



UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia Química

CAMILA CORDEIRO MANDU

EFEITO DA ADIÇÃO DE EMULSIFICANTES INDUSTRIAIS DE GRAU ALIMENTAR
NA ESTRUTURAÇÃO DE ORGANOGÉIS DE CERA DE CANDELILLA EM ÓLEO
DE SOJA

CAMPINAS

2018

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DE SOJA

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DEDICATÓRIA

*Dedico esse trabalho em honra e memória
a meu querido, amado pai e eterno amigo.
Pássaro azul, por favor me guie enquanto
eu viver, e me encontre quando eu partir.*

RESUMO

Lipídios são ingredientes fundamentais na dieta humana. Porém devido a necessidade de adequação tecnológica via modificação desses lipídios na indústria de alimentos, a quantidade de gordura *trans* e saturada tem aumentado substancialmente em produtos industrializados nas últimas décadas. Ao mesmo tempo, vem aumentando o número de estudos que comprovam os efeitos maléficos desses alimentos na saúde humana. Neste contexto, os organogéis surgem como uma alternativa promissora na substituição de gordura *trans* e saturada em alimentos processados. Dentre os principais desafios de se preparar um organogel, está a dificuldade de encontrar estruturantes compatíveis e viáveis para a indústria de alimentos. As ceras vêm sendo estudadas para este fim e se encontram entre os estruturantes mais promissores. A cera de candelilla é muito conhecida devido ao seu poder de estruturação de óleos vegetais. Entretanto, algumas dificuldades como custo e disponibilidade podem limitar sua aplicação nível industrial. Além disso, a literatura aponta diversos estruturantes com potencial de atuação sinérgica quando utilizados em combinação. Devido a isso, o principal objetivo desse estudo foi avaliar o efeito da substituição total e parcial da cera de candelilla como estruturante para óleo de soja por emulsificantes industriais compostos basicamente de mono, di e triacilgliceróis. Dependendo do tipo e da combinação de estruturantes empregados, os mesmos podem ter efeito sinérgico ou antagônico, podendo prejudicar ou estimular o efeito do outro. O conteúdo de sólidos convencionalmente aplicado pela indústria de óleos e gorduras não tem relação direta com a dureza e consistência do organogel, e por mais que um gel possua alto teor de sólidos não necessariamente seja o mais estruturado e resistente. Por outro lado, a forma e organização das partículas sólidas e cristais formados no organogel estão entre os fatores mais determinantes em relação à dureza e consistência do organogel, e por isso devem ser cautelosamente estudados e avaliados antes da aplicação em cada formulação.

Palavras chave: organogel, estruturante, cera de candelilla, emulsificantes.

ABSTRACT

Lipids are key ingredients in the human diet. However due to the requirements for technological adaptation through modification of these lipids in the food industry, the amount of trans and saturated fatty acids have increased substantially in industrialized products in the last decades. At the same time, the number of studies showing the harmful effects of these foods on human health has increased. In this context, organogels appear to be a promising alternative in the substitution of trans and saturated fat in processed foods. Among the main challenges of preparing an organogel is the difficulty of finding compatible and viable structures in the food industry. Waxes have been studied for this purpose and are among the most promising structuring agents. Candelilla wax is well known due to its power of structuring vegetable oils. However, some difficulties such as cost and availability may limit its industrial application. In addition, the literature points out several structuring agents with potential for synergistic action. In this context, the main objective of this study was to evaluate the effect of the total and partial substitution of candelilla wax as structuring agent for soybean oil by industrial emulsifiers basically composed of mono, di and triacylglycerols. Depending on the type and the combination of structurers employed, they may have a synergistic or antagonistic effect, one of which may impair or stimulate the effect of the other. The solid fat content, conventionally applied by the oils and fats industry is not directly related to the hardness and consistency of the organogel, and even a gel presents a high solid fat content it is not necessarily the most structured and resistant one. On the other hand, the shape and organization of the solid particles and crystals formed in the organogel are among the most determinant factors with respect to the hardness and consistency of the organogel, and therefore should be carefully studied and evaluated before application in each formulation.

Key words: organogel, structuring agent, candelilla wax, emulsifiers.

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CAPÍTULO 1 – APRESENTAÇÃO

Esta dissertação de mestrado está estruturada nos seguintes capítulos:

O capítulo 1 contempla a introdução geral com a contextualização do assunto, contribuições deste trabalho e objetivo.

O capítulo 2 apresenta um artigo de revisão bibliográfica que aborda os principais mecanismos de estruturação e estruturantes estudados na literatura, as principais ceras utilizadas como estruturantes para organogéis, seus mecanismos de estruturação.

O capítulo 3 apresenta em forma de artigo o material experimental desenvolvido ao longo do mestrado, apresentando as metodologias utilizadas, os resultados, discussões e conclusões encontradas quanto ao efeito da adição dos emulsificantes industriais nos organogéis de óleo de soja e cera de candelilla.

O capítulo 4 apresenta as conclusões gerais desenvolvidas ao longo da dissertação.

O capítulo 5 contempla o histórico escolar de graduação como requisito obrigatório.

O capítulo 6 apresenta as referências bibliográficas.

1.1 – Introdução

Os lipídios são componentes importantes da dieta, não somente em aspectos nutricionais, mas também tecnológicos. Os lipídios afetam a estrutura, estabilidade, qualidade, as características sensoriais e a vida de prateleira dos alimentos. Na indústria de alimentos os lipídios podem ser empregados como um meio de transferência de calor, em processos de fritura, por exemplo, ou até mesmo facilitando a difusão de sabor e aroma, proporcionando consistência e características específicas de fusão aos produtos que os contenham. . Para tecnologia de alimentos, os lipídios são considerados como óleos (líquidos à temperatura ambiente) ou gorduras (sólidas à temperatura ambiente), cuja principal diferença é o ponto de fusão. Esta propriedade física é um reflexo da composição em ácidos graxos e conseqüentemente da composição em triacilgliceróis de cada fonte lipídica (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018). Ácidos graxos saturados apresentam ponto de fusão superior em relação ao ácido graxo correspondente, de mesmo tamanho de cadeia, com uma ou mais duplas ligações. Ácidos graxos insaturados podem existir nas configurações *cis* e *trans*, com diferentes propriedades físico-químicas. Por suas características estruturais, os ácidos graxos na forma *trans* têm ponto de fusão mais elevado quando comparado com seu isômero *cis* correspondente, mas, inferior ao ponto de fusão do ácido graxo saturado com mesmo número de átomos de carbono (MOURA, 2007). As propriedades sensoriais de alimentos à base de gordura, principalmente as de textura, são determinadas principalmente pela rede tridimensional formada através da cristalização dos triacilgliceróis compostos de ácidos graxos *trans* (AGT) e saturados (TORO-VAZQUEZ et al., 2013).

