when analyzed together with other structuring agents, such as in organogels 4 (3% S, 3% H), 7 (2% S, 2% H, 2% M), 9 (1% S, 4% H, 1% M) and 11 (2% S, 2% H, 2% M) showed satisfactory results, capable of being used as organogels for application in lipid based products. The characteristic hardness of organogels was suitable for spreadable products, with the exception of organogels 1, 2 and 5, composed of 6% S, 6% M and 3% and 3% S and M, respectively. The most positive results were obtained when the 3 structuring agents were used at a concentration of 2% each (organogels 7 and 11). The set of evaluated properties characterize the analyzed organogels as lipid bases with potential to be used as fat

substitutes in industrial processes, in order to meet a demand of consumers looking for healthier foods.

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7. TABLES AND FIGURAS

Table 1. Induction time, maximum solids, solid content at 25ºC and hardness

Samples	S (%)	H (%)	M (%)	IT (minutes)	SCmáx (%)	SC a 25ºC (%)	Hardness (N)
1	6	0	0	6	2.66	3.04	32.00±0.15
2	0	6	0	15	5.70	4.87	0.56±0.10
3	0	0	6	3	5.44	5.60	1.52±0.02
4	3	3	0	7	1.66	4.33	4.55±0.09
5	3	0	3	3	3.62	4.56	0.82±0.04
6	0	3	3	6	5.38	5.19	3.45±0.18
7	2	2	2	4	4.61	4.63	3.56±0.17
8	4	1	1	5	2.60	3.59	1.76±0.10
9	1	4	1	12	4.94	4.77	4.01±0.38
10	1	1	4	3	5.34	5.44	1.18±0.02
11	2	2	2	4	4.83	5.11	3.49±0.16

S - sorbitan monostearate; H - palm oil *hardfat*; M - Monoglycerides Grindsted Crystallizer 100; IT – Induction time; SC - solid content.

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

Table 2. Thermal behavior of oleogels

Samples	Crystallization							
	Peak 1				Peak 2			
	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)
1	47.95	18.30	3.11	1.98	-10.34	-36.47	-50.48	50.39
2	26.06	22.15	0.27	6.31	-26.06	-33.06	-48.76	49.95
3	56.48	48.07	7.12	7.61	-26.22	-33.43	-47.96	49.33
4	24.61	16.95	5.89	1.72	-28.86	-32.92	-46.24	47.54
5	24.39	18.37	2.67	1.29	-31.84	-36.11	-50.74	59.91
6	48.13	21.61	5.99	5.01	-25.71	-34.76	-48.58	51.86
7	26.14	18.93	3.37	2.32	-30.15	-33.95	-46.97	51.98
8	21.69	14.42	5.09	0.74	-31.63	-36.12	-47.70	51.55
9	27.79	20.52	7.62	3.47	-27.7	-34.32	-48.17	57.86

10	52.14	21.92	-1.92	6.36	-31.13	-34.42	-46.26	47.82
11	25.53	19.75	7.07	1.90	-30.38	-33.95	-50.04	53.31

	Melting							
Samples	**Peak 1**				**Peak 2**			
	T_{oc} (ºC)	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**	**T_{oc} (ºC)**	**T_{pc} (ºC)**	**T_{fc} (ºC)**	**ΔH_c (J/g)**
1	-15.99	-3.38	11.34	49.94	26.26	45.26	58.08	5.81
2	-15.48	-3.38	17.59	51.07	25.26	45.29	59.20	6.08
3	-15.99	-3.54	11.54	57.73	35.96	53.40	70.15	7.78
4	-14.40	-3.47	9.51	50.61	16.46	36.75	48.29	2.35
5	-14.80	-4.76	8.14	54.67	21.72	28.10	36.86	0.66
6	-15.14	-4.21	8.69	50.28	23.92	47.03	64.03	7.67
7	-14.80	-4.64	7.15	56.65	28.61	38.46	53.32	3.14
8	-14.94	-4.77	9.96	57.98	19.29	31.06	48.33	1.26
9	-15.09	-4.45	11.60	55.02	27.14	40.78	49.52	4.20
10	-14.24	-4.32	5.41	57.85	29.12	48.31	62.95	5.48
11	-15.00	-4.40	8.16	55.85	28.28	38.49	51.81	2.93

T_{oc} and T_{om}: onset crystallization and melting temperature; **T_{pc} and T_{pm}** peak crystallization and melting temperature; **T_{fc} and T_{fm}:** final crystallization and melting temperature; **ΔH_c, ΔH_m and ΔH_{total}:** crystallization, melting and total enthalpies.

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

Table 3. Lipid phase diffractograms of oleogels

Oleogels	**Short spacings**			**Polymorphic form**
	4.6	**4.2**	**3.8**	
1	liquid	liquid	liquid	-
2	4.43	4.14	3.75	β' + β
3	4.57	n.d.	n.d.	β
4	4.59	n.d.	n.d.	β
5	4.54	n.d.	n.d.	β
6	4.54	4.18	3.81	β' + β
7	4.50	n.d.	n.d.	β
8	4.42	n.d.	n.d.	β

9	4.58	4.32	4.02	β' + β
10	4.58	n.d.	n.d.	β
11	4.52	n.d.	n.d.	β

n.d.: not detected

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

Table 4. Microstructure of oleogels

Samples	Crystalline elements	Mean density (µm)	Mean D crystals (µm)	Agglomerated crystals (%)
1	13256.00	68.24	1.76	32.30
2	611.00	69.45	2.24	29.79
3	38229.00	17.07	1.34	13.28
4	210.00	52.10	1.94	25.24
5	48810.00	10.00	1.19	11.93
6	5988.00	42.60	1.34	19.82
7	31934.00	18.94	1.36	13.99
8	4416.00	62.73	1.52	25.45
9	73.00	51.87	2.14	23.29
10	28125.00	12.43	1.44	16.06
11	18940.00	18.28	1.40	19.20

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

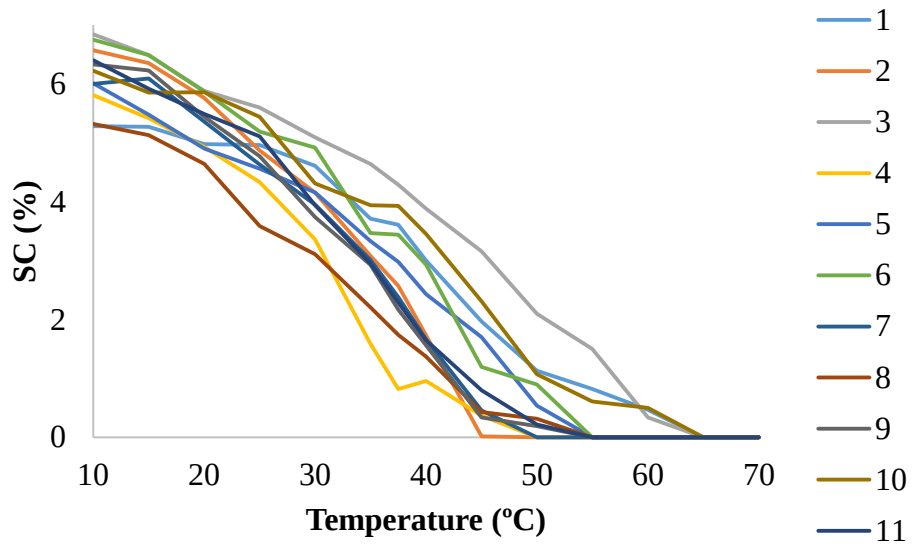
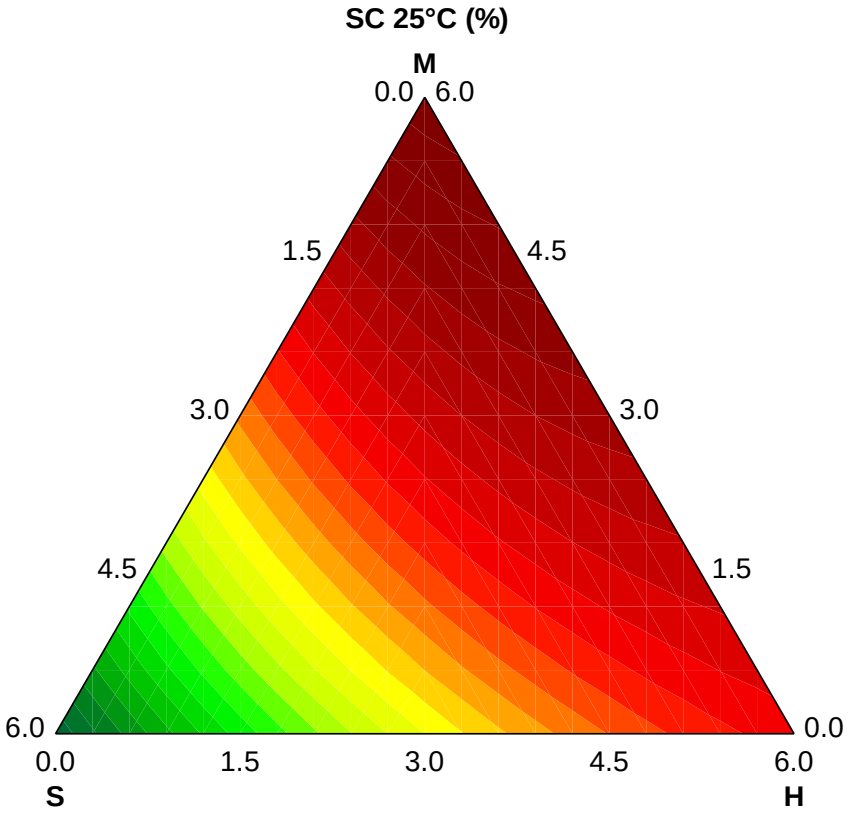
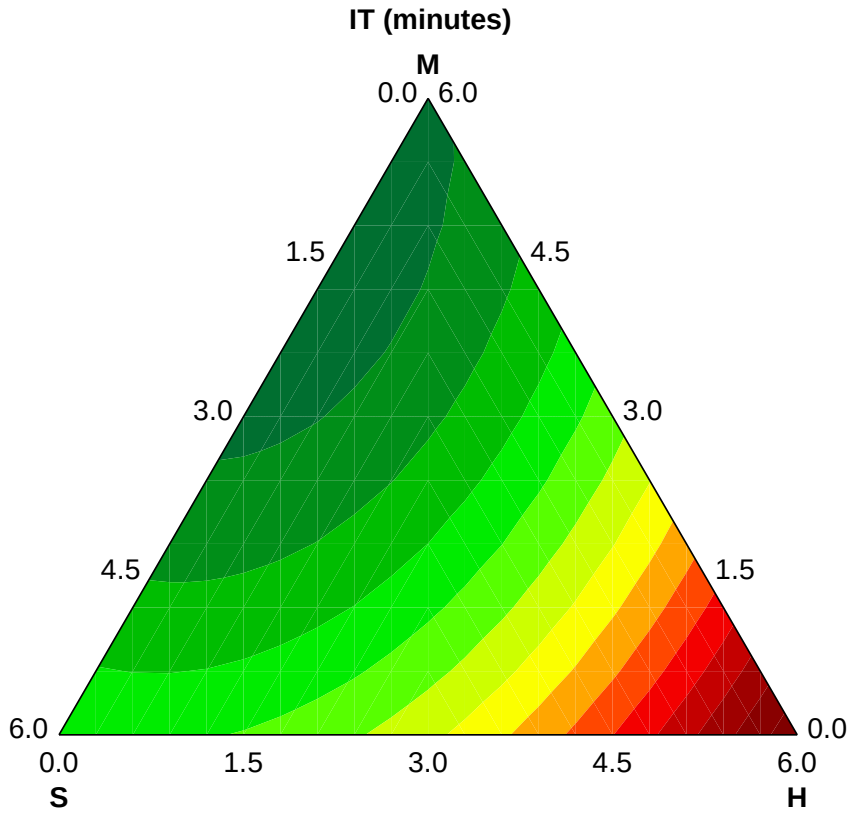


Figure 1. Fat solids profile of oleogels.

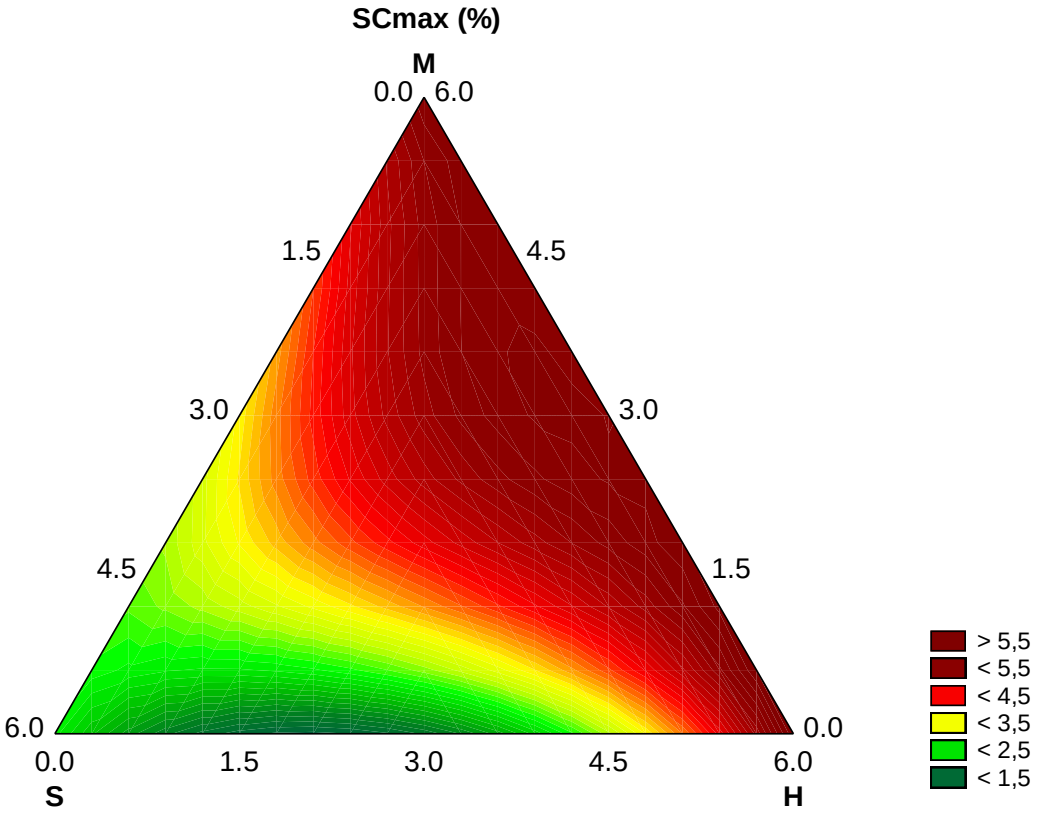
1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.