No passado, a formação de isômeros *trans* de ácidos graxos foi considerada uma vantagem tecnológica, uma vez que, devido ao seu ponto de fusão intermediário em relação aos correspondentes isômeros *cis* e saturados, eles conferem uma plasticidade adequada às gorduras comerciais para diversas aplicações. Estes ácidos graxos favorecem o estabelecimento do teor de sólidos desejáveis das gorduras parcialmente hidrogenadas e conseqüentemente melhor texturização e características sensoriais. Historicamente, o consumo mundial de

AGT tem aumentado desde a década de 20, em paralelo com o aumento da produção comercial de margarinas e “*shortenings*”, constituindo, ainda hoje, uma proporção significativa da dieta nos países ocidentais (MOURA, 2007).

Ao longo dos últimos anos, estudos demonstraram que dietas com consumo elevado desse tipo de gordura têm consequências negativas para a saúde, contribuindo para diversas doenças em seres humanos, mas principalmente na ocorrência de doenças cardiovasculares que reduzem significativamente a qualidade de vida dos indivíduos portadores e podem levar a morte (HUNTER; ZHANG; KRIS-ETHERTON, 2009). As consequências adversas à saúde, associadas ao consumo dessas gorduras, levaram as agências reguladoras internacionais a promulgar leis proibindo o uso industrial de gorduras parcialmente hidrogenadas com altos teores de AGT, estipulando, inclusive, um tempo finito para remover ou limitar esses ingredientes em alimentos processados (SINGH; AUZANNEAU; ROGERS, 2017; FDA-2013-N-1317).

Entretanto para que a indústria conseguisse manter seu padrão de textura e palatabilidade, a quantidade de ácidos graxos saturados (AGS) vem sendo aumentada consideravelmente, o que também tem se mostrado negativo para a saúde humana, tendo sua redução recomendada pelas diretrizes de alimentação de diversos países, inclusive o Brasil (WANG; ROGERS, 2015, SAÚDE, 2014). A secretaria de saúde e serviços humanos e a secretaria de agricultura dos Estados Unidos (HHS and USDA), publicaram em 2016 um guia conhecido como Diretrizes Dietéticas para Americanos (*Dietary Guidelines for Americans*) que fornece ferramentas importantes para que a população tenha condições de fazer escolhas mais saudáveis aproveitando a diversidade de produtos disponíveis. Dentre as recomendações está a sugestão de optar por alimentos com redução de gordura saturada e *trans* ou redução de gorduras totais (ESTADOS UNIDOS, 2016). Estas medidas têm exigido de pesquisadores e indústrias uma busca por alternativas a esse tipo de gordura para uso em alimentos processados. A Associação Brasileira das Indústrias da Alimentação (Abia) em parceria com o governo federal publicou um estudo que mostra considerável redução de gordura *trans* nos alimentos circulados no país no ano de 2009. As metas foram estabelecidas em 2007 e previam redução para até 5% de gordura *trans* no total de gorduras em alimentos

processados, e 2% do total de gorduras em óleos e margarinas. Nas categorias de margarinas, cremes vegetais, bolos e biscoitos, no entanto, o ministério afirmou que os resultados foram "menos expressivos". A associação calculou que, com a redução verificada, cerca de 230 mil toneladas de gordura *trans* deixaram de ir para os alimentos no ano de 2009, em comparação com 2008 (TITO, 2010).

Segundo a sociedade brasileira de cardiologia, a ingestão recomendada de ácidos graxos saturados para adultos sem comorbidades deve ser < 10% do valor energético total (VET). Para indivíduos adultos que apresentam fatores de risco associados a doença cardiovascular como: hipertensão arterial sistêmica, diabetes, sobrepeso ou obesidade, circunferência da cintura aumentada, hipercolesterolemia, hipertrigliceridemia, síndrome metabólica, intolerância à glicose ou aterosclerose significativa o consumo deve ser < 7% do valor energético total (VET). Para crianças maiores de dois anos e adolescentes com perfil lipídico alterado, a ingestão de gorduras deve se manter entre 25% e 35% para manutenção de ganho de peso e para crescimento normal. A recomendação de ácidos graxos saturados deve, nesses casos, ser 7% do VET. O consumo de ácidos graxos saturados para gestantes com dislipidemia prévia ou desenvolvida durante a gestação deve ser limitado a < 7%. A substituição de ácidos graxos saturados por ácidos graxos monoinsaturados e poli-insaturados é recomendada por ocasionar melhora no perfil lipídico (I DIRETRIZ SOBRE O CONSUMO DE GORDURAS E SAÚDE CARDIOVASCULAR, 2013)

O potencial dos organogéis como uma alternativa de estruturação de óleos foi identificada e investigada intensamente com diferentes sistemas de óleo comestíveis nos últimos anos. Os organogéis são um grupo de materiais que possuem características de gel, porém a fase imobilizada é um composto orgânico, o que o difere de outros géis formados basicamente por compostos hidrossolúveis. Quando a fase imobilizada se trata de um óleo vegetal, podemos adotar o termo oleogel para designar o sistema. Os organogéis são mais adequados nutricionalmente do que as opções tecnológicas usadas atualmente, pois não causam nenhuma mudança química na estrutura do triacilglicerol e mantém as características nutricionais do óleo inalteradas. Em especial ocorre a manutenção dos teores de ácidos graxos insaturados e das suas posições no glicerol,

deixando-os na sua forma original e sem incremento no teor de ácidos graxos saturados e/ou *trans* (ROCHA, 2012).

Os estudos recentes têm demonstrado que os organogéis podem ter seu desempenho tecnológico comparado à uma gordura sólida, viabilizando seu uso em algumas aplicações, conforme serão apresentadas no artigo de revisão. Um organogel é geralmente preparado pelo aquecimento e solubilização de um ou mais estruturantes sólidos em óleo, até seu ponto de fusão. Após completa homogeneização a solução é resfriada até abaixo da temperatura de gelificação. O material resultante é um gel ou geleia dependendo da sua capacidade de estruturação ou espessamento (GUEDES, 2012). A capacidade dos agentes estruturantes em estruturar organogéis em baixas concentrações é extremamente importante para a indústria de alimentos, uma vez que algumas substâncias para este fim possuem legislação de controle de utilização em alimentos, e além disso podem produzir um sabor residual indesejável (Hughes et al., 2009).

A cera candelilla é um aditivo alimentar bem conhecido, destaca-se pelo seu reconhecimento como aditivo seguro para uso em alimentos pelo órgão americano FDA (Food and Drug Administration) - Generally Recognized as Safe (GRAS) – FDA e usado sem outras limitações além das já conhecidas boas práticas de fabricação (BPF). A cera candelilla é usado principalmente como agente de texturização para a base da goma de mastigação, agente de revestimento de superfície, portador para aditivos alimentares (incluindo sabores e cores) e agente de turvação. Segundo estudos recentes da literatura ela é capaz de se estruturar em uma rede tridimensional com alta capacidade de se aprisionar o óleo e por isso podem formar géis em concentrações relativamente baixas (TAVERNIER et al., 2017). As ceras formam naturalmente através de seus componentes, cristais com morfologia de hastes ou plaquetas, resultando em géis de estrutura mais forte. Durante o resfriamento, os *clusters* formados pelos agregados cristalinos aprisionam o óleo líquido em uma estrutura tridimensional autossustentada com características macroscópicas semelhantes a um sólido. As interações inter e intramoleculares, dipolo-dipolo, ligação de hidrogênio e interação polar-polar entre os constituintes de cera estabilizam a estrutura da rede cristalina, resultando na formação de um gel forte (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018).

Os géis monocomponentes utilizam um único estruturante para estruturar o óleo líquido, e géis mistos requerem interações sinérgicas entre múltiplos estruturantes. Em sistemas mistos a estruturação é influenciada pela nucleação heterogênea, aumento da nucleação e fortalecimento da rede cristalina através de seus estruturantes. Além disso, para melhora de características sensoriais, sistemas mistos possuem vantagens quando por exemplo, um de seus estruturantes apresenta característica sensorial acentuada, como é o caso das ceras. Nesse contexto, o desenvolvimento de sistemas mistos tem ganhado interesse de muitos pesquisadores (Patel & Dewettinck, 2016).

Mono e diacilgliceróis, utilizados como aditivos comerciais em alimentos, são moléculas que possuem um ou dois ácidos graxos esterificados em uma molécula de glicerol, respectivamente. Sua composição em ácidos graxos é bastante variada, é possível encontrar desde os ricos em ácidos graxos saturados, até os ricos em ácidos graxos insaturados. Em concentração suficiente os organogéis estruturadas com esse grupo de aditivos possuem firmeza relativamente alta, entretanto, sua dosagem e combinação deve ser muito cautelosa uma vez que eles podem influenciar na eficiência de cristalização um do outro e até atrapalhar a estruturação (PERNETTI et al., 2007).

Já é constatado na literatura o grande potencial da cera de candelilla e de compostos como mono, di e triacilgliceróis como estruturantes para organogel, entretanto a literatura ainda é deficiente quanto aos efeitos da combinação dessas duas classes de estruturantes.

1.2 – Objetivo

Esse estudo tem como objetivo estudar a possível sinergia entre cera de candelilla e emulsificantes industriais de grau alimentício composto basicamente de mono, di e triacilgliceróis como estruturantes para organogéis de óleo de soja.

CAPÍTULO 2 – REVISÃO BIBLIOGRÁFICA

WAXES USED AS STRUCTURING AGENTS FOR FOOD ORGANOGELS.

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ABSTRACT

Lipids are key ingredients in the human diet. Because of the manipulation and modification of these lipids in the food industry, the amount of *trans* and saturated fat has been substantially increasing in industrialized products. At the same time, the number of studies demonstrating the harmful effects of these foods on human health has increased. Organogels arise as a promising alternative in replacing *trans* and saturated fat in processed foods. Among the main challenges of preparing an organogel is the difficulty of finding compatible and viable structuring agents in the food industry. Waxes have been studied for this purpose and are one of the most promising organogelators. This article brings a bibliographical review on the recent studies regarding the use of waxes as structuring agents for edible vegetable oils.

Key words: Organogel, organogelator, Wax

1. INTRODUCTION

Currently a series of problems are being associated with the use of traditional fats in modern human food, mainly related to the presence of high content of saturated and *trans* fatty acids (YđLMAZ; ÖđÜTCÜ, 2015). Over the last few years, studies have shown that diets with high intakes of this type of fat have negative consequences for health, contributing to several diseases in humans but mainly increasing the risk of developing cardiovascular diseases and diabetes, which significantly reduce quality of life and can lead to death (SOUZA et al., 2015, HUNTER, ZHANG and KRIS-ETHERTON, 2009). Public organizations around the world are implementing regulations to restrict and inhibit the use of this type of fat in the food industry. Trans fat has already been banned in some countries or has restricted its use in others (Executive Order No. 160, 2003; OVIEDO, 2010; Medical Officer of Health); FDA Federal Register - 68 FR 41433 July 11, 2003; New York City Health Code, 2006; Resolutions RDC No. 359 - Technical Regulation of Portions of Packaged Foods for the Purposes of Nutrition Labeling and RDC No. 360 - Technical Regulation on Labeling Nutrition of Packaged Food, incorporating the norms approved in the Mercosul to the national legal order - Anvisa, 2009).

However, in order for the industry to maintain its pattern of texture and palatability, the amount of saturates has been increased considerably which has also been shown to be incompatible with human health. Saturated Fatty Acids are also having their recommended reduction in the dietary guidelines of several countries (WANG; ROGERS, 2015; HHS ad USDA - UNITED STATES, 2016). These measures have required researchers and industries to search for alternatives to this type of fat applied in processed foods. In this context, in recent years one of the research themes in evidence are edible organogels as substitutes for saturated and trans-fat in foods. Organogels are semi-solid systems, predominantly high in unsaturated fatty acids, structured by a three-dimensional network formed by a structuring agent. An oil by itself does not have sufficient structure to confer texture and palatability to foodstuffs in a similar way to the fats with high levels of trans and saturated fatty acids, which does not allow their direct application in several processed foods. Recently studies have shown that organogels can present a

technological performance very similar to a solid fat, making it possible to use them in some food applications (HWANG; SINGH; LEE, 2016; KOUZOUNIS; LAZARIDOU; KATSANIDIS, 2017; CHAVES, BARRERA-ARELLANO; RIBEIRO, 2018).

In food, the main challenge is to identify the best oil structuring agent that are food grade, availability, affordable, and especially that they are able to structure liquid lipids at relatively low concentrations and produce organogels with fat-like physical characteristics for food purposes. This challenge versus the real necessity to reduce saturated fats in the food industry has exponentially increased research in the prospection of new structuring agents for this purpose (SINGH; AUZANNEAU; ROGERS, 2017). The waxes appeared among the several structuring agents studied and appear to be among the most promising ones (TAVERNIER et al., 2017).

Waxes are found on the surface of leaves, seeds and fruits. They their main function is the protection of the plant in relation to the loss and absorption of water, gases and biological volatile compounds. They have a very particular lipid composition, commonly known by their long fatty acids esterified with long chains alcohols. They are generally classified according to their origin, animal, vegetable and mineral. Among the benefits of using natural wax as a structuring agent for oils is its recognition as a safe additive for use in food by the FDA (Food and Drug Administration) - Generally Recognized as Safe (GRAS) – FDA. They are able to structure in a three-dimensional network with high capacity to bind with oil and can form gels at relatively low concentrations (TAVERNIER et al., 2017). The objective of this study is to collect as a bibliographical review a selection of articles from the last years referring to the types and diversity of waxes used as structuring agents for application in food grade organogels.