SC 25°C (%)
M
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
S
H
> 5,6
< 5,6
< 5,2
< 4,8
< 4,4
< 4
< 3,6
< 3,2

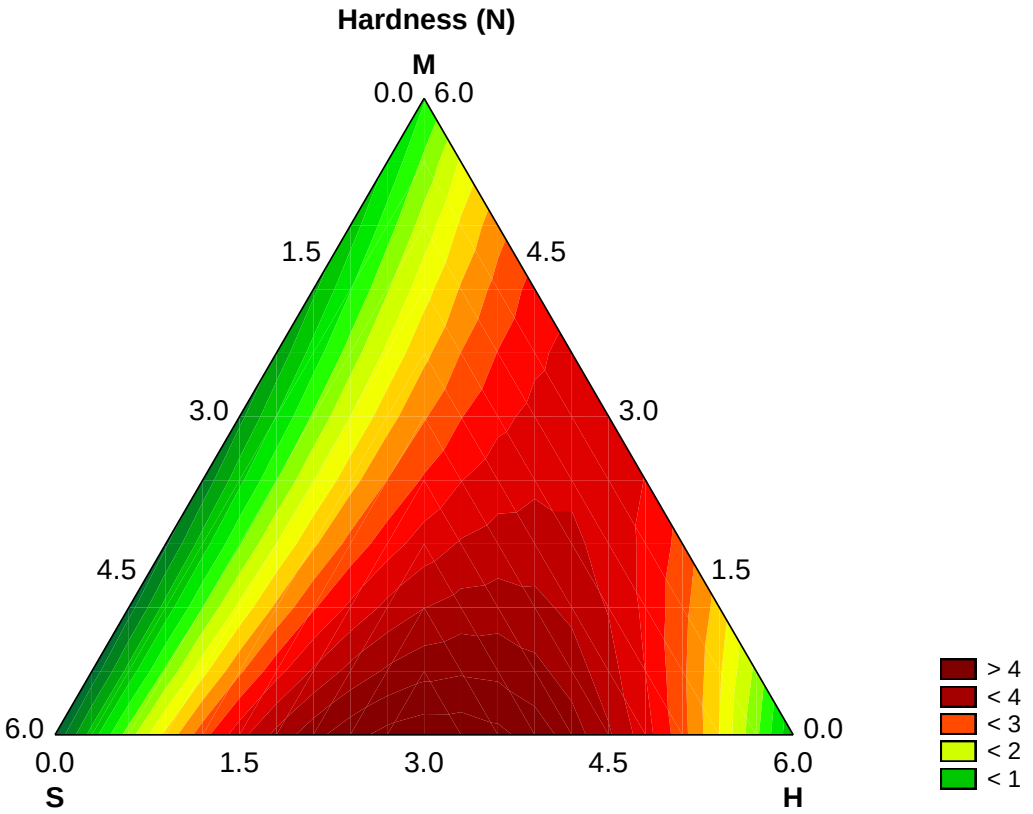
1 **(A)**


IT (minutes)
M
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
S
H
> 14
< 14
< 12
< 10
< 8
< 6
< 4

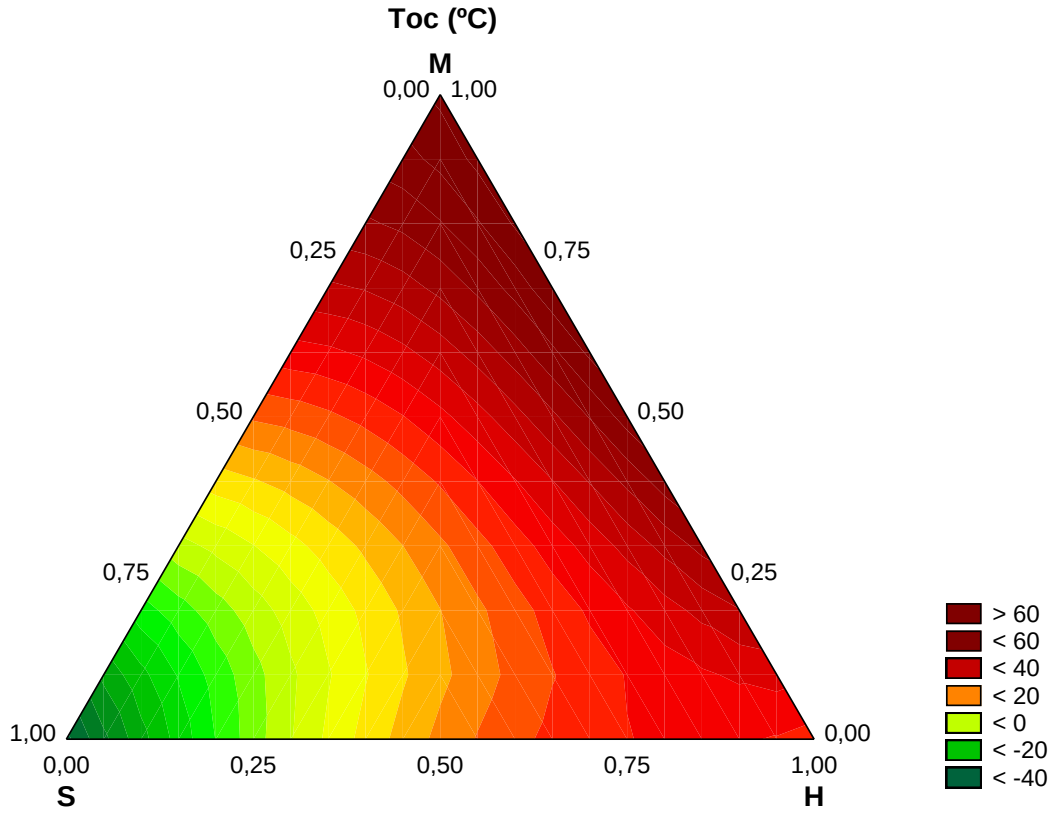
2 **(B)**


SCmax (%)
M
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
S
H
> 5,5
< 5,5
< 4,5
< 3,5
< 2,5
< 1,5

(C)

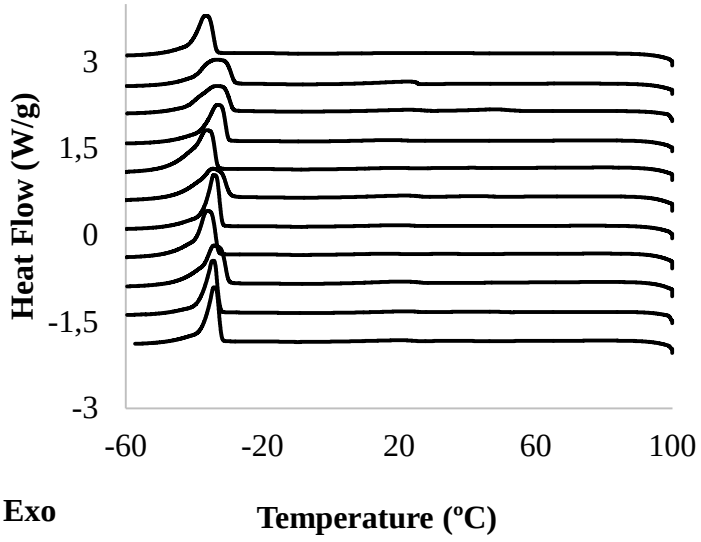

Hardness (N)
M
0.0
6.0
1.5
4.5
3.0
3.0
4.5
1.5
6.0
0.0
0.0
1.5
3.0
4.5
6.0
S
H
> 4
< 4
< 3
< 2
< 1

(D)



(E)

Figure 2. Triangular diagrams for solid content at 25ºC (A), induction time (IT) (minutes) (B), maximum solid content (SCmax) (%) (C), hardness (N) (D) and onset crystallization temperature (Toc) (ºC) (E) as a function of sorbitan monostearate (S), palm oil *hardfat* (H) and monoglycerides (M).



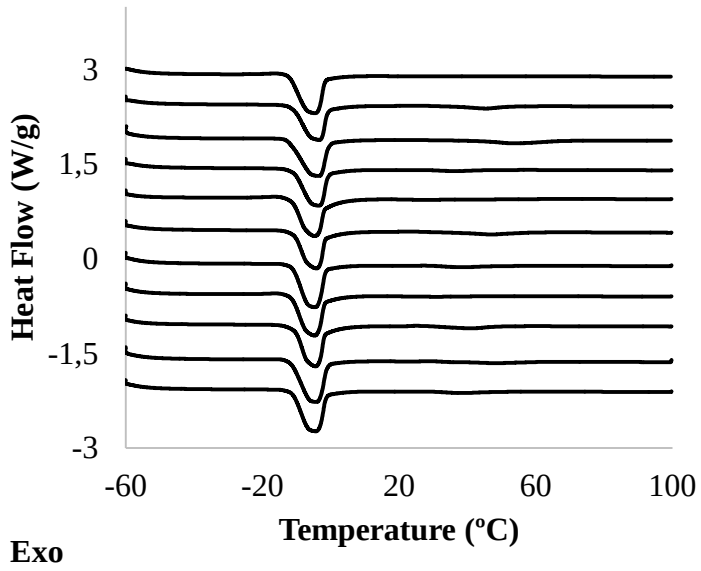


Figure 3. Melting and crystallization curves of organogels.

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

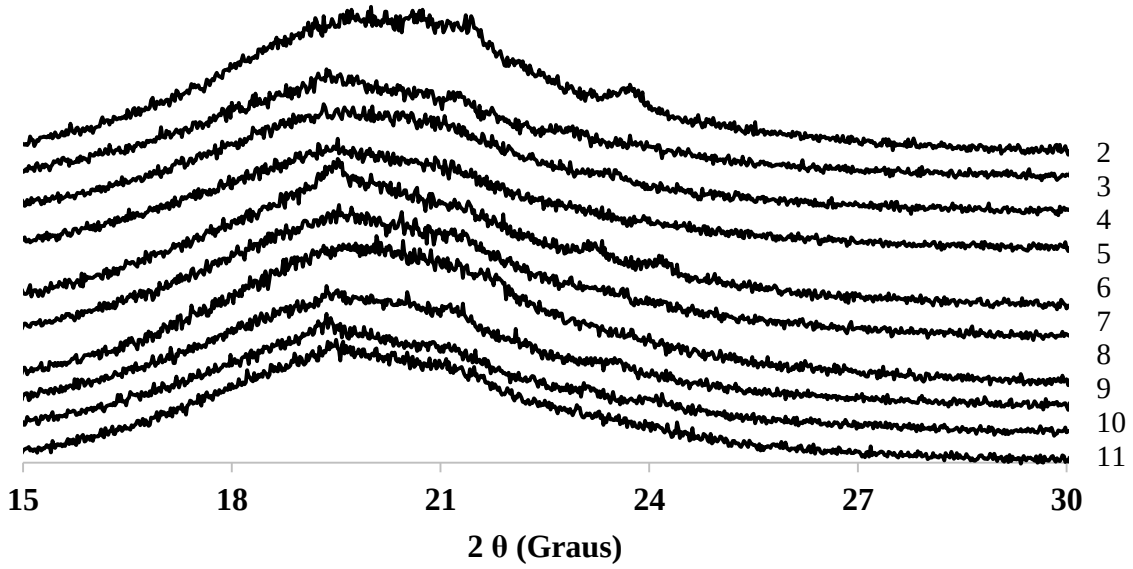


Figure 4. Lipid phase diffractograms of oleogels.

2: 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

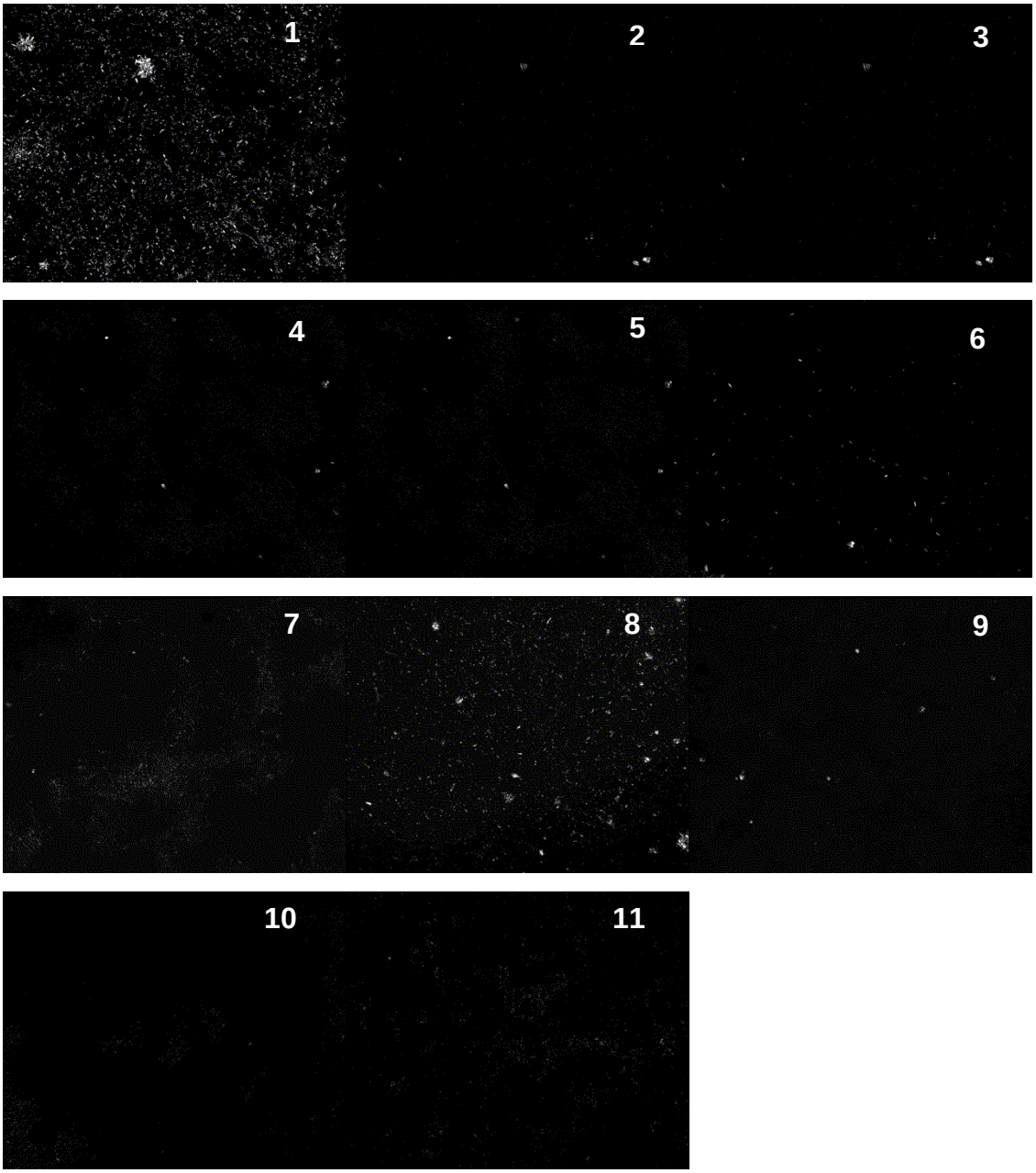
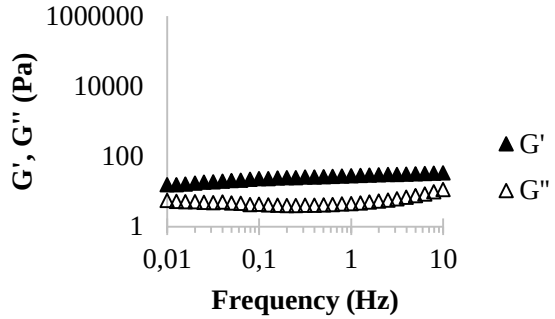


Figure 5. Microstructure of oleogels at 20x magnification and 25°C

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

1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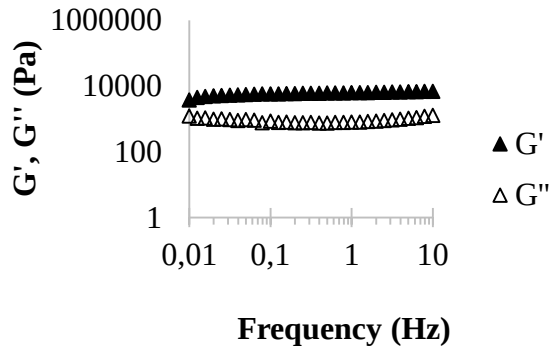
G', G'' (Pa)
0,01
0,1
1
10
Frequency (Hz)
G'
G''

1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1
10
Frequency (Hz)
G'
G''

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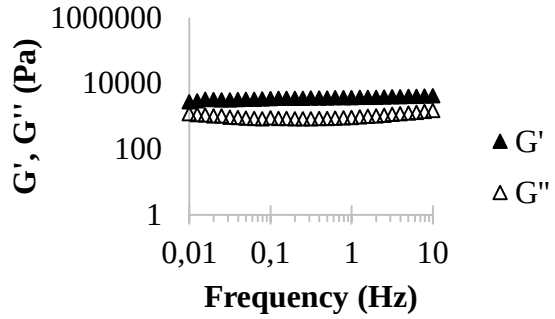
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1
10
Frequency (Hz)
G'
G''

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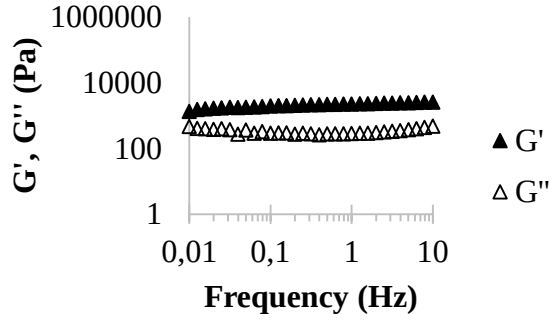
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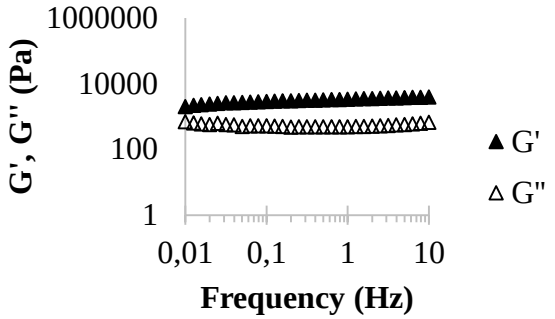
1
10
Frequency (Hz)
G'
G''

4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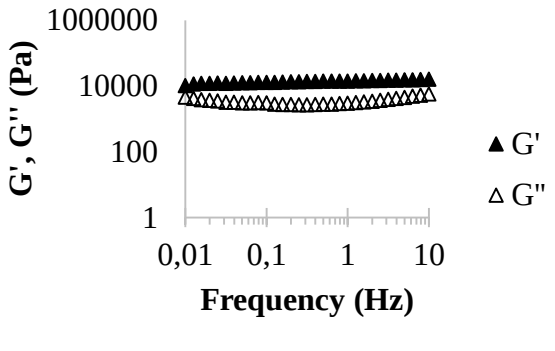
0,1
1
10
Frequency (Hz)
G'
G''

5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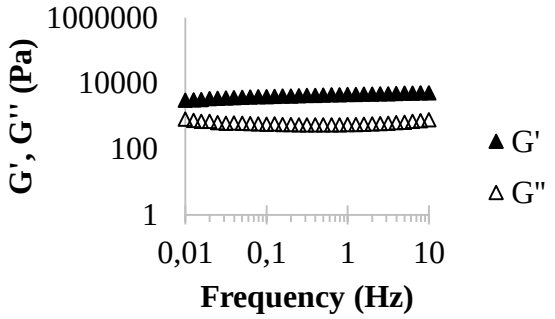
1
10
Frequency (Hz)
G'
G''

6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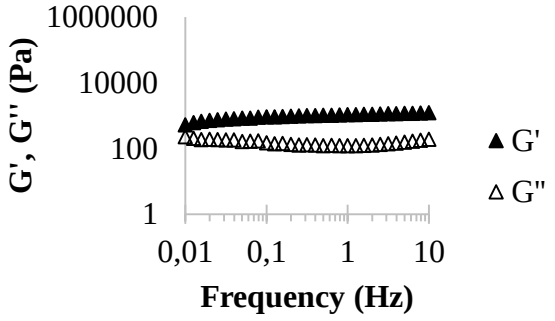
10
Frequency (Hz)
G'
G''

7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Frequency (Hz)
G'
G''

8

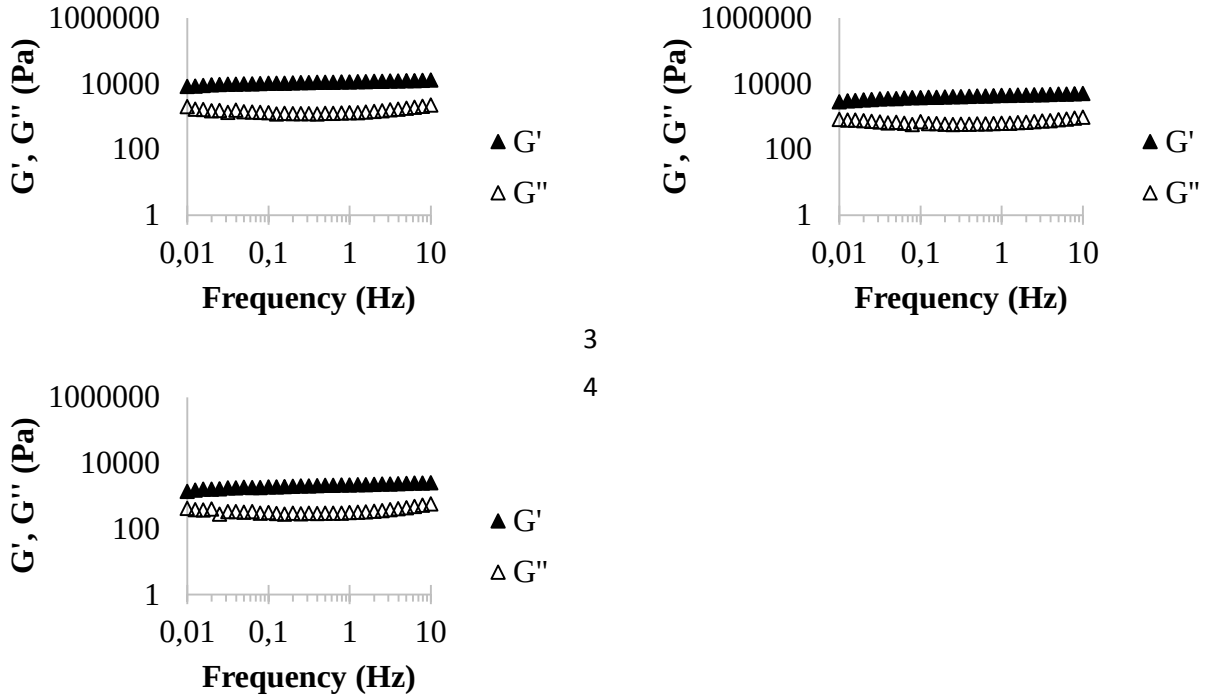
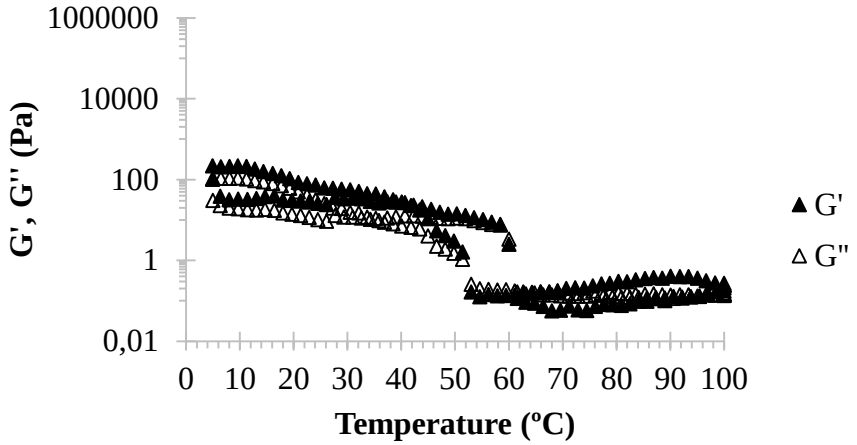


Figure 6. G’ (a) and G’’ (b) of the frequency scanning of oleogels.

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.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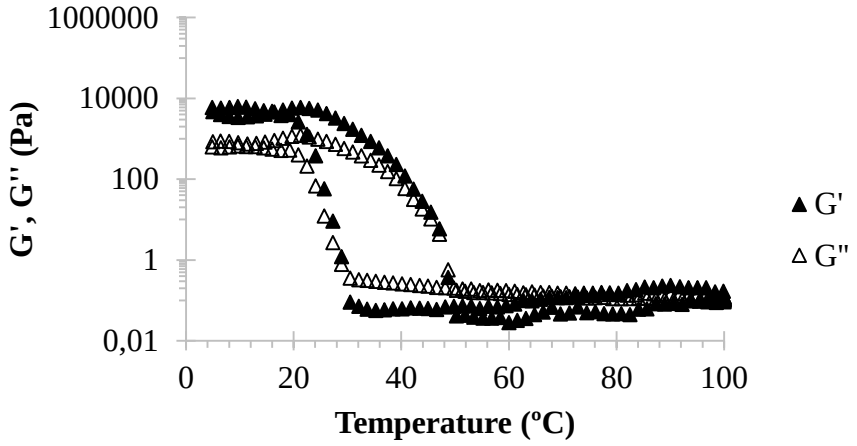
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G', G'' (Pa)
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Temperature (ºC)
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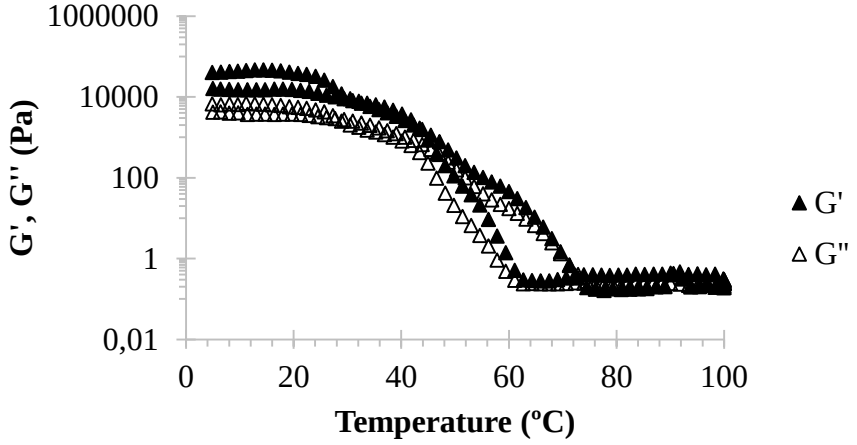
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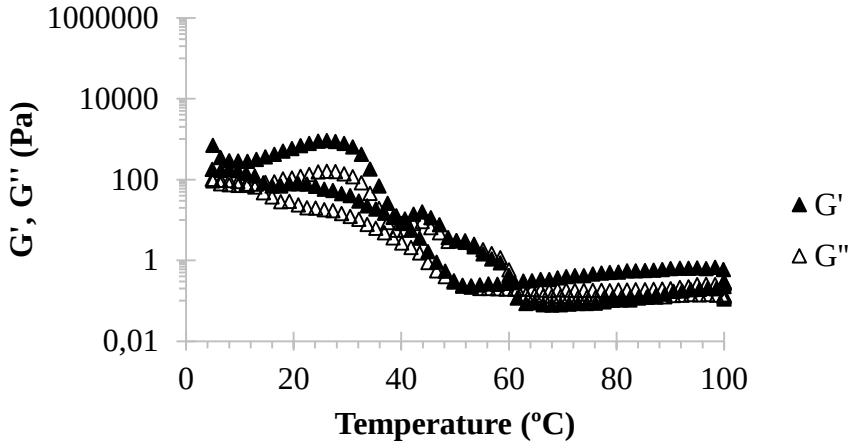
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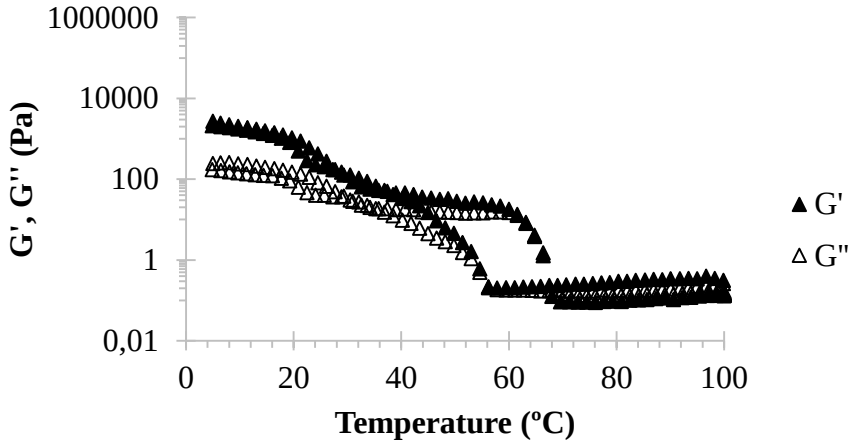
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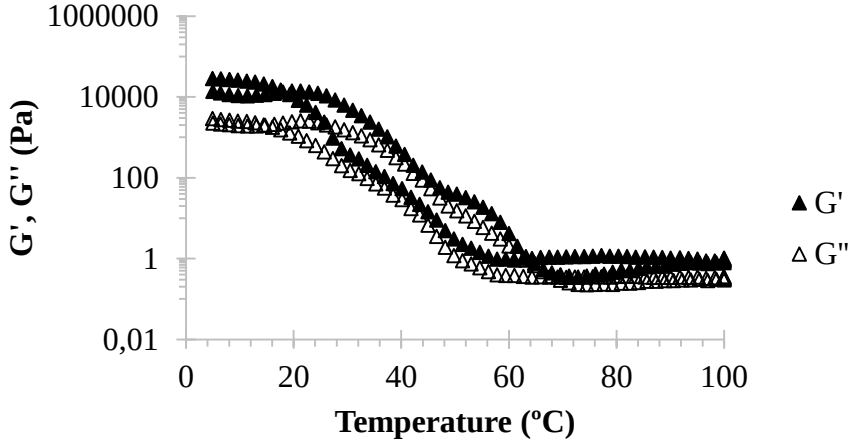
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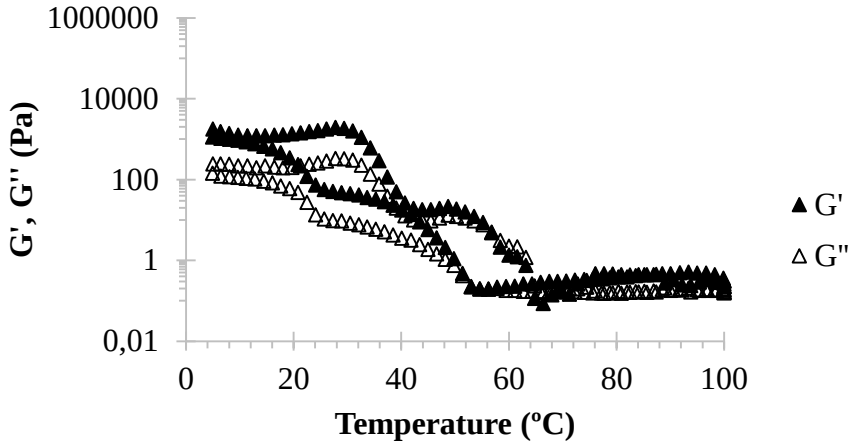
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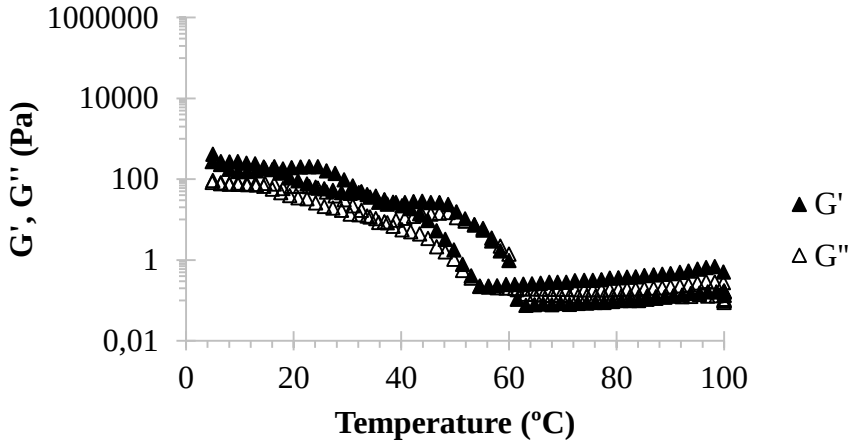
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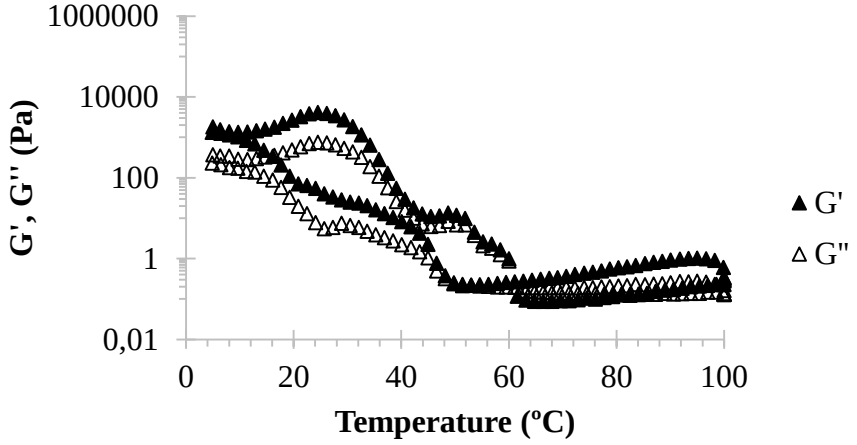
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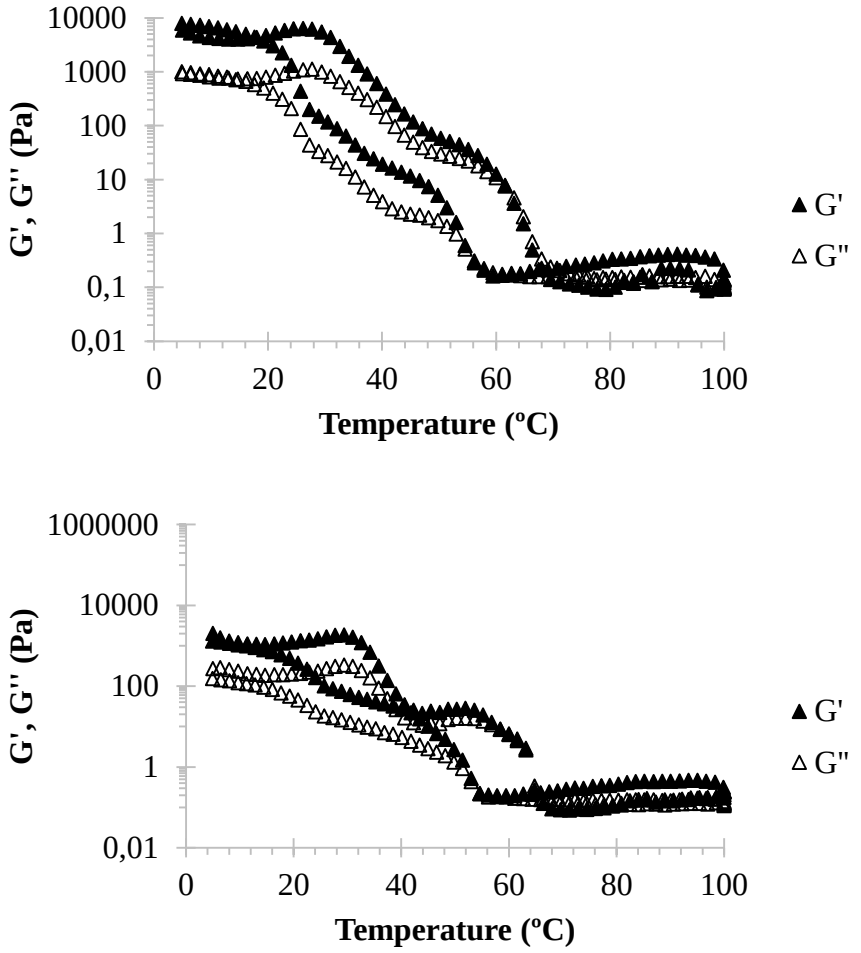