1.1 - Fundamentals and mechanisms for the establishment of organogels

Regarding to the concept of gels, based on material science, a more sensible definition should be limited to systems that attend the following phenomenological characteristics: (1) consisting of two or more components, of which the major component is liquid; (2) the minor components must be solid or semi-solid. Other

very important features that contribute to the concept of gels are presented through dynamic mechanical properties. Rheologically, the energy storage module, elastic modulus G' exhibits a steep plateau extending to times of the order of seconds and a modulus of loss, viscous modulus G'' , which is considerably smaller than the storage modulus. The most important is the solid gel character, and the deformations will be elastic or recoverable (TERECH, WEISS, 1997) (ALMDAL et al., 1993). Pseudoplastic flow is most common for food gel formulations. These compounds have their apparent viscosity decreased gradually as the shear stress increases. Since viscosity is an expression of fluid resistance to flow, the higher the viscosity, the higher the resistance. The pseudoplastic solutions have behavior resulting from a structure in fully organized network (GUEDES, 2012).

An organogel is generally prepared by heating an oil and one or more solid structuring agent until the melting point of all the components is overcome and complete homogenization occurs. The solution is then cooled until the gelling temperature is reached. The resulting material is a gel or jelly depending on its hardenability or thickening, but the formal description and classification are based on the rheological properties as previously mentioned (GUEDES, 2012).

Different approaches have been studied as potential for the structuring of lipid-based organogels in order to reduce the amount of saturated and trans-fat. Structures may vary widely, but in relation to structural organization they fall into the categories of crystalline particles, polymer chains, particulate networks, and liquid crystal (SINTANG et al., 2017). Because of this, recent studies describe the use of various components for structuring oils. These structuring agents would be capable of acting on lipid systems modulating characteristics such as thermal behavior, polymorphic stability and microstructure. These effects would consequently be observed at a macroscopic level, such as appearance, rheology and consistency (CHAVES, BARRERA-ARLENANO, RIBEIRO, 2018). The structuring agents commonly used include fatty acids, fatty alcohols, mixtures of fatty acids and fatty alcohols, mixtures of phytosterols / orizanol, sorbitan monostearate, mixtures of lecithin, sorbitan tristearate, celluloses, methylcelluloses and waxes (ROGERS; WRIGHT; MARANGONI, 2009). In addition, the blend of some ingredients may result in a synergistic effect on the structuring potential of the oils when compared to the use of pure materials (CHAVES, BARRERA-ARELLANO; RIBEIRO, 2018). In

conventional lipid structuring when a complex mixture of triacylglycerol is subjected to cooling, the molecules with the high melting point start the nucleation event interacting with each other through non-covalent forces. After the nucleation, these formed nuclei aggregate and form agglomerates that transform into large structures finally reaching the macroscopic three-dimensional crystalline network. This structure of plastic fats characterizes the conventional crystallization process based on triacylglycerols (Figure 1-A). The unconventional structuring process, explored in the organogels, deals with lipid systems mostly high in unsaturated fatty acids that are structured in gels through continuous networks of small molecules (structuring agents) that interact in different ways imprisoning the liquid fraction (Figure 1 -B) (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018).

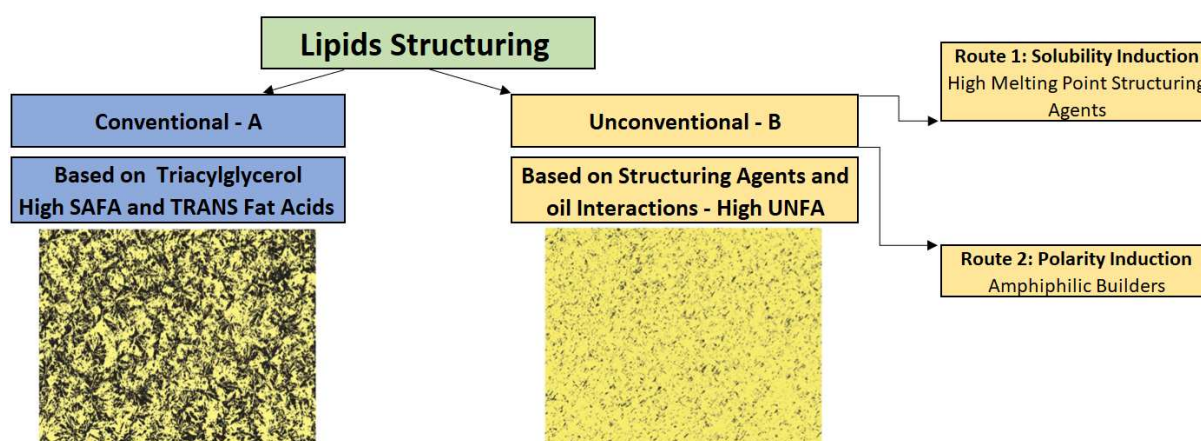


Figure 1 - Lipid microstructure through the conventional process A, and unconventional B, adapted from (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018).

Generally, two routes can be found for unconventional lipid structuring, solubility induction and polarity induction. The definition of these routes depends basically on the structuring agent, the oil and the process applied. The solubility induced pathway occurs mainly for high melting point structuring agents. With the reduction of temperature occurs a supersaturation of the system forming small crystals that are organized in a self-structured network and imprison the oil in gel format. The polarity-induced pathway occurs for amphiphilic builders as in the case of phospholipids, where the fractions of similar polarity, for example the polar heads of

the phospholipids, are associated forming a self-structuring network of structuring agents that imprison the oil in gel form. These routes promote the formation of so-called structuring elements that have the action of building blocks in structuring the three-dimensional network of the organogel. The size, shape of these structuring blocks and the interaction between them will determine the final macrostructure of the product and therefore its application properties (PERNETTI et al., 2007).

According to Marangoni, 2011 the structuring of gels can occur in 5 different ways depending on the structuring agent employed.

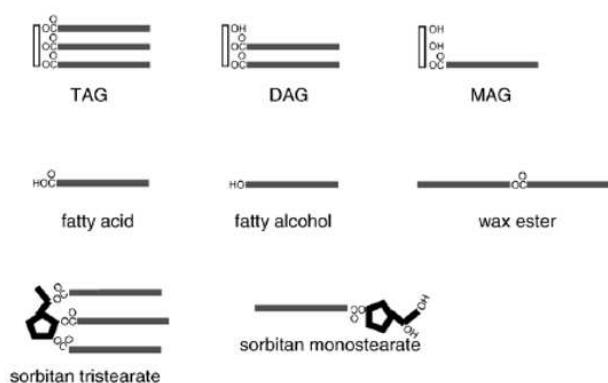
- a) **Crystalline particles:** are systems formed through the route of solubility. Crystalline particles formed are able to "trap" the oil within its crystals causing gelation. The size, shape, and interaction of these crystals will determine the mechanical properties of the organogel. This type of structuring can be activated with diacylglycerols, monoacylglycerols, fatty acids, waxes, sorbitans being widely used in foods (Marangoni, 2011).
- b) **Crystalline fibers:** these are self-assembled networks through crystalline fibers hundreds of microns in length. Structuring agents that can cause this type of structuring are those known as low molecular weight builders such as phytosterols with oryzanol, 12-hydroxystearic acid and ricinoleic acid. They generally use the polarity route for their structuring in oils (Marangoni, 2011).
- c) **Polymer chains:** Through the polarity route, the polymers promote the gelation of a medium. They may be gels formed by covalent bonding or gels formed by self-assembly. In the literature the great majority of studies using this type of structuring are for aqueous systems and still little used for oils (Marangoni, 2011).
- d) **Particulate Chain:** formed by the solubility route are lipid-based products structured by a dispersion of a large number of inert particles, such as silica, in a continuous phase. They can be solid (suspension) or liquid (emulsion). The structuring agent must be present in sufficiently high concentration to promote gelation. The

disadvantage is that it requires a high amount of structuring agents which is still not favorable for food application (Marangoni, 2011).