Figure 7. G’ (a) and G’’ (b) of the temperature scanning of oleogels.

1: 6% S; **2:** 6% H; **3:** 6% M; **4:** 3% S, 3% H; **5:** 3% S, 3% M; **6:** 3% H, 3% M; **7:** 2% S, 2% H, 2% M; **8:** 4% S, 1% H, 1% M; **9:** 1% S, 4% H, 1% M; **10:** 1% S, 1% H, 4% M; **11:** 2% S, 2% H, 2% M.

CAPÍTULO 9

CARACTERIZAÇÃO DE FASE LIPÍDICA E REFORMULAÇÃO DE SPREADS DE CHOCOLATE PARA REDUZIR ÁCIDOS GRAXOS SATURADOS

Artigo Submetido

Revista Brasileira de Ciências Agrárias

Caracterização de fase lipídica e reformulação de *spreads* de chocolate para reduzir ácidos graxos saturados

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RESUMO

O objetivo desse estudo foi caracterizar organogéis e reformular *spreads* de chocolate para redução de ácidos grados saturados. A caracterização dos organogéis foi realizada quanto ao conteúdo de sólidos, cinética de cristalização, comportamento térmico, polimorfismo, microestrutura, dureza e propriedades reológicas. Os *spreads* de chocolate foram caracterizados quanto à consistência, distribuição do tamanho de partículas, propriedades reológicas e turbidimetria dinâmica. A faixa de consistência dos *spreads* variou entre 13.83 a 631.60 gF, sendo que na formulação contendo apenas óleo de girassol alto oleico não foi possível detectar consistência, pois os *spreads* apresentavam exsudação de óleo. Todos os *spreads* de chocolate formulados com organogel mostraram tamanho de partícula entre 14.59 e 46.23 µm em índice d 0.5 e d 0.9, respectivamente. Quanto aos parâmetros reológicos, foi observado valor do G' (módulo viscoso) superior ao G'' (módulo complexo), indicando propriedades de materiais sólidos. Dessa forma, os *spreads* de chocolate apresentaram comportamento elástico

predominante, sem exsudação de óleo líquido. Esta caracterização mostra-se fundamental para reformulação de produtos em termos de maior saudabilidade.

Palavras-chaves: organogéis; tamanho de partículas; turbidimetria dinâmica; agentes estruturantes

Lipid base characterization and reformulation of chocolate spreads to reduce saturated fatty acids

ABSTRACT

The aim of this study was to characterize organogels and reformulate chocolate spreads to reduce saturated fatty acids. The organogels characterization was performed as to the solids content, crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness and rheological properties. Chocolate spreads were characterized for consistency, particle size distribution, rheological properties and dynamic turbidimetry. The consistency range of the spreads ranged from 13.83 to 631.60gF, and in the formulation containing only high oleic sunflower oil it was not possible to detect consistency, because the spreads showed oil exudation. All chocolate spreads formulated with organogel showed particle size between 14.59 and 46.23 μm at index d 0.5 and d 0.9, respectively. Regarding rheological parameters, G' (viscous modulus) value higher than G" (complex modulus) was observed, indicating properties of solid materials. Thus, chocolate spreads showed a predominant elastic behavior without liquid oil exudation. This characterization is essential for product reformulation in terms of greater healthiness.

Keywords: organogels; particle size; dynamic turbidimetry; structuring agents

Introdução

Spreads de chocolate são dispersões de pó de cacau e partículas de açúcar em fase lipídica contínua, com uma proporção considerável de gordura sólida. Os *spreads* de chocolate possuem propriedades reológicas características, uma vez que se comportam como um sólido que evita a sedimentação de partículas dispersas e a separação de fases (óleo líquido), mas também exibem elevada capacidade de espalhamento (Patel et al., 2014). Os *spreads* de chocolate são muito populares, devido ao sabor e alto valor nutritivo e energético. A popularidade destes alimentos está associada principalmente ao seu potencial para despertar o prazer sensorial e emoções positivas (Macht e Dettmer, 2006).

Os *spreads* de chocolate são alimentos à base de gorduras cujas propriedades físicas são dependentes de sua estrutura cristalina. Consequentemente, os óleos e gorduras utilizados na formulação apresentam efeito significativo sobre a qualidade do produto (Mayfield et al., 2015).

Há um grande risco de exsudação e oxidação da fase lipídica nos *spreads* de chocolate, uma vez que a gordura utilizada possui uma fase líquida; e o processo tecnológico de obtenção destes produtos inclui moagem dos ingredientes. Assim, todos os sistemas de aprisionamento da gordura precisam ser eficientes, para que o óleo seja efetivamente retido na rede cristalina (Norberg, 2006). Sendo assim, para reduzir o teor de ácidos graxos saturados (AGS), as propriedades plásticas dos lipídios com diferentes propriedades de cristalização devem ser exploradas (Manzocco et al., 2014).

Óleo de palma e suas estearinas, com altos teores de AGS, são parcialmente sólidos à temperatura ambiente, e estão entre as matérias-primas lipídicas mais amplamente utilizadas para a formulação de *spreads* de chocolate (El-Hadad et al., 2011; Shin et al., 2010). Gorduras sólidas (óleos vegetais parcialmente hidrogenados ou gorduras naturais com elevados teores de AGS) são utilizados para formulações de *spreads* de chocolate. Além disso, estabilizadores lipídicos (triacilgliceróis de alto ponto de fusão à base de óleos totalmente hidrogenados) são geralmente incorporados à formulação para evitar a exsudação de óleo nas temperaturas de armazenamento, distribuição e comercialização (Patel et al., 2014).

A gordura saturada é a principal causa alimentar de elevação de colesterol plasmático, cuja redução na dieta é mundialmente amparada para a redução do risco de doenças cardiovasculares (Wassell et al., 2010). Diante dos efeitos nocivos dos AGS e *trans* à saúde foram realizadas ações que direcionam as indústrias para aumentar a saudabilidade dos

alimentos, através da alteração das matérias primas empregadas convencionalmente (Santos et al., 2013).

A tecnologia de organogéis é potencialmente viável em comparação aos processos convencionais de modificação lipídica, pois não causa nenhuma mudança química na estrutura de ácidos graxos e triacilgliceróis (TAGs), mantendo as características nutricionais do óleo inalteradas, em especial os teores de ácidos graxos insaturados (AGI) e distribuição estereoespecífica natural (Sundram et al., 2007).

No escopo da estruturação não convencional de fases orgânicas, sistemas lipídicos compostos por AGI, como óleos vegetais líquidos, podem ser estruturados como géis, tais estruturas formam redes contínuas de pequenas moléculas que se reúnem em cristais líquidos, micelas ou redes fibrilares, desenvolvendo diversos arranjos estruturais descritos na literatura (Chaves et al., 2019; Okuro et al., 2018; Pernetti et al., 2007; Siraj et al., 2015). Este tipo particular de estruturação caracteriza sistemas conhecidos como organogéis, materiais viscoelásticos compostos por agentes estruturantes e uma fase líquida apolar, que os diferem de outros géis formados basicamente por compostos hidrossolúveis, também denominados hidrogéis. São sistemas semi-sólidos onde uma fase oleosa é imobilizada por uma rede tridimensional autossustentada do estruturante (Dassanayake et al., 2009; Rogers et al., 2009, 2007).

Um estruturante é eficiente quando não é necessário utilizar altas concentrações para obter o resultado desejado, visto que haverá aumento de custo do produto final. Além de não se ter total conhecimento dos efeitos causados por esses aditivos em altas concentrações no organismo (Co e Marangoni, 2012).

O potencial das ceras como estruturantes tornou-se uma técnica alternativa para a estruturação dos óleos, e diferentes sistemas óleo-estruturante comestíveis têm sido intensamente pesquisados (Marangoni e Garti, 2011). A cera de candelilla é aprovada como um aditivo alimentar pela Food and Drug Administration (FDA), sendo reconhecido como seguro (GRAS) e como ingrediente alimentar na dieta humana (FDA, 2016).

Outros componentes descritos recentemente pela literatura científica com potencial de estruturar matrizes lipídicas complexas referem-se aos emulsificantes (SIRAJ et al., 2015). Os monoacilgliceróis são moléculas lipídicas que possuem apenas um ácido graxo esterificado a molécula de glicerol, que pode variar quanto ao tamanho de cadeia e grau de insaturação (Chen e Terentjev, 2010). A estruturação de óleos vegetais por monoacilgliceróis ocorre através do mecanismo *self-assembly*, pela formação de micelas ou fases lamelares

inversas durante o resfriamento do sistema formado (Lopez-Martínez et al., 2015; Valoppi et al., 2016; Wang et al., 2016).

O monoestearato de sorbitana é um surfactante emulsificante não-iônico hidrofóbico, utilizado para modificar as propriedades de cristalização em sistemas lipídicos (Marangoni e Narine, 2002). Demonstra capacidade para formação de dispersões viscosas em solventes orgânicos e óleos comestíveis, através do mecanismo de *self-assembly* (Co e Marangoni, 2012; Smith et al., 2011). Estudos recentes sobre o efeito estruturante do monoestearato de sorbitana sugerem seu uso como potencial elemento de estruturação em óleos vegetais, além de uma interação positiva com os TAGs (Cerqueira et al., 2017; De Oliveira et al., 2015; Sonwai et al., 2017).

Uma opção de baixo custo e alto potencial para estruturação de fases lipídicas são os óleos vegetais totalmente hidrogenados, denominados *hardfats.* Estes componentes são considerados sistemas-modelo em termos de composição em ácidos graxos e TAGs, que representam fatores importantes na determinação do efeito estruturante e modificador dos processos de cristalização em fases lipídicas contínuas ou emulsionadas (Omonov; Bouzidi; Narine, 2010). Além da função como agentes primários de cristalização, os *hardfats* atuam modificando as propriedades físicas de sistemas gordurosos contínuos, possibilitando diversas adequações relativas ao desenvolvimento de organogéis, propriedade que tem justificado uma série de estudos sobre a estruturação convencional de TAGs (Wassell et al., 2010; Smith et al., 2011).

Esses agentes estruturantes permitem que duas fases distintas se combinem em um estado quase homogêneo, modificando inclusive o comportamento térmico. Os estruturantes específicos, utilizados isoladamente ou em conjunto, bem como suas interações, determinam a estrutura do produto final, e consequentemente, suas propriedades de consistência e plasticidade (Cerdeira et al., 2006; Silva et al., 2018).

O uso de organogéis em produtos alimentícios é uma alternativa bastante atrativa, uma vez que tais materiais podem conferir características como textura e consistência, sem aumento do teor de ácidos graxos *trans* e com redução significativa do teor de AGS incorporado ao produto final, representando produtos com forte apelo nutricional e tecnológico (Rogers, 2009).

O óleo de girassol alto oleico (OGAO) é considerado uma matéria-prima *premium*, geralmente utilizado em aplicações alimentícias que requerem o emprego de óleo líquido com estabilidade oxidativa excepcional. Possui sabor e aroma neutros, característica

associada ao seu alto potencial de aplicação em alimentos, cosméticos e fármacos (Gunstone, 2005).

No desenvolvimento de novos produtos à base de gordura, o maior desafio é mimetizar as propriedades sensoriais da gordura original, que são dependentes na microestrutura cristalina lipídica (Rush et al., 2008). Os fatores estruturais como teor de gorduras sólidas e microestrutura determinam as propriedades reológicas macroscópicas do sistema lipídico final (Rombaut et al., 2009). Informações sobre o comportamento reológico e propriedades térmicas (comportamento de cristalização e fusão) da fase lipídica presente nos produtos alimentícios pode fornecer um melhor controle de qualidade em cada etapa de processamento (Gonçalves e Lannes, 2010).

O objetivo desse estudo foi caracterizar diferentes organogéis e seus respectivos *spreads* de chocolate, visando a reformulação da fase lipídica desta categoria de produtos para redução do teor de ácidos graxos saturados.

Material and methods

Material

Para formulação das bases lipídicas utilizou-se óleo de girassol alto oleico (OGAO) fornecido pela Cargill Agrícola S.A. (Brasil); óleo de palma (PO) refinado fornecido por Agropalma (Brasil); cera de candelilla (C) (Light Special Candelilla REAL®), fornecida pela empresa Multiceras S.A., García - NL, México; óleo vegetal totalmente hidrogenado (*hardfat*), obtido a partir do óleo de palma (HP), fornecido pela Cargill Alimentos Ltda, Brasil; monoglicerídio (M) Grindsted Crystallizer 100, fornecido pela DuPont do Brasil S.A., São Paulo – SP, Brasil; e monoestearato de sorbitana (S), fornecido pela Sigma Aldrich, EUA. Para a elaboração das formulações dos *spreads* de chocolate foram utilizados os seguintes ingredientes: glaçúcar União, cacau em pó alcalino Cargill cal 70, lecitina de soja padrão, fornecida pela Bunge Alimentos S.A. e essência de avelã Arcolor.

Delineamento experimental

Caracterização dos organogéis

Os organogéis foram preparados mediante a mistura da fase imobilizada (OGAO) e do estruturante (CSM, CSH e SHM a 6%), sob agitação e aquecimento acima do ponto de fusão do estruturante. Em seguida, o aquecimento foi desligado mantendo-se a agitação por mais 3 minutos para completa homogeneização (Rocha et al., 2013; Stahl et al., 2018).

Conteúdo de sólidos (SC). Foi determinado utilizando Espectrômetro de Ressonância Magnética Nuclear (RMN) Bruker pc120 Minispec, com auxílio de banhos secos de alta precisão (0 - 70°C) Tcon 2000 (Duratech, EUA). Método AOCS Cd 16b- 93: método direto, leitura das amostras em série, temperagem para gorduras não estabilizadas (AOCS, 2009).

Cinética de cristalização. Foi obtida através de temperagem inicial das amostras (100ºC por 15 min, 1 h a 70ºC) e o teor de sólidos foi monitorado a 25ºC (± 0,5ºC) em Espectrômetro de Ressonância Magnética Nuclear Mq20 NMR AnalyzerBruker (Wassell e Young, 2007). Os dados foram adquiridos automaticamente, com medida a cada minuto, durante 1h. A caracterização da cinética de cristalização foi realizada segundo o período de indução (τSC) - relativo ao início da formação dos cristais, teor máximo de sólidos (SCmáx) (Campos, 2005; Stahl et al., 2018).

Comportamento térmico. A análise térmica das amostras foi realizada em calorímetro diferencial de varredura (DSC) TA Q2000, acoplado ao RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). O sistema de processamento de dados utilizado foi o Universal V4.7A (TA Instruments, Waters LLC, New Castle). As condições de análise foram: massa da amostra: ~ 10 mg; método AOCS Cj 1-94 (AOCS, 2009) modificado: temperatura entre -60 e 100°C, com rampa de 5°C/min (cristalização e fusão). Foram utilizados os seguintes parâmetros para avaliação dos resultados: temperatura *onset* de cristalização e fusão (T_{oc} e T_{of}), temperaturas de pico de cristalização e fusão (T_{pc} e T_{pf}), entalpias de cristalização e fusão (ΔH_c e ΔH_f) e temperatura de conclusão de cristalização e fusão (T_{fc} e T_{ff}) (Barbosa et al., 2018; Campos, 2005).

Polimorfismo. Determinados por difração de raios-x, segundo procedimentos de Sawalha e colaboradores (Sawalha et al., 2012). As análises foram realizadas em difratômetro Philips (PW 1710), utilizando a geometria Bragg-Bretano (θ:2θ) com radiação de Cu-ka (l = 1.54056Å, tensão de 40 KV e corrente de 30 mA). As medidas foram obtidas com passos de 0,02° em 2θ e tempo de aquisição de 2 segundos, com scans de 15 a 30° (escala 2θ) à temperatura de 25ºC. A identificação das formas cristalinas foi realizada a partir dos short spacings (SS) característicos dos cristais (AOCS, 2009).

Microestrutura. A microestrutura dos organogéis foi avaliada por microscopia sob luz polarizada. Com o auxílio de um tubo capilar, uma gota de amostra foi colocada sobre uma lâmina de vidro, que foi coberta com uma lamínula e mantida a temperatura de 5ºC por 24 horas e posteriormente a 25ºC por 24 horas. Os organogéis foram analisados a 25°C usando ampliação de 20x. A microestrutura dos cristais foi avaliada com o uso de microscópio de

luz polarizada (Modelo BX51, Olympus America Inc., Estados Unidos) acoplado a câmara de vídeo digital (Media Cybernetics). As imagens foram capturadas pelo aplicativo Image Pro-Plus 7.0 (Media Cybernetics, Estados Unidos) em quatro diferentes campos visuais de cada lâmina para cada amostra e o resultado de diâmetro médio de partícula foi expresso pela média e desvio padrão desses valores (Cindio e Cacace, 1995; Toro-Vazquez et al., 2013).

Dureza. Determinada utilizando texturômetro (TA-XTi2, Stable Microsystems, Inglaterra), controlado por microcomputador. Para as análises, 30 mL dos organogéis foram colocados em béqueres de 50 mL e acondicionados em estufa B.O.D., em temperatura de 5ºC, por 24 h. Foi realizado um teste de compressão/extrusão usando probe cilíndrico de acrílico de 25 mm de diâmetro e 35 mm de comprimento, com velocidade de 1.0 mm/s, e uma distância fixa para penetração do probe de 15 mm. O valor considerado foi a força máxima obtida (Rocha et al., 2013).

Propriedades reológicas. As análises reológicas foram realizadas de acordo com metodologia proposta por (Rocha et al., 2013) utilizando um reômetro de tensão controlada (Physica MCR 301, Anton Paar, Alemanha). Foi utilizada a geometria de placas paralelas de aço inox de superfície rugosa (50mm de diâmetro e gap de 200μm). A temperatura foi controlada usando um sistema Peltier. Varreduras de temperatura na taxa de 5°C/min foram feitas de 5°C a 100°C, posteriormente, resfriadas de 100 até 5°C e reaquecidas de 5 a 100ºC. Foram utilizadas frequência (f) de 1Hz e deformação de 1%, dentro do intervalo de viscoelasticidade linear. Os organogéis foram analisados por varredura de tensão e de frequência, de forma a avaliar sua resistência mecânica e o comportamento frente a diferentes tempos de observação, respectivamente. As varreduras de tensão foram realizadas de 0,1 a 10 Pa (f = 1Hz) a 25ºC. As varreduras de frequência foram obtidas entre 0,01 e 10 Hz, dentro do intervalo de viscoelasticidade linear, e na mesma das varreduras de tensão. Foram determinados como parâmetros: módulo elástico (G'), módulo viscoso (G"), módulo complexo (G*), ângulo de fase (δ) e viscosidade complexa (η*).

Caracterização dos spreads de chocolate

Foram produzidas três formulações de *spreads* de chocolate de 3 Kg de acordo com as três formulações de organogéis (Tabela 1). A caracterização físico-química dos organogéis foi realizada quanto ao conteúdo de sólidos, cinética de cristalização, comportamento térmico, polimorfismo, microestrutura, dureza e propriedades reológicas.

Os organogéis foram aplicados em *spreads* de chocolate, visando a incorporação adequada, a formulação e o processamento do *spreads* de chocolate padrão foram baseados em Ambiel (2013). Os ingredientes e as respectivas porcentagens da formulação padrão e dos *spreads* de chocolate com organogel estão apresentados na Tabela 2.

Tabela 1. Formulações das fases lipídicas dos s*preads* de chocolate

Ingrediente (%)	F1	F2	F3	F4	F5	F6	F7	F8
PO	99.60	-	-	-	-	-	-	-
OGAO	-	96.60	93.60	96.60	93.60	96.60	93.60	99.60
C	-	1.00	2.00	1.00	2.00	-	-	-
HP	-	-	-	1.00	2.00	1.00	2.00	-
M	-	1.00	2.00	-	-	1.00	2.00	-
S	-	1.00	2.00	1.00	2.00	1.00	2.00	-
Lecitina de soja	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

PO: óleo de palma; OGAO: óleo de girassol alto oleico; C: cera de candelilla; HP: *hardfat* de óleo de palma; M: monoglicerídio Grindsted Crystallizer 100; S: monoestearato de sorbitana.

Tabela 2. Composição (%) da formulação dos *spreads* de chocolate.

Ingrediente	Percentual (%)
Açúcar refinado	39.50
Cacau em pó alcalino	25.00
Óleo de palma ou organogel	35.03
Lecitina de soja	0.40
Aroma de chocolate	0.07

O processamento para obtenção dos *spreads* de chocolate ocorreu através da mistura dos ingredientes secos (açúcar e cacau em pó), que foram previamente misturados em moinho de esferas Caotech (Wormerveer, Holland) tipo CAO - B5. Nesta etapa, a fase lipídica considerada nas diferentes formulações foi adicionada à mistura seca. Os ingredientes foram homogeneizados e refinados em moinho de esferas Caotech (Wormerveer, Holland) tipo CAO - B5, para diminuição do tamanho das partículas à temperatura de 60ºC. O produto refinado foi acondicionado em recipientes plásticos com tampa e posteriormente, foi resfriado em BOD à temperatura de 5ºC durante 24 horas e à temperatura de 25ºC por 24 horas pela realização das análises. A caracterização físico-química dos *spreads* de chocolate foi realizada quanto à consistência, distribuição do tamanho de partículas, propriedades reológicas e turbidimetria dinâmica.

Consistência. As consistências dos *spreads* de chocolate foram determinadas pelo analisador de texturas TA-XT Plus (Stable Micro Systems, Surrey, Reino Unido). O probe

cone com ângulo de ponta não truncado de 45° foi utilizado e a profundidade de penetração aplicada foi de 10 mm com velocidade de sonda de 2 mm/s. A força de compressão obtida é dada em força gramatical (gF) (Campos, 2005). As amostras foram analisadas em quadruplicado e os resultados expressos como as médias das repetições.

Distribuição de tamanho de partículas (DTP). A distribuição do tamanho de partículas (DTP) dos *spreads* de chocolate foi determinada utilizando o equipamento Horiba LA-960 Laser Particle Size Analyser – High Performance Laser Diffraction Analyser. Os *spreads* de chocolate pré-dispersos (0.4g em 10 mL de óleo de girassol) à temperatura ambiente (25ºC), foram adicionadas diretamente na unidade de amostragem até obter valor de obscuração de 15%. As amostras foram dispersas com auxílio de banho ultrassônico por 2 minutos para assegurar que as partículas fossem dispersas e suspensas individualmente, e posteriormente mantidas sob agitação. A distribuição de tamanho das partículas foi expressa pelos valores de diâmetro médio volumétrico D[4,3], mediana D(0.5) e moda. Também foram avaliados os parâmetros D(0.5) e D(0.9), os quais representam os valores de diâmetro abaixo dos quais se situam 50 e 90% da distribuição acumulada (em volume), respectivamente (Afoakwa et al., 2009). Como indicativo da amplitude da distribuição de tamanho de partículas, foi utilizado o índice *Span*, calculado pela equação 1.

$$Span = \frac{D(0.9) - D(0.1)}{D(0.5)} \text{ (Equação 1)}$$

Propriedades reológicas. As análises reológicas foram realizadas de acordo com metodologia proposta por Rocha et al., (2013) utilizando reômetro de tensão controlada (Physica MCR 301, Anton Paar, Alemanha). Foi utilizada a geometria de placas paralelas de aço inox de superfície rugosa (50mm de diâmetro e gap de 200μm). A temperatura foi controlada usando um sistema Peltier. Foram utilizadas frequência (f) de 1Hz e deformação de 1%, dentro do intervalo de viscoelasticidade linear. Os *spreads* de chocolate foram analisados por varredura de tensão e de frequência, de forma a avaliar sua resistência mecânica e o comportamento frente a diferentes tempos de observação, respectivamente. As varreduras de tensão foram obtidas de 0.1 a 10 Pa (f = 1Hz) à temperaturas de 25ºC. As varreduras de frequência foram obtidas entre 0.01 e 10 Hz, dentro do intervalo de viscoelasticidade linear, e na mesma das varreduras de tensão. Foram determinados como parâmetros: módulo elástico (G'), módulo viscoso (G"), módulo complexo (G*), ângulo de fase (δ) e viscosidade complexa (η*).

Técnica de Turbidimetria Dinâmica

A estabilidade cinética do *spread* de chocolate foi acompanhada usando um retroespalhamento de luz de infravermelho (NIR) próximo a 880 nm (TurbiscanTM LAB, Formulaction, Ramonville St. Agne, França). Para isso, 15 mL de *spread* de chocolate foram transferidos imediatamente após o processamento a para tubos de vidro borossilicato (12 mm de diâmetro interno e 30 mm de altura). A fonte de luz digitalizou as amostras nos intervalos de 0, 8, 15, 22, 29 e 36 dias e mediu a porcentagem de luz retroespalhada à temperatura de 25°C. A variação de retroespalhamento (*backscatter variation* - ΔBS) foi determinada a partir da diferença de retroespalhamento entre amostras de controle (*spreads* de chocolate produzidos com OP e OGAO sem estruturante) e *spreads* de chocolate com organogel. Os dados foram analisados usando o software Turbisoft 2.0 (Zhao et al., 2014).

Análise estatística

Os resultados objetivos foram avaliados através do programa estatístico Statistica 8.0 - Statsoft, USA (STAT SOFT, 2007) para cálculo do coeficiente de regressão, probabilidades (p-valor) e análise de variância (ANOVA) com nível de significância de 5%. Com o mesmo programa foi realizada a comparação de médias, para a comparação dos *spreads* de chocolate, através de teste de Tukey.

Resultados e discussão

Caracterização dos organogéis

Conteúdo de sólidos (SC)

A Figura 1 apresenta os perfis de sólidos determinados para as os organogéis e entre 10 a 50ºC. Os organogéis apresentaram SC variando entre 5.22 a 6.20% a 10ºC, todos mostraram fusão completa a 45ºC e SC inferior a 2.34% a 37.5ºC. Nesta faixa, o SC deve ser inferior a 5%, para minimizar possível sensação cerosa (De Oliveira et al., 2014; Masuchi et al., 2014). O organogel SHM mostrou SC de 6.20% à temperatura de 10ºC, superior ao teor total de estruturantes (6%) adicionados na formulação, seguido do CSM (5.70% de sólidos). Estas formulações contém 2% de M e provavelmente devido às diferentes composições em classes triacilglicerólicas do M, este estruturante pode ter induzido a cristalização da fração triacilglicerólica saturada do OGAO. O organogel CSH apresentou menor SC à temperatura de 10ºC (5.22% de sólidos), percentual inferior aos 6% de estruturantes adicionados à fase lipídica Esta formulação contém 2% de S, que em estudos prévios mostrou menores teores de SC na forma isolada ou combinada com C ou M.