- e) Liquid cristas:** occurs with the formation of organogel through a semicrystalline scaffold formed inside the oil with gel properties through the polarity route. These scaffolds are formed by the crystalline mesophasic liquid which are substances in the intermediate state between the liquid and the solid (Marangoni, 2011).

These structuring agents are differentiated by their molecular mass, such as high or low, where molecules with low molecular weight are those with less than 3000 Da. They can be used for example to trap liquid oil inside its self-assembled structure by promoting a macroscopic structure (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018). The most common structuring agents for food applications (Figure 2) and their structuring mechanisms are briefly described below.

Crystalline particles:



Crystalline fibers:

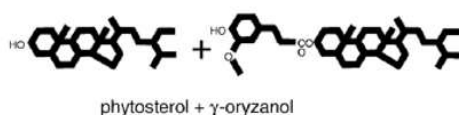


Figure 2 - Scheme of the molecular chains of the main structuring agents for organogels for food applications, according to their structuring behavior, adapted from (PERNETTI et al., 2007).

These structuring agents can be found in their purified form or as commercial mixtures commonly applied as food ingredients. For instance, saturated triacylglycerols can be found as hardfats or fully-hydrogenated oils, mono and diacylglycerols, as well as lecithins and sorbitan stearates, can be found as food emulsifiers and/or stabilizers. On the other hand, long chain fatty acids and alcohols plus their esters (wax esters) can be found in natural waxes, along with some natural hydrocarbons which can also contribute to the structuring effects.

As previously mentioned, natural waxes are one of the main structuring agents applied in food organogels. The wax organogels are the central focus of the review, thus, the physicochemical characteristics of natural waxes, their structuring properties, as well as their applications will be extensively discussed during the next topics.

2. CHEMICAL COMPOSITION OF WAXES

Waxes are considered natural mixtures of lipidic and can be differentiated by the proportion of the following compounds: n-alkanes or hydrocarbons (HC), free fatty acids (FFAs), free fatty alcohols (FAL), wax esters (WEs), ketones and sterols (DOAN, 2017). Waxes with different chemical compositions have different melting points and, therefore, different crystallization behaviors and consequently different oil structuring abilities (DOAN, 2017b). Therefore, to understand the behavior of a wax as structuring agent for an organogel, a better understanding of its chemical composition is essential (DOAN, 2017).

The chemical composition of the wax is closely linked with its origin and extraction methodology, but solid information about the proportion of its constituent and chain size are still deficient (DOAN, 2017). As they are derived from natural sources their composition and characteristics are intrinsic to each material (CHAVES, et al 2018).

In 2017, DOAN et al. carried out a characterization study of the main waxes applied for food organogels and the differences between them. Among the most studied waxes are those from rice, sunflower, bee, candelilla, carnauba and berry wax (DOAN, 2017). Carnauba wax is derived from the leaves of the palm tree,

Copernicia cerifera. The wax of candelilla is obtained from the leaves of the Candelilla shrub, *Euphorbia cerifera*. The rice bran wax (*Oryza sativa*) and sunflower wax (*Helianthus annuus*), are obtained through the winterization process of their respective oils (Chaves, et al., 2018). The berry wax is derived from the fruit peels of the *Rhus verniciflua* tree, native to China. Beeswax of natural origin produced by bees during the formation of hives (DOAN, 2017).

For the chemical characterization the authors have previously characterized the major lipid class such as hydrocarbons, free fatty acids, free fatty alcohols and wax esters through HPLC analysis. In this study ketones and sterols were not evaluated because of their minority participation in the composition of the waxes.

During the following characterization steps, the authors have determined the composition of each lipid class. Regarding the wax esters, the composition of esterified fatty alcohols and fatty acids were studied, indicating that the chain length ranges from 32 to 68 carbon atoms. The esters were formed by a medium/long chain fatty acid bonded with a long chain fatty alcohol. The hydrocarbon fraction was present only in bees' wax and candelilla wax. The free fatty acid portion of the natural waxes constitute a large part of components having an alkyl chain of 16 and 32 carbon atoms, while the free fatty alcohols chains varies from 24 to 34 carbons (Table 1) (DOAN, 2017).

Sunflower wax, rice bran wax, and berry wax may be considered one-component. This happens because for sunflower waxes and rice bran the composition is mostly (> 90%) wax esters. And for berry wax the composition is mostly free fatty acids (~95%) (DOAN, 2017). The esters of sunflower wax are composed predominantly of chains from 44 to 50 carbons and their major free fatty acids have chains from 16 to 24 carbons, also confirmed by Kanya et al. (Kanya et al., 2007; DOAN, 2017).

Rice bran wax also has the major free fatty acids from 16 to 24 carbons, also confirmed by Kanya et al. Their esters wax chains were from 44 to 50 carbons, which is a similar composition to sunflower wax (Kanya et al., 2007; DOAN, 2017). In berry wax, palmitic and stearic acid are the major free fatty acids (approximately 90%)(DOAN, 2017).

The other waxes can be considered of less uniform composition. Carnauba waxes from different origins, one commercial wax from Brazil and one wild sample, do not have significant differences in their composition. Both have wax esters as the main component (61% on average) and free fatty alcohols (33% on average) as the second component. For both samples, the major fatty acids in wax ester molecules was C24:0 and the major fatty alcohol was C32:0 (DOAN, 2017).

The waxes from bees and candelilla were those that have a greater amount of long chain hydrocarbons. Beeswax is among the most heterogeneous composition waxes, but, wax esters are still the predominant ones (~58%) followed by hydrocarbons (~26%). The hydrocarbons have C27, 29 and 31 chains, such observations have also been reported by Toro-Vazquez in 2013. The fatty acid fraction of wax esters was basically of palmitic acid (C16:0, ~80%), while in the the fatty alcohols fraction were found chains from 18 to 32 carbons. For the free fatty acid they varied from 12 to 34 carbons constitute approximately 95% of their composition, with predominant chains from 16 to 24 carbons and the results are compatible with those of Tulloch et al. The portion of free fatty alcohols was composed of C40 to 48 alkyl chains in beeswax (DOAN, 2017).

The candelilla wax is the only one among the evaluated waxes that has hydrocarbons as its main component (~72%), followed by wax esters (~15%). Around 82% of this hydrocarbon fraction was of 31 carbons, which is a very particular characteristic of candelilla wax. Palmitic acid was the main fatty acid in the wax ester fraction, while C30:0 was the main fatty alcohol in this fraction. Its portion of free fatty alcohols was composed mainly of C16:0 and C30:0 (DOAN, 2017). Due to the differences in chemical composition, the different waxes present different behaviors when applied in organogels, these structuring behavior will be extensively discussed in the following chapters.

Table 1 presents all of these compiled composite data.

Table 1 – Average of the main composition of waxes, adapted from (DOAN, 2017)

	RBW	SW	BW	CLW	CRBW	CRWW	BEW
HC	0,29	0,17	26,84	72,92	0,41	0,16	0,03
WE	93,49	96,23	58,00	15,76	62,05	58,99	0,02
FFA	6,00	3,29	8,75	9,45	6,80	6,32	95,70

FAL	0,22	0,32	6,42	2,20	30,74	34,53	4,24
	RBW	SW	BW	CLW	CRBW	CRWW	BEW
Hydrocarbon							
C ₂₇	//	//	40,28	0,15	//	//	//
C ₂₉	//	//	25,63	6,26	//	//	//
C ₃₁	//	//	18,05	82,48	//	//	//
C ₃₃	//	//	3,12	7,68	//	//	//
FFA							
C ₁₆	20,76	33,18	30,11	17,84	16,17	28,57	82,13
C ₁₈	5,54	20,42	6,84	1,90	17,78	5,38	11,26
C ₂₀	8,70	22,12	1,74	1,31	10,57	9,14	0,39
C ₂₂	17,52	9,73	4,33	1,60	6,28	5,50	//
C ₂₄	28,33	4,00	27,80	0,64	15,47	13,37	//
C ₂₆	3,36	2,26	6,55	0,90	6,17	5,25	//
C ₂₈	3,34	2,85	7,20	3,13	10,58	9,78	//
C ₃₀	3,03	0,99	6,60	31,38	4,70	4,50	//
FAL							
C ₂₄	25,30	25,25	12,01	1,77	//	//	//
C ₂₆	26,15	24,11	9,46	4,53	//	//	//
C ₂₈	16,16	17,74	12,95	11,48	6,38	5,67	//
C ₃₀	15,39	10,46	29,23	42,28	13,94	13,22	//
C ₃₂	9,47	5,92	20,40	14,57	65,39	68,29	//
C ₃₄	4,70	//	3,94	2,83	11,17	10,95	//
Wax Ester Fraction							
FA Moieties							
C ₁₆	8,63	4,89	80,27	34,16	15,16	19,35	79,89
C ₁₈	4,97	4,84	9,54	17,44	16,69	19,00	13,25
C ₂₀	22,95	48,65	0,51	6,31	16,02	13,06	//
C ₂₂	26,75	24,14	0,43	10,87	12,19	9,47	//
C ₂₄	27,83	5,87	0,47	1,20	22,38	18,65	//
FAL Moieties							
C ₁₈	2,80	1,82	2,93	20,70	13,69	21,48	66,02
C ₂₂	6,92	9,48	//	//	3,08	2,55	//
C ₂₄	29,46	34,41	25,19	2,05	3,61	4,41	8,77
C ₂₆	23,94	30,07	14,20	8,18	1,73	1,46	3,82
C ₂₈	13,70	12,15	13,45	18,72	3,15	1,60	3,32
C ₃₀	11,16	4,01	26,12	26,64	11,95	10,37	3,05
C ₃₂	5,31	2,20	13,57	7,99	51,74	47,89	3,53

HC: hydrocarbon, WE: wax easter, FFA: free fatty acid and FAL: free fatty alcohol

RBW: rice bran wax, SW: sunflower wax, BW: bees wax, CLW: candelilla wx, CRBW: carnauba Brazilian wax and BEW: berry wax.

3. WAXES AND STRUCTURING

The physical properties of a wax organogel are not only result of the wax and oil composition but are also influenced by the interactions between the components of the structuring agents and the crystals formed by them. The number

of these interactions is directly proportional to the number and structure of the crystals that form the self-structured crystalline network resulting in the alteration of the rheological properties of the gel (DOAN, 2017).

The waxes naturally form through their components, crystals with morphology of stems or platelets, resulting in gels of stronger structure. They use the route of solubility in this process. Due to their high melting point, when the system is cooled, the wax components crystallize into small platelets forming a self-sustaining network. Some partially polar fractions of the wax constituents contribute to the stability of the system through the polarization path but are not the key points in this case (DOAN, 2017).

The strength, consistency, fragility and stability of the wax-based organogels can be governed changes in the concentration of wax, the type of oil used, the rate of cooling and shear during the preparation of the organogel. Modification of these factors will alter the structuring agent/oil interaction leading to a change in the gelling properties. To exemplify these differences, in 2017, Doan et al. Carried out comparative studies of gels made up of 7 different waxes in rice bran oil. The waxes were used in different proportions according to their minimum gelation capacity: 1.0% for sunflower, candelilla and berry waxes; 1.5% for bees' wax, 5% for rice bran wax, 2% for imported carnauba wax and 4% for Brazilian carnauba wax. The behavior of all gels was compared through analysis of microstructure, rheology, DSC (differential calorimetry scanning), and XRD (X-ray diffraction) (DOAN, 2017). These analyzes are the most mentioned and cited in the characterization of organogels in the literature. The first observation made by the authors, through DSC analysis, was that the predominant components in the composition of the wax govern crystallization. In the case of rice bran and sunflower wax, the wax esters crystallized at a temperature considered to be very high (approximately 50°C). For beeswax the most relevant peak is also wax esters at approximately 40°C, followed by the second largest component of the beeswax, the hydrocarbons at approximately 30°C. For the organogel with candelilla wax the strongest crystallization peak occurred in approximately 40°C due to the large presence of hydrocarbons (mainly 82.48% of the hentriacontane, C₃₁), also a smaller peak appeared at approximately 43°C, probably due to the portion of esters of wax. The organogels with Brazilian and wild carnauba

wax (nationality not mentioned clearly) presented similar crystallization due to similar chemical composition. However, organogel with imported carnauba wax exhibited only a minor peak at approximately 33°C due to the presence of free fatty alcohols. The organogel with berry wax obtained the crystallization at the lowest temperature, with first peak around 10°C, probably due to the presence of 82% of medium chain free palmitic acid (C16:0) in its composition (DOAN, 2017).

In the rheology analysis, it was observed that the organogels prepared with the sunflower, bees, candelilla and berry wax presented the largest G' modulus (elastic modulus) and critical stress comparing to the rice bran, Brazilian and wild carnauba wax organogels. The organogels with candelilla and berry wax showed the highest consistencies and lower yield stress. In the group of gels considered to be weaker, the organogel with rice bran wax was the one that presented higher G' but less critical stress compared to the gels of carnauba wax. As expected the carnauba wax gels obtained very similar weakness independent of their minimum gelling concentration (4% for Brazilian carnauba and 2% for imported carnauba) (DOAN, 2017).