O SC em diferentes temperaturas descreve os perfis de fusão dos organogéis, portanto, qualifica os organogéis para aplicação em *spreads* de chocolate. Esta análise permitiu determinar a fração sólida de cada organogel durante a fusão. A baixas temperaturas (4 a 10°C), os valores de SC tipificam a espalhabilidade dos organogéis refrigerados. À temperatura ambiente (20 a 22°C), é essencial mínimo de 10% de gordura sólida para garantir a resistência à exsudação de óleo e estabilidade dos produtos quando nos referimos a uma gordura convencional. O SC dos organogéis variou entre 4.69 (CSM) a 5.43% (SHM) a 20ºC, uma vez que são bases lipídicas não convencionais. Em temperaturas entre 30 e 35°C, as gorduras de uso geral, a exemplo do óleo de palma, distinguem-se pela fusão, com concomitante liberação do sabor; e o SC fornece uma estimativa dos atributos sensoriais na degustação (Wassell e Young, 2007). A temperatura corporal (37.5ºC) é crítica para qualidade sensorial de produtos de base lipídica. Durante a cristalização da fase lipídica, materiais com composição química e estrutura molecular semelhante são mais propensos à co-cristalização (Marangoni, 2005).

Cinética de cristalização

A Tabela 3 mostra o tempo de indução (TI) e o SCmáx a 25ºC dos organogéis. O TI variou entre 4 a 8 minutos e o SCmáx entre 2.29 a 4.72%. O organogel CSH apresentou cristalização mais lenta, com TI de 8 minutos, seguido dos organogéis CSM (6 minutos) e SHM (4 minutos). O SCmáx à 25ºC do organogel SHM foi o mais elevado, provavelmente devido às diferentes composições em classes triacilglicerólicas do M, em concordância com os resultados de SC.

Tabela 3. Tempo de indução, máximo de sólidos e SC à 25ºC, *short spacings* e dureza dos organogéis

Organogel	TI (minutos)	SCmáx. à 25ºC (%)	SS	Dureza (N)
CSM	6±0.71	3.50±0.05	β	4.35±0.47
CSH	8±1.41	2.29±0.03	β	4.58±0.43
SHM	4±0.00	4.72±0.16	β	3.53±0.15

TI: tempo de indução; SCmáx. à 25ºC: conteúdo de sólido máximo à 25ºC; SS: *short spacings;* CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio.

Comportamento térmico

O comportamento térmico dos organogéis é mostrado na Tabela 4. As curvas de fusão e cristalização podem ser subdivididas em diferentes regiões, refletindo as diferentes classes triacilglicerólicas presentes nos organogéis.

Quanto ao comportamento térmico na cristalização, os organogéis CSM, CHS e SHM mostraram menor temperatura *onset* de cristalização (T_{oc}) (46.22, 36.79 e 25.53ºC, respectivamente), provavelmente pela alta concentração de TAGs de maior ponto de fusão do H, que é o estruturante presente em maior concentração. Os *hardfats* desempenham um papel interessante na estruturação de TAGs devido à sua insolubilidade, ou solubilidade limitada, em óleos vegetais poliinsaturados, e a capacidade de formar uma rede sólida de cristais. Os diferentes tipos de *hardfats* apresentam características de fusão semelhantes, com alto ponto de fusão, elevada entalpia de fusão em comparação à gorduras convencionais e capacidade de formar uma matriz que irá cristalizar em altas temperaturas, formando uma dispersão fina de cristais estáveis (Alander e Lidefelt, 2007; Norberg, 2006; Talbot, 1989). A presença de *hardfats* como aditivos modificam o hábito cristalino e alteram o comportamento de cristalização, reduzindo o período de indução da cristalização, atuando como germens de cristalização (Oliveira, 2011). Este comportamento está relacionado às características físicas e da composição em ácidos graxos do óleo a partir do qual o *hardfat* foi obtido (Alander e Lidefelt, 2007).

As curvas de cristalização mostraram dois picos largos (representando frações de componentes de baixo e alto ponto de fusão) e sobrepostos, que são associados à presença destas diferentes classes triacilglicerólicas (Manzocco et al., 2014; Saberi et al., 2011). Todos os estruturantes apresentaram co-cristalização entre si e com OGAO. Os parâmetros de comportamento térmico dos organogéis indicaram um primeiro pico de fusão, relativo aos TAGs mais insaturados, provavelmente do OGAO, e um segundo pico de fusão relativo ao(s) estruturante(s) utilizado(s) e também à fração saturada do OGAO.

Tabela 4. Comportamento térmico dos organogéis

	Cristalização							
Amostras	Pico 1				Pico 2			
	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)
CSM	46.22	26.23	7.80	2.85	-32.33	-37.93	-49.92	40.36
CHS	36.79	15.14	3.05	3.47	-31.70	-38.30	-50.60	42.81
SHM	25.53	19.75	7.07	1.90	-30.38	-33.95	-50.04	53.31
	Fusão							
Amostras	Pico 1				Pico 2			

	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)	T_{oc} (ºC)	T_{pc} (ºC)	T_{fc} (ºC)	ΔH_c (J/g)
CSM	-14.24	-4.24	7.23	52.25	18.18	26.37	59.73	2.49
CHS	-15.51	-4.95	11.45	53.69	26.31	33.71	46.56	1.49
SHM	-15.00	-4.40	8.16	55.85	28.28	38.49	51.81	2.93

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio.

Polimorfismo

O conteúdo de gordura típico dos *spreads* de chocolate é de aproximadamente 30%, caracterizando o produto como um sistema gorduroso contínuo, onde o açúcar e outras partículas ficam dispersos, fazendo com que as propriedades da gordura tenham grande influência sobre o comportamento sensorial, proporcionando cremosidade e maciez. Normalmente, gorduras com tendência polimórfica β formam cristais grandes e fornecem *snap* desejável em produtos de chocolate, enquanto gorduras tipo β' promovem cristais pequenos e sensação de suavidade durante a degustação (Norberg, 2006).

As formas cristalinas são caracterizadas por *short spacings* (SS) específicos (Tabela 3). Os SS característicos correspondem a 4,15 Å para as formas polimórficas α, 3,8 e 4,2 Å para β' e 4,6 Å para β, respectivamente; e são usados para determinar a proporção relativa e tipo de polimorfos presentes nas bases lipídicas (Stahl et al., 2017).

Para todos os organogéis foram verificados SS iguais a 4.6 Å, caracterizando o hábito polimórfico β. Apesar do estruturante M possuir elevada concentração de ácido behênico (C22:0) (Silva et al., 2018), que favoreceria o hábito polimórfico β, sua estrutura molecular é heterogênea devido à distribuição regioespecífica típica dos monoacilgliceróis. A tripalmitina e os monoacilgliceróis do ácido behênico agem como aceleradores do processo de cristalização do óleo de palma, muito importante na indústria do chocolate, onde o cristal do tipo β é desejado (Basso et al., 2010). Entretanto, em fase lipídica de *spreads* e margarinas, os cristais devem ser estabilizados preferencialmente na forma β' para favorecer a espalhabilidade do produto (Wassell e Young, 2007), porém, como os organogéis contém elevada quantidade de OGAO na formulação, já possuem uma maior espalhabilidade devido ao elevado teor de AGI, independente da forma polimórfica.

Microestrutura

A composição lipídica e as condições de cristalização influenciam o formato do cristal, e diferentes formas polimórficas e morfologias cristalinas são possíveis. Os cristais são agregados em estruturas maiores formando uma rede, o que caracteriza o nível

microestrutural da gordura. O conceito de microestrutura inclui informações sobre o estado, quantidade, forma, tamanho, relação espacial e interação entre todos os componentes da rede cristalina, e tem uma enorme influência nas propriedades macroscópicas das gorduras (Marangoni e Hartel, 1998; Oliveira et al., 2015; Ribeiro et al., 2009; Shi et al., 2005).

TAG cristalizam-se geralmente como esferulitos, que correspondem à agregação de lamelas cristalinas, que crescem radialmente a partir dos mesmos núcleos centrais e podem desenvolver ramificações durante o amadurecimento (Rousset, 2002). Eventualmente, dependendo das condições de resfriamento ou mesmo do perfil característico de fusão de cada gordura, os TAGs também podem cristalizar em outras morfologias, como agulhas e discos (Oliveira et al., 2015).

Organogéis podem se estruturar formando uma rede fibrosa 3D, onde o solvente é aprisionado na matriz estruturante, evitando o fluxo de solvente. A rede é estabilizada por interações fracas entre as cadeias, como ligação de hidrogênio, forças de van der Waals e π staking (Huang, Chen, Huang, & Xu, 2014; Lupi et al., 2016; Pirner, Dulle, E. J. Mauer, & Förster, 2016; Simsolo, Eroglu, Tanriverdi, & Ozer, 2018). Embora já se saiba que os organogéis são formados através de interações fracas intermoleculares entre as moléculas dos estruturantes, o que gera redes tridimensionais (Steed, 2011), ainda há uma falta de compreensão fundamental considerando o tipo de interações que são necessárias (Nikiforidis, Gilbert, & Scholten, 2015). A associação entre o *self-assembly* e estruturação de partículas cristalinas em óleos vegetais constituem sistemas híbridos com alto potencial para a formação de organogéis.

A Tabela 5 mostra o número de elementos cristalinos, densidade média (µm), D média dos cristais (µm), cristais aglomerados (%) e D média dos cristais individuais (µm) dos organogéis, após estabilização isotérmica estática a 25ºC, durante 24 horas. A Figura 2 mostra imagens da microestrutura dos organogéis com estabilização a 25ºC e ampliação de 20x.

Os organogéis CSM e CHS mostraram maior número de elementos cristalinos (43845 e 47367, respectivamente) e menor densidade média (13.95 e 11.76 µm, respectivamente), que está diretamente relacionado com maiores TI e dureza e menores valores de SCmáx à 25ºC (Tabela 3). O diâmetro médio dos organogéis foi inferior a 30 µm, variando entre 1.30 (CSM) a 1.93 (CHS) µm, minimizando a percepção de arenosidade na boca resultante da fase lipídica (Beckett, 2008).

Os organogéis contendo C mostraram a menor densidade de rede em contraste com os organogéis de SHM, que apresentaram densidade média e D média cristais mais elevada

(18.94 μm). Os organogéis CSM e SHM mostraram menores diâmetro médio de cristais (1.30 e 1.36 μm, respectivamente), evidenciando que a presença de M como estruturante induz a formação de cristais menores. Geralmente, o tamanho dos cristais está relacionado à firmeza resultante das bases lipídicas, sendo que cristais menores são mais resistentes que cristais maiores (Hwang et al., 2012), porém como os cristais dos organogéis apresentam tamanho inferior a 1.93 μm e pouca variação entre eles, foi possível correlacionar a dureza com a densidade média dos cristais, sendo que quanto maior a dureza dos organogéis, maior a densidade média.

Hardfats específicos, provenientes de uma determinada fonte oleosa, apresentam perfil triacilglicerólico único e diferenciado, que caracterizam estes materiais como indutores de hábitos polimórficos particulares. Após o resfriamento de uma mistura lipídica adicionada de *hardfats*, seus TAGs trissaturados, de alto ponto de fusão (65-75°C), promovem a formação de núcleos de cristalização para a ordenação de uma rede cristalina altamente estruturada a partir do sistema líquido (Pernetti et al., 2007), como foi possível observador para o organogel com CSH, pois apresentou o maior percentual de cristais aglomerados (23.64%) e maior dureza (4.58N) (Tabela 3).

Tabela 5. Microestrutura dos organogéis

Amostras	Elementos cristalinos	Densidade média (μm)	D média dos cristais (μm)	Cristais aglomerados (%)
CSM	43845.00	13.95	1.30	12.38
CHS	47367.00	11.76	1.93	23.64
SHM	31934.00	18.94	1.36	13.99

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio.

Dureza

A dureza é a resistência de um material contra uma deformação permanente, e sua magnitude mede a resistência mecânica de uma estrutura como resultado das forças de interação entre os componentes do material (Walstra, 2003). Os valores de dureza dos organogéis estão apresentados na Tabela 3. Os valores variaram entre 3.53 e 4.58 N, onde o valor mínimo correspondeu ao organogel com SHM e o máximo ao organogel com CSH. Esta característica pode ser favorável com relação às aplicações lipídicas, onde a dureza e a textura são importantes e podem ser benéficas em substituição às gorduras que requerem aumento de fluidez para produtos, como *spreads* (Manzocco et al., 2014).

Os resultados de dureza foram inversamente proporcionais aos teores de SC a 25ºC. Maiores percentuais de cristais aglomerados (Tabela 5) podem induzir a formação organogéis com maior dureza. Os organogéis com estas propriedades também exibiram maior número de elementos cristalinos (Tabela 5), conforme características típicas de redes cristalinas mais coesas e de maior dureza (Campos, 2005). No geral, os parâmetros dureza dos organogéis podem ser associados à formação de pequenos cristais de gordura dispersos em elevada proporção de óleo líquido, promovendo a formação de redes cristalinas menos coesas.

Os organogéis formulados com C (CSM e CSH) resultaram em géis com maior dureza (4.35 e 4.58 N, respectivamente), sendo indicados para uso em sistemas híbridos, essas informações são compatíveis com o observado na literatura (Doan et al., 2015; Rocha et al., 2013; Toro-Vazquez et al., 2009).

Propriedades reológicas.

As análises de varredura de frequência para investigação do comportamento de deformação dos organogéis dentro da região de viscoelasticidade linear estão apresentadas na Figura 3. Todos os organogéis mostraram comportamento similar e propriedades independentes da frequência, sendo observado valor do G’ superior ao G’’, indicando comportamento de material sólido (Steffe, 1996). As análises de varredura de frequência mostraram que o organogel CSH apresentaram valores de G’ e G’’ superiores aos organogéis SHM e CSM, resultados condizentes com dureza (Tabela 3) e número de elementos cristalinos (Tabela 5).

As análises de varredura de temperatura dos organogéis são apresentadas na Figura 4, indicando comportamento similar, através da viscosidade aparente, com um aumento do módulo elástico (G’) e módulo viscoso (G”) dentro da mesma faixa de temperatura, sendo observado valor do G’ superior ao G’’, como observado para análise de varredura de frequência (Figura 3).

Os resultados obtidos nas análises de varredura de temperatura dos organogéis mostraram que o comportamento foi o mesmo observado para o comportamento térmico com valores de início e final de cristalização e fusão (Tabela 4) similares. Os resultados indicam que o primeiro pico observado para análise térmica está relacionado à ruptura da rede de organogéis, resultados condizentes com outros trabalho que avaliaram o comportamento reológico para organogéis (Rocha et al., 2013).

As temperaturas de cristalização também foram superiores às temperaturas encontradas para fusão, podendo ser observado o cruzamento de G'-G'', que pode ser usado como um critério simples para o ponto de gel (Rocha et al., 2013). A temperatura de gelificação refere-se aos fenômenos de cristalização e à agregação de cristais em clusters (Lupi et al., 2012). Quando o sistema fundido é resfriado, cristais de gordura no formato α são obtidos e seu tamanho e número aumentam com a diminuição da temperatura. Além disso, durante o processo de resfriamento, potenciais transições (α → β ′ transformação polimórfica) e agregações ocorrem formando uma rede cristalina tridimensional (Campos, 2005; Marangoni, 2005).

Durante o aquecimento, os valores de G' e G'' diminuíram com o aumento da temperatura, porém durante o resfriamento dos organogéis os valores aumentaram, provavelmente pela recristalização e reorganização da rede tridimensional dos organogéis. A varredura de temperatura mostra que os organogéis podem ser usados como substitutos de gordura em processos industriais usando cisalhamento, mesmo que a temperatura de processo seja superior à temperatura de formação do organogel, uma vez que são termorreversíveis, conforme relatado por outros pesquisadores (Alvarez-Mitre et al., 2012; Dassanayake et al., 2011; Pernetti et al., 2007; Smith et al., 2011).