In X-ray diffraction analysis, all wax organogels (except berry wax) showed peaks at high diffraction angles, which are indicative of orthorhombic crystal structure. All wax-based organogels showed peaks in the low-angle region, providing evidence of lamellar packaging. It was also mentioned by the authors a difference of intensity between high and low angle peaks (as is the case of the organogel of candelilla) is the result of a strong anisotropy in the crystal growth rates between the vertical directions to the lamellar and different directions inside of the lamellar plane. The Van der Waals interaction between the long hydrocarbon chains and the ester group chains (the molecular interactions in the lamellar plane) are much stronger than the molecular interactions across the terminal CH₃ groups. This mechanism leads to the appearance of stem or platelet morphologies, which are desirable for the formation of a strong organogel. This is confirmed by the strong gelling property of sunflower, candelilla, bee and berry wax in rice bran oil, which exhibited small needle-like crystals (Figure 3) (DOAN, 2017).

The activity of crystallization and gelation of waxes is usually explained by the difference of their composition. The presence of wax esters resulted in a strong but brittle gel, which is expressed in a high storage modulus and flow stress of

the organogels and their waxes. Hydrocarbons and free fatty acids contribute to the consistency and stability of wax-based organogels (high critical stress and high consistency). However, chain length needs to be taken into account because the shorter the chain fatty acids (<16 carbons) the more they result in the crystallization of wax at low temperatures, which may limit their ability in food application, similar to what happens in berry wax organogels. Free fatty alcohols also have an important contribution to the structuring of wax organogels. However, they have strong hydrocarbon linkages and may entail gelation. The results of the studies provide important information about the need to know and understand the role of each component of the waxes in the structuring of oils, which should be explored to select a wax with desirable properties for application in food (DOAN, 2017).

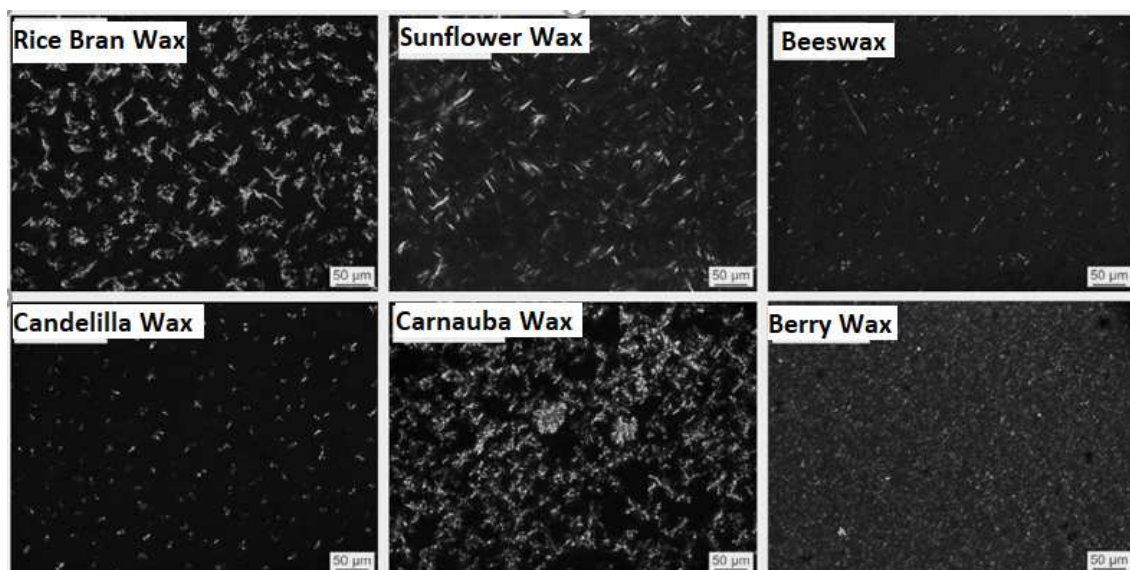


Figure 3: Microstructure of structured organogels with different waxes, adapted from (DOAN, 2017)

4 – APPLICATIONS OF WAX ORGANOGELS IN FOODS

4.1 – Margarine

In a study in 2013, Hwang and colleagues used 3 different types of waxes (candelilla, sunflower and rice bran) as soybean oil scrapers for margarine application. It was discovered in a previous study by the same group that sunflower

wax, candelilla and rice bran are the most promising as structuring for soybean oil and therefore were selected for this study (HWANG et al, 2012). Soybean oil was used because of its wide availability, low cost and high polyunsaturated content. In this study margarines made with organogels from the three different waxes had characteristics such as firmness, melting behavior and spreadability compared to commercial margarine. The authors started with the evaluation of organogels. All gels had their consistency measured in texturometer and formed gels consistent in the following growing order of firmness, rice bran wax, candelilla wax and sunflower wax in soybean oil. The authors have also observed significant differences for gels of the same wax obtained from different suppliers, noting that the difference in composition and purity of the wax directly influences the consistency and firmness of the organogel.

For the margarine production, first the fatty and aqueous phase were prepared separately and heated to the complete melting of the lipid phase. The emulsion was then formed by agitation and cooled to below the melting point of its fatty phase. The main results presented in this study were that the margarine formulated with an organogel of sunflower wax and soybean oil obtained a higher value of firmness being considered the most successful organogel. The margarine formulation with candelilla wax organogel showed phase separation after formation of the emulsion and therefore did not have its texture evaluated. The authors believed that the emulsifiers used in the margarine formulation may be incompatible with candelilla wax interfering in the stability of the emulsion. They suggest that for the use of this wax in margarine formulations the emulsifiers should be replaced with compatible ones. Rice bran wax did not allow margarine to achieve adequate consistency and showed the least firmness.

Even after forming stable and consistent gels, most of the wax organogels studies by Hwang and coworkers were not effective in the formation of margarine with compatible characteristics to commercial margarines due to their interactions with the other components of the formula. Then, the author compared the best results of the margarines formulated with sunflower wax to margarines produced with partially hydrogenated soybean oil (18 - 30%), structuring agent the commonly fat applied in industrial margarine production. After application to the margarine formulation, the wax organogels had slight decreases in firmness over margarine

prepared with partially hydrogenated soybean oil. This result provides important information that in terms of hardness characteristics, about 2-6% sunflower wax can replace 18-30% partially hydrogenated soybean oil in a margarine formulation, waxes being considered much more suitable for diet when compared to a partially hydrogenated oil. Firmness results were also compared with commercial products like spreads and margarines. The results also indicated that sunflower wax gels (2% w/w) were effective in achieving the firmness and consistency of commercial products. The author also suggests that gels with higher amounts of wax can also be produced to achieve the consistency of harder commercial margarines.

In addition, analyzes of DSC, SFC and drop point were applied to margarine formulations with organogel structured with sunflower wax (more promising result according to firmness analysis). Dropping point analysis showed that margarine formulated with wax organogel > 2% wax had higher melting point than commercial margarines and spreads. This dropping point increased as the concentration of wax in the organogel was increased. Based on the DSC analysis they concluded that the highest melting point of margarine containing sunflower wax organogel is due to the higher melting point of the wax present in that margarine. In solid fat content (SFC) analyzes, margarine samples prepared with sunflower wax obtained slightly higher values of solids than margarine prepared with hydrogenated soybean oil, mainly at 35°C, human body temperature that directly implies the sensorial characteristic of fusion in the mouth. Even so, the author does not clarify how this interferes with sensory quality and suggests that a sensory panel be made in future studies. The study concluded that sunflower wax organogels in soybean oil have the potential for total substitution of trans fats and / or saturated fats in margarines and spreads (HWANG, 2013).

4.2 – Ice Creams

Typically, ice creams have about 10-14% fat in their formulations of which 60-70% are commonly composed of saturated fatty acids. Banupryia et al, 2016. studied the partial and total substitution of milk fat by sunflower oil organogels structured with rice bran wax in ice cream. Ice creams with 10% of fat phase were prepared and five different proportions of milk fat substitution were evaluated (0% - control, 2.5%, 5%, 7.5% and 10% - total substitution). Sunflower oil and rice bran

wax were used to prepare the organogel in a ratio of 90 and 10% respectively. After preparation, the organogel was added as the oily phase in the different ratios mentioned above with respect to milk fat. The authors showed that the substitution of organogel in up to 100% of total fat did not change parameters such as pH and acidity, important parameters that interfere in the capacity of aeration of the ice creams. Regarding the viscosity the range obtained a considerable variation of 60cP (100% organogel) to 66cP (100% milk fat). The addition of the organogels as the fatty phase in the ice cream promoted the reduction of the viscosity and consequently of the overrun, proportionally the added amount. However, for substitutions of up to 50% of milk fat by organogels the differences were insignificant (Banupryia, 2016).

During the same period, the research group presented a very similar study to the previous one, replacing the sunflower oil with linseed oil. The authors emphasize the health of the product, since linseed oil has high levels of alpha linolenic acid (C18:3, Omega3) that contributes directly to the reduction of cardiovascular diseases, arterial problems, tumors and low triglyceride levels in the blood. The researchers presented that the costs for producing ice cream with organogel from linseed oil with rice bran wax were more expensive than the formulation made with milk fat, basically due to the cost of the ingredients. Regarding the physico-chemical characteristics, it was concluded that the substitution can be made up to a maximum of 25% of the total fat of the ice cream formulation.. Thus, it is believed that the industry can use organogels in ice cream preparations as partial substitutes for fat and consumers can be served with healthier products and with the same product quality (Banupryia, 2016).

Botega and coworkers in 2013 developed formulations of ice cream with different wax organogels, high oleic sunflower oil with carnauba, candelilla and rice bran waxes using milk fat as a control. In addition, they have also investigated the influence of emulsifiers, fat concentration and process variations on the final quality of the ice cream with organogels. In the production of ice cream, emulsifiers are essential in the formation of appropriate lipid structures. The correct distribution of structuring agents and emulsifiers ensure the correct ice cream's blasting and consequently a soft texture to the palate as well as good quality and melting characteristics. This is achieved through the perfect emulsification and structuration of the oily, aqueous and air phases, leading to a soft product that melts with the

desired uniformity and speed in the mouth of its consumers. Thus, as the ice cream has both emulsion (emulsified droplets) and foam (emulsified air) properties, the nature and quality of emulsifiers employed are important factors for the success of a formulation. In the study, the authors emphasized through the evaluation of microstructure and melting curves of the ice cream. For the wax organogels formulations, the emulsifiers, which are basically mixtures of saturated mono and diglycerides (~80%) and polysorbate 80 (~20%) directly interfering in the stability, melting and fat aggregation and therefore should be carefully tested and selected when the oil phase changes. The size and shape of the crystals formed by the wax during the structuring of the organogel may lead to synergistic and beneficial crystallization in the presence of emulsifiers but may also have a detrimental effect if not correctly selected. For this it is necessary an evaluation of the compatibility and synergy of crystals formed between all the structuring agents. The authors presented different possibilities in the freezing process for the formulations in order to evaluate the best quality ice cream. The resistance to melting of the ice cream, is correlated with the formation of fat network in the emulsion. In general, rice bran wax organogel ice creams had lower melt rates and better melt strength. The use of continuous freezing led to better structural stability than batch freezing in organogel formulations. The author does not make it clear whether the results are similar and compatible with formulations of ice cream prepared with milk fat but states that rice bran wax in high oleic sunflower oil presented promising results for application in ice cream. As structuring agent for the organogels the rice bran wax obtained the most promising result in comparison to candelilla and carnauba, providing more stable ice cream with lower percentage of melting and with distribution of size of emulsion particles more homogeneous comparable to the characteristics of the ice cream used as control. (BOTEGA, 2013).

4.3 – Cookies

For this kind of products the organogels are used as replacers of shortenings. Shortening consists of a technical, crystallized and plasticized fat applied mainly in bakery products such as cakes, cookies and biscuits. Its main effect is to prevent the cohesion of gluten nets during mixing, and also plays a key role in the texture of

these foods. In addition, it contributes positively to issues of stability and shelf life of the products. As is well known, conventional fats applied in this type of market also have high levels of saturated and/or trans fats. Some previous studies have applied vegetable oil to replace shortenings, but the final products have lost their crispness, become greasier and have had their stability and shelf life decreased, a fact that justifies the use of organogels for these types of product (Jang, 2015).

Jang and colleagues in 2015 investigated organogels of candelilla wax (3% and 6%) in canola oil as substitute for shortening in cookies. Regarding the firmness tests, the authors reported that shortening fat had higher firmness values than the organogel samples. Organogels tended to be firmer with increasing levels of candelilla wax. The viscosity of all samples had a tendency to decrease non-linearly with increasing temperature. Organogels clearly exhibited higher viscosity values than shortening fat at temperatures between 50 and 70°C. Furthermore, it became clear that the viscosity of the organogels depended on the concentration of wax in the formulation. The rheology of the cookies mass was also evaluated for each formulation. The storage (G') and loss (G'') modules have tended to increase with increasing frequency. In addition, all samples presented higher values of G' than G'' , showing more elastic characteristics. The cookie dough prepared with shortening presented higher values of G' and G'' compared to the samples prepared with organogel. These rheological properties can be attributed to the firmer texture of shortening as already mentioned. The samples of cookies prepared with organogel were also evaluated for their composition in fatty acids in comparison to the sample prepared with shortening. The percentage of total saturated and unsaturated fatty acids in shortening fat was 52.8% and 47.2%, respectively. In the case of cookies prepared with organogel, the level of unsaturated fatty acids clearly increased to about 90-92% and the level of saturated fatty acids was reduced to 8-10%. The predominant component cookies prepared with shortenings was palmitic acid (C16:0: 0.41.9%), while samples prepared with organogel were rich in oleic (C18:1, 62%), linoleic (C18:2, 20%) and linolenic acid (C18:3, 7%) acids, considered to be much healthier than shortening saturated fatty acids. Regarding the physical characteristics more related to the sensorial, the authors presented