Caracterização dos spreads de chocolate

Distribuição de tamanho de partículas (DTP)

A Tabela 6 apresenta os parâmetros de distribuição de tamanho das partículas sólidas, incluindo Span (µm), d 0.5 (µm) e d 0.9 (µm), que corresponde ao diâmetro máximo para 50 e 90%, respectivamente, da distribuição das partículas dos *spreads* de chocolate.

Tabela 6. Distribuição do tamanho de partícula e consistência de *spreads* de chocolate.

Amostras	*Span* (µm)	d 0.5 (µm)	d 0.9 (µm)	Consistência (gF)
F1	11.61±1.34	18.05±1.68	212.09±8.57	631.60±207.45
F2	2.95±0.13	14.59±0.36	46.23±1.32	15.13±1.65
F3	2.48±0.05	9.43±0.39	26.57±0.65	16.90±1.04
F4	2.78±0.19	11.66±0.45	35.60±3.26	13.83±1.07
F5	3.09±0.47	13.50±0.58	45.03±0.80	14.07±1.86
F6	3.02±0.28	11.21±0.33	36.98±2.87	15.27±1.55
F7	2.29±0.05	9.34±0.19	24.47±0.80	15.20±3.38
F8	2.72±0.11	11.10±0.26	33.27±0.77	n.d.

F1: Padrão PO; F2: 3% (C, M e S); F3: 6% (C, M e S); F4: 3% (C, HP e S); F5: 6% (C, HP e S); F6: 3% (HP, M e S); F7: 6% (HP, M e S); F8: Padrão OGAO e n.d.: não detectado

Spreads de chocolate são refinados para um tamanho de partícula inferior a 30 µm (Beckett, 2008) e o tamanho final das partículas influencia criticamente as propriedades reológicas e sensoriais (Afoakwa et al., 2007). As partículas lipídicas acima de 30 µm ocasionam textura arenosa na boca e partículas menores que 20 µm são sensivelmente lisas e cremosas. A distribuição do tamanho de partícula desempenha função clara na fluidez do produto, mas é geralmente restrita ao conhecimento empírico baseado na experiência (Beckett, 2008). Os valores do índice *Span* apresentaram diferença significativa, ao nível de 5%; e variaram entre 2.29 (F7) e 11.61 (F1) µm.

Todos os *spreads* de chocolate com organogel mostraram tamanho de partículas inferiores a 14.59 e 46.23 µm em d 0.5 e d 0.9, respectivamente; desta forma os *spreads* de chocolate com organogel avaliados provavelmente apresentam baixa percepção sensorial para arenosidade, uma vez que a distribuição do tamanho de partícula tem influência direta nesta característica e nas propriedades reológicas em chocolates (Afoakwa et al., 2007).

Tan e Kerr (2018) avaliaram o tamanho e distribuição de partículas chocolate mostraram d 0.5 de 11.63, 7.64, 5.93 e 5.16 µm para os tempos 15, 30 minutos, 4 e 8 horas de refino, respectivamente e d 0.9 de 46.41, 30.67, 15.50 e 11.75 µm para os tempos 15, 30 minutos, 4 e 8 horas de refino, respectivamente.

Consistência

Os valores de consistência dos *spreads* de chocolate estão apresentados na Tabela 6. Os valores variaram entre 13.83 e 631.60 gF, sendo que o valor máximo corresponde ao *spread* de chocolate com fase lipídica padrão contendo óleo de palma (F1), o *spread* padrão com OGAO apresentou instabilidade e portanto não foi possível detectar um valor mínimo de leitura no equipamento. Os spreads de chocolate com organogel mostraram consistência variando entre 13.83 e 16.90 gF, esta característica é favorável com relação à fluidez que o produto requer. Estes resultados condizem com os valores de Span, d 0.5 e d 0.9 dos *spreads* de chocolate (Tabela 6).

Varredura de frequência

As análises de varreduras de frequência para investigação do comportamento de deformação dos *spreads* de chocolate dentro da região de viscoelasticidade linear estão apresentadas na Figura 5. Os *spreads* de chocolate mostraram comportamento similar, sendo observado valor do G’ superior ao G’’, indicando comportamento de material sólido, dessa

forma, os *spreads* de chocolate com organogel apresentaram comportamento elástico predominante, como nos resultados observados por Doan et al. (2016), Fayaz et al. (2017b) e Patel et al. (2014). A F1 apresentou consistência mais elevada entre as amostras, impossibilidade a análise de varredura de frequência para este *spread* de chocolate padrão de óleo de palma.

Os *spreads* de chocolate com organogel apresentaram valores de G' e G'' similares e a F8 mostrou o menor valor para G' e G'', resultados condizentes com a consistência dos *spreads* de chocolate (Tabela 6).

Técnica de turbidimetria dinâmica

A estabilidade da fase lipídica dos *spreads* de chocolate pode ser acompanhado por varredura em comprimento de onda do infravermelho próximo (880nm). Os valores de retrodifusão foram determinados a partir do fundo (5 mm) até ao topo (35 mm) dos tubos. Durante o tempo de análise da estabilidade dos *spreads* de chocolate, a diferença máxima de *backscatter variation* - ΔBS foi de 1%, indicando baixa dispersão do leituras em todo o tubo. Assim, os valores médios de BS obtidos nas varreduras de 5 a 35 mm foram utilizados, e esses valores foram plotados ao longo do experimento para obtenção das curvas (Figura 6).

A instabilidade da F8 observada pelo ΔBS ocorreu provavelmente devido à ausência de estruturantes, resultados condizente com consistência (Tabela 6) e varredura de frequência (Figura 5) dos *spreads* de chocolate. Os demais *spreads* mostraram baixa variação no ΔBS, indicando que o uso de organogéis como substituto de bases lipídicas convencionais em *spreads* de chocolate foi eficiente, uma vez que apresentaram comportamento similar ao padrão (F1) que continha óleo de palma, durante o tempo de análise (0, 8, 15, 22, 29 e 36 dias), além disso a concentração de 3 ou 6% de estruturantes foi indiferente com relação a estabilidade e estes resultados condizem com a consistência e a varredura de frequência dos *spreads* de chocolate, dessa forma é possível produzir um produto de qualidade utilizando baixa concentração de estruturante.

Spreads de chocolate padrão e *spreads* preparados por substituição total e parcial de óleo de palma (27%) por organogéis de goma laca foram avaliados quanto à viscosidade, com parâmetros similares aos *spreads* comerciais. Os *spreads* padrão e formulado com organogel não mostraram exsudação quando armazenado a 30ºC por mais de 4 semanas (Patel et al., 2014).

Conclusão

Os *spreads* de chocolate com organogéis analisados neste estudo mostraram estabilidade característica, uma vez que não ocorreu exsudação de óleo líquido no período de estabilização a 25ºC por 36 dias, resultado que pode ser associada as demais propriedades dos *spreads* de chocolate, como SCmáx à 25ºC, consistência, tamanho e distribuição de partículas, polimorfismo β, densidade média dos cristais e comportamento de material sólido, uma vez que as mesmas apresentaram G' maior que G''. Além disso, o polimorfismo β, juntamente com tamanho de partículas inferior a 14.59 µm em d 0.5, presente nos *spreads* de chocolate com organogel deste estudo proporciona partículas que não são perceptíveis sensorialmente e foi possível desenvolver um produto que atender a demanda de consumidores que buscam por alimentos mais saudáveis, utilizando organogéis com baixa concentração de estruturantes. Todas os organogéis (CMS, CHS e HMS) podem ser utilizados para aplicação em *spreads* de chocolate nas concentrações de 3 e 6% de estruturante. A utilização destes organogéis em substituição ao óleo de palma ocasionou uma redução de AGS variando entre 69,79 a 76,04%.

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FIGURAS

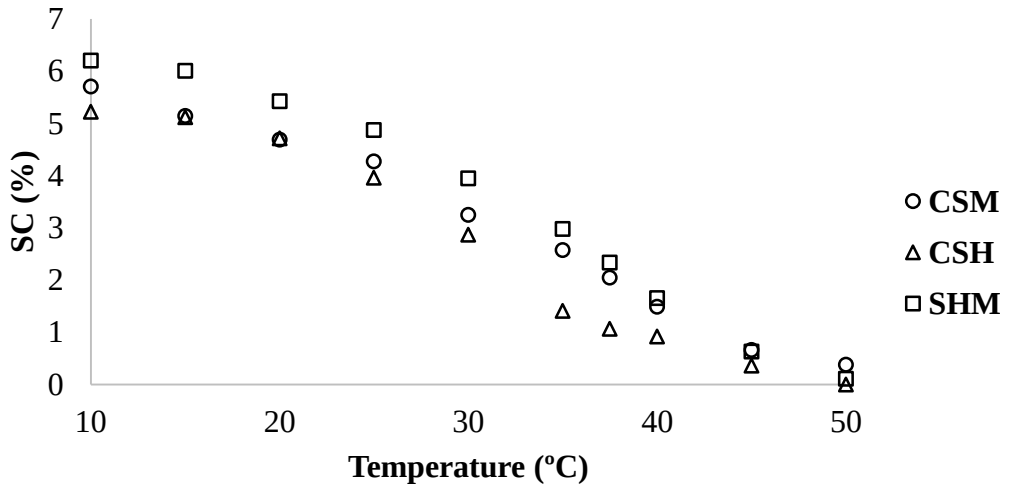

Figura 1. Perfil de sólidos dos organogéis.

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio

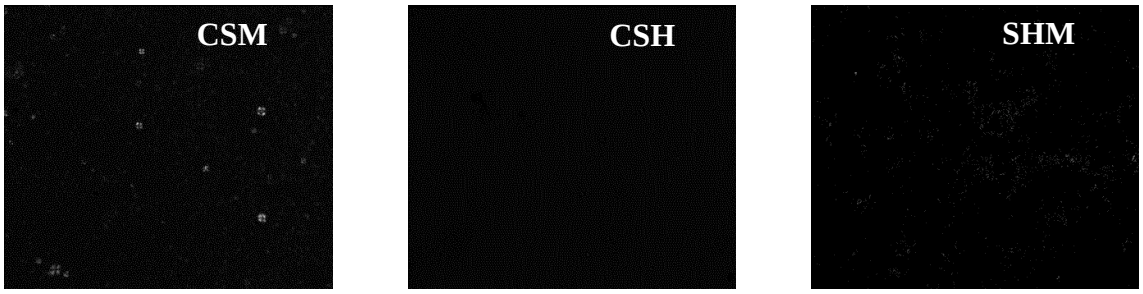

Figura 2. Microestrutura dos organogéis com 20x de ampliação a 25°C.

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio

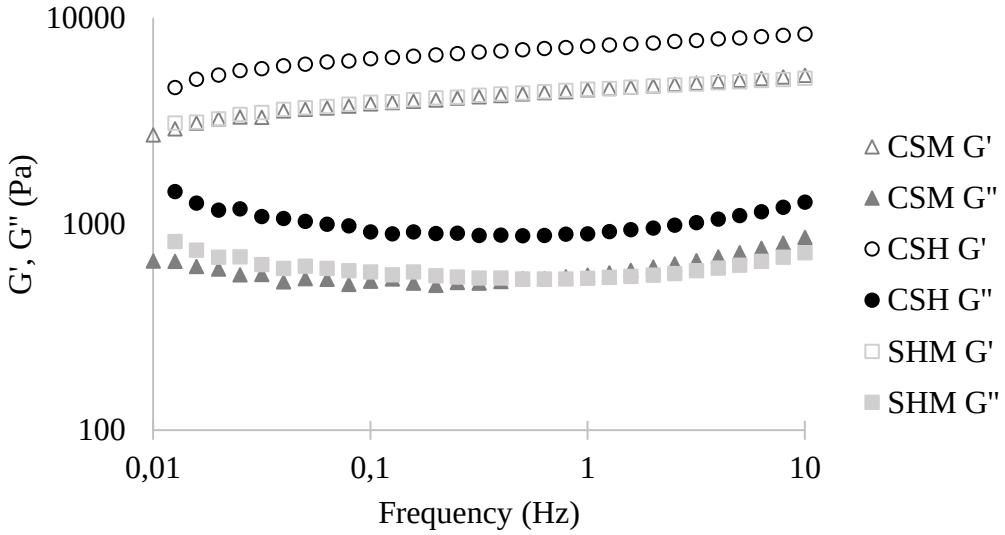

Figura 3. G’ (a) e G’’ (b) da varredura de frequência de organogéis.

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio

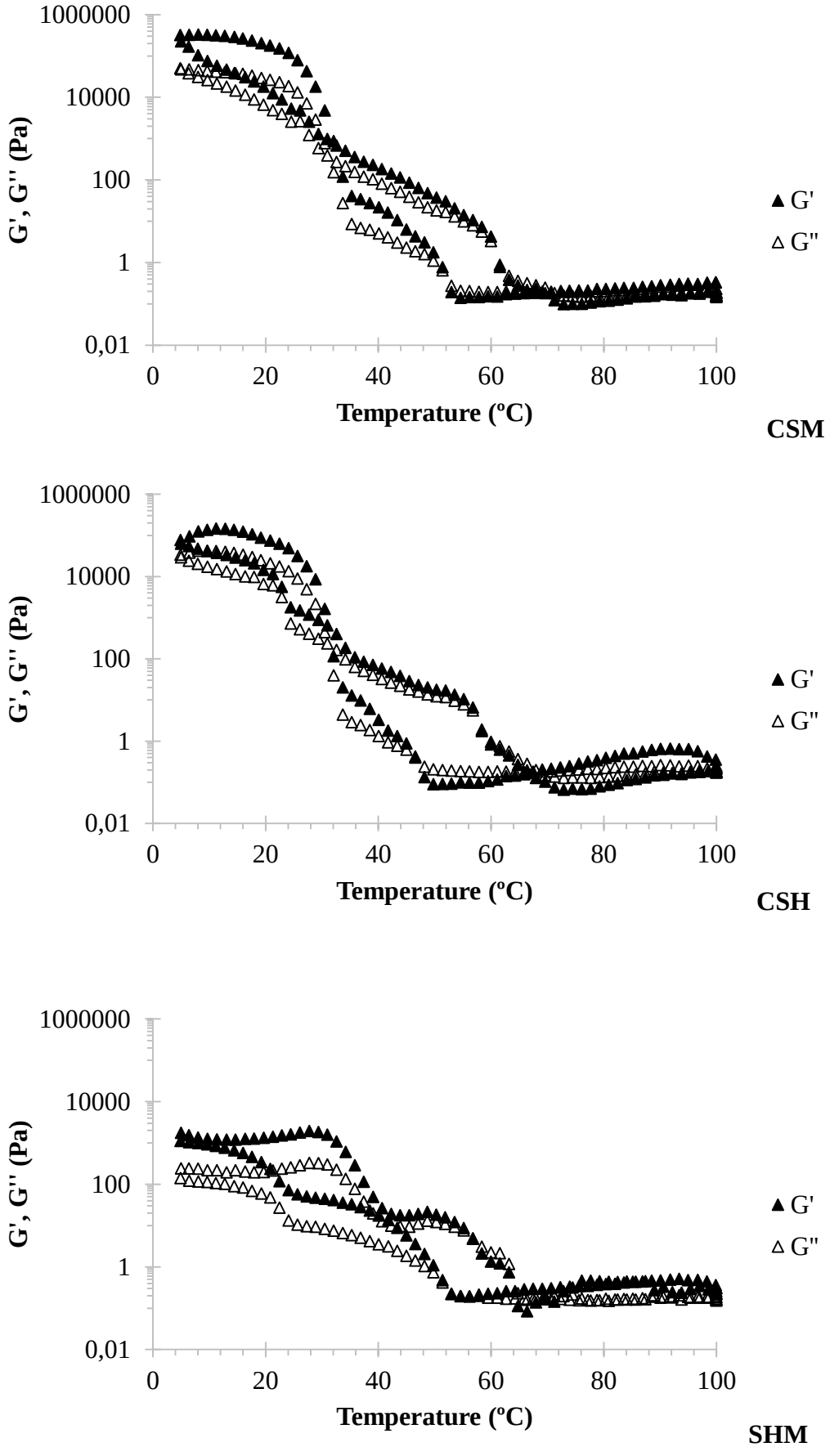

Figura 4. G’ (a) e G’’ (b) da varredura de temperatura de organogéis..

CSM: cera de candelilla, monoestearato de sorbitana, monoglicerídio; CSH: cera de candelilla, monoestearato de sorbitana, *hardfat* de óleo de palma; SHM: monoestearato de sorbitana, *hardfat* de óleo de palma, monoglicerídio

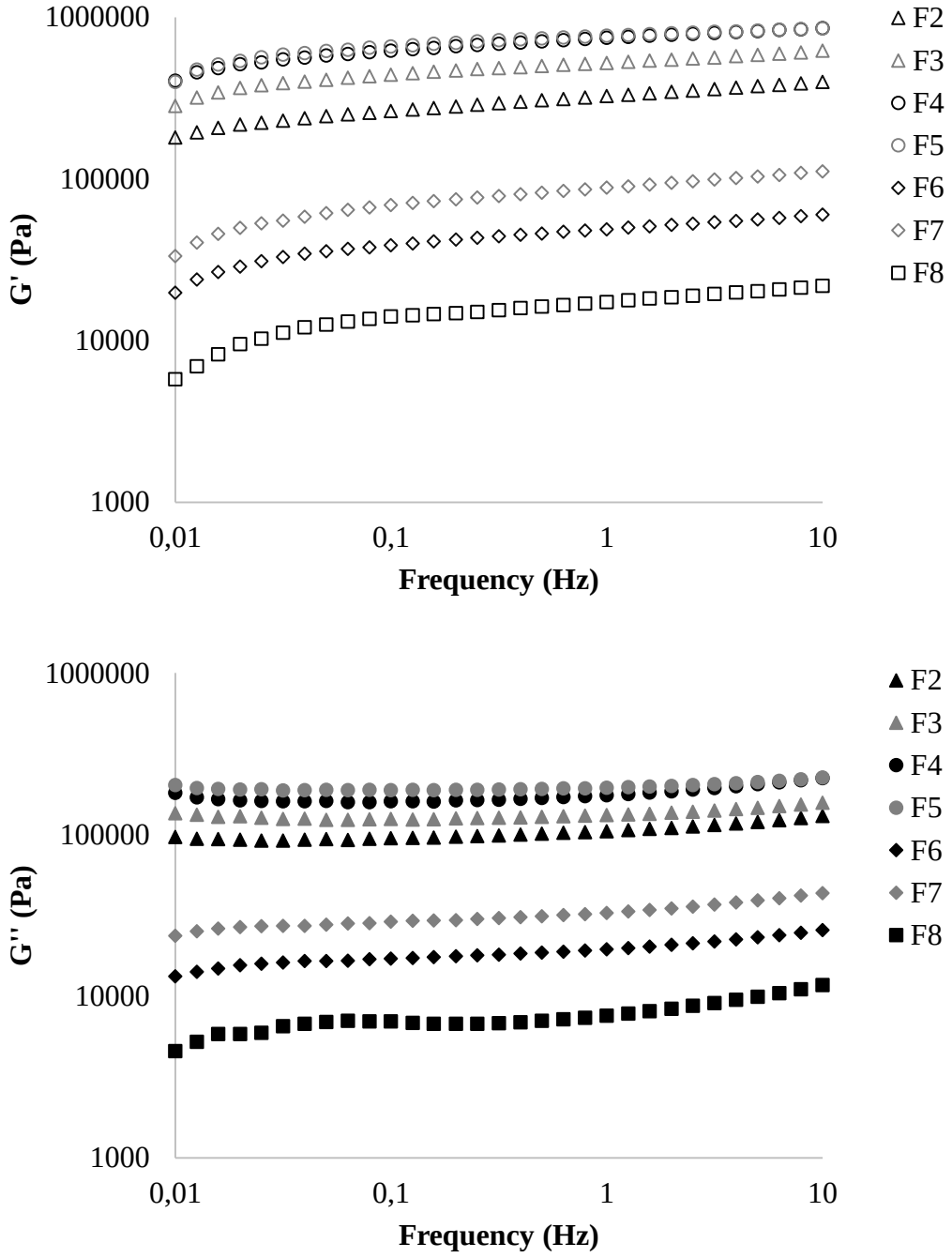

Figura 5. G’ (a) e G’’ (b) da varredura de frequência de *spreads* de chocolate.

F2: 3% (C, M e S); F3: 6% (C, M e S); F4: 3% (C, HP e S); F5: 6% (C, HP e S); F6: 3% (HP, M e S); F7: 6% (HP, M e S); F8: Padrão OGAO

4. DISCUSSÃO

O conteúdo lipídico dos *spreads* comerciais variou de 17,66 a 24,90%. O teor de AGS variou de 19,31 a 35,05%; Os ácidos graxos insaturados variaram de 64,80 a 80,24% e os ácidos graxos trans apresentaram 0,44% como concentração máxima, evidenciando a ausência de gorduras parcialmente hidrogenadas. O tempo de indução (TI) da cristalização das fases lipídicas variou de 16,5 a 71 minutos; e o SCmax variou de 2,17 a 5,35%. A TI das fases lipídicas dos *spreads* foi semelhante aos parâmetros de caracterização isotérmica das amostras de óleo de palma. Curvas de fusão e cristalização mostraram duas regiões diferentes, refletindo as diferentes classes de triacilgliceróis presentes na fase lipídica dos *spreads* de chocolate. A fase lipídica cristalina de todos os *spreads* foi caracterizada pelo polimorfismo β'. Algumas gorduras mostraram cristais esferulíticos típicos de óleo de palma. Outras gorduras não apresentaram cristais individualizados sob as condições de análise (25°C), uma vez que a temperatura de cristalização inicial ocorreu entre 14,99 e 18,30°C. A dureza variou de 1,34 a 3,01 Kg e a capacidade de propagação de 1,10 e 2,78 Kg.s. Todos os *spreads* mostraram tamanho de partícula entre 12,01 a 34,80 μm em d 0,5 e 0,9, respectivamente. Em relação aos parâmetros reológicos, o valor de G' (módulo de elasticidade) e superior a G'' (módulo viscoso) foi observado, indicando propriedades dos materiais sólidos. Assim, os *spreads* de chocolate apresentaram um comportamento elástico predominante, sem exsudação de óleo. Essa caracterização é fundamental para a reformulação dos *spreads* de chocolate com melhor composição nutricional. Os estruturantes cera de candelilla (C), óleos totalmente hidrogenados de soja (HS), crambe (HC) e palma (HP), monoacilgliceróis (M) e monoestearato de sorbitana (S) foram caracterizados quanto às suas características físico-químicas através da composição em ácidos graxos, teor de sólidos, cinética de cristalização, comportamento térmico na fusão e cristalização, polimorfismo e microestrutura. Todos os estruturantes exibiram propriedades similares, como elevada concentração de ácidos graxos saturados, alto teor de sólidos na temperatura de avaliação, baixo TI da cristalização, elevada resistência térmica, hábito preferencial β' e parâmetros similares quanto à morfologia e dimensões cristalinas. Assim, selecionamos os estruturantes C, HP, L, M e S, como potenciais estruturantes, juntamente com o óleo de girassol alto oleico (OGAO). Organogéis foram formulados nas concentrações de 4, 5 e 6% de C, HP, L, M e S como estruturantes isolados e analisados quanto dureza e estabilidade. Organogéis com 6% de estruturantes foram elaborados segundo um

diagrama de possíveis combinações de até cinco componentes e analisados quanto ao conteúdo de sólidos, cinética de cristalização, microestrutura, dureza e estabilidade. Os teores de ácidos graxos saturados dos estruturantes foram elevados, com exceção da lecitina. Nas concentrações avaliadas, os organogéis formulados com C resultaram em maior dureza; os organogéis com estruturantes HP e M promoveram valores intermediários de dureza; e os organogéis contendo L e S mostraram menor dureza. O trabalho abrangeu grande variedade de organogéis, portanto, obtivemos desde organogéis com que apresentaram 100% de estabilidade e sistemas instáveis em todas as concentrações. O M apresentou um efeito acelerador do início da cristalização, quando combinado com os demais estruturantes. A presença da C como estruturante induz a formação de cristais menores. As combinações com maior dureza e estabilidade dureza foram CH, HM, HS, CHS, CMS, HMS e CHMS. Os organogéis (CSM, CSH e SHM) na concentração total de 6% dos estruturantes, foram obtidos organogéis de OGAO com variação dos teores de cada agente estruturante, através de um planejamento centróide simples. Os organogéis apresentaram fusão completa a 65ºC. Os parâmetros de comportamento térmico dos organogéis indicaram um primeiro pico de fusão, relativo aos triacilgliceróis mais insaturados do OGAO, e um segundo pico de fusão, relativo aos estruturantes utilizados. Todos os estruturantes apresentaram co-cristalização entre si e com OGAO, com exceção do S. Todos os organogéis avaliados foram verificados short spacings (SS) iguais a 4.6 Å, caracterizando o hábito polimórfico β. Todos os organogéis mostraram propriedades reológicas similares e independentes da frequência e da temperatura, sendo observado valor do G’ superior ao G’’, indicando comportamento de material sólido. Os resultados obtidos nas análises de varredura de temperatura dos organogéis mostraram comportamento correspondente às propriedades térmicas. Os organogéis analisados neste estudo mostraram-se estáveis, com exceção do organogel com 6% de S, esse resultado pode ser associado às demais propriedades de dureza, estabilidade térmica e mecânica e polimorfismo dos organogéis. Os resultados mais positivos com relação à estabilidade térmica e mecânica foram obtidos quando os estruturantes (CSM, CSH e SHM) foram utilizados na concentração de 2% cada. Portanto, utilizou-se estes 3 organogéis para produzir as formulações de *spreads* de chocolate com 3 e 6% de estruturantes. Todos os *spreads* de chocolate com organogel mostraram tamanho de partículas inferiores a 14,59 e 46,23 µm em d 0,5 e d 0,9, respectivamente; desta forma os *spreads* de

chocolate com organogel avaliados provavelmente apresentam baixa percepção sensorial para arenosidade. Os *spreads* de chocolate com organogel mostraram consistência variando entre 13,83 e 16,90 gF, esta característica é favorável com relação à fluidez que o produto requer. Os *spreads* de chocolate mostraram comportamento similar, sendo observado valor do G' superior ao G'', indicando comportamento de material sólido, dessa forma, os *spreads* de chocolate com organogel apresentaram comportamento elástico predominante, na análise varredura de frequência. Os *spreads* de chocolate com organogel mostraram baixa variação no ΔBS, indicando que o uso de organogéis como substituto de bases lipídicas convencionais em *spreads* de chocolate foi eficiente, uma vez que apresentaram comportamento similar ao padrão produzido com óleo de palma, além disso a concentração de 3 ou 6% de estruturantes foi indiferente com relação a estabilidade, dessa forma é possível produzir um produto de qualidade utilizando baixa concentração de estruturante e reduzindo o teor de AGS.

5. CONCLUSÃO GERAL

Os resultados obtidos nesse estudo são importantes para o desenvolvimento da tecnologia de organogéis na indústria de alimentos e áreas afins. Com base nas pesquisas realizadas, verificou-se que diferentes estruturantes, utilizados em baixas concentrações, são comprovadamente eficazes nos processos de modificação lipídica e representam uma opção altamente viável, em termos econômicos e de processo, para modular as propriedades de cristalização de óleos e gorduras industriais. Além disso, o uso de organogéis em aplicações alimentícias como substitutos de ácidos graxos *trans* e saturados é altamente viável. Várias matérias-primas lipídicas podem ser utilizadas, a fase imobilizada (óleo vegetal) pode variar de acordo com a região geográfica, disponibilidade e custo. Agentes estruturantes são usados em pequenas proporções, estão comercialmente disponíveis, seguros para consumo e acessíveis.

Os *spreads* de chocolate comerciais analisados neste estudo mostraram estabilidade, uma vez que não houve exsudação de óleo líquido durante o período de estabilização. A dureza e a espalhabilidade foram adequadas para produtos espalháveis; no entanto, eles poderiam apresentar menores níveis de AGS para atender a demanda de consumidores que buscam alimentos mais saudáveis.

Todos os estruturantes (C, HP, HC, HS, M, e S) apresentaram propriedades semelhantes, como alta concentração de ácidos graxos saturados, alto teor de sólidos na temperatura analisada, baixo tempo de indução de cristalização, alta resistência térmica, bem como parâmetros uniformes quanto à morfologia e dimensões cristalinas.

A formação de organogéis a base de óleo de girassol alto oleico estruturados com C, HP, M, S e L foi efetiva para os parâmetros avaliados nos estruturantes isolados, misturas binárias, ternárias e até os quatros estruturantes juntos, principalmente em concentrações elevadas. Porém, a presença da L, isolada ou com qualquer outro estruturante, independente da concentração utilizada não apresentou formação eficiente da estrutura de organogel.

O estruturante S apresenta resultados negativos isoladamente, porém quando analisado juntamente com outros estruturantes mostra resultados satisfatórios, capazes de serem usados como organogéis para aplicação em produtos de base lipídica. A dureza dos organogéis foi adequada à produtos espalháveis, com exceção do organogel composto por 6% e C, que é adequado para produtos mais firmes, como chocolate. Essas características tornam os organogéis analisados bases lipídicas com

potencial para serem usados como substitutos de gordura em processos industriais, para atender uma demanda de consumidores que buscam alimentos mais saudáveis.

Os *spreads* de chocolate com organogéis analisados neste estudo mostraram estabilidade, assim como os *spreads* de chocolate comerciais, sendo viável o desenvolvimento de um produto que atenda a demanda de consumidores que buscam por alimentos mais saudáveis, utilizando organogéis com baixa concentração de estruturantes. Todas os organogéis (CMS, CHS e HMS) podem ser utilizados para aplicação em *spreads* de chocolate nas concentrações de 3 e 6% de estruturante. A utilização destes organogéis em substituição ao óleo de palma ocasionou uma redução de AGS variando entre 69,79 a 76,04%.

6. SUGESTÕES PARA TRABALHOS FUTUROS

Os resultados obtidos nesta tese ampliam as informações disponíveis sobre o uso de organogéis como bases lipídicas para aplicação em alimentos, especialmente em *spreads* de chocolate. Para aprofundamento científico desta linha de pesquisa, sugere-se para trabalhos futuros avaliar outros estruturantes com diferentes solventes orgânicos, além da aplicabilidade em outros tipos de alimentos.

Em termos de aplicabilidade tecnológica, outras fatores podem ser utilizados e analisados, como a utilização de alta pressão hidrostática e ultrassom para a estruturação dos organogéis e também o custo para implementação e manutenção destes processamentos.

Por fim, sugere-se a continuidade do trabalho com a produção de diversos tipos de produtos com bases lipídicas (chocolate, sorvete, chantilly, margarina, biscoitos, bolos) utilizando diferentes estruturantes, solventes e tecnologias associadas ao uso de organogéis, visando o desenvolvimento de alimentos mais saudáveis para atender a demanda dos consumidores.

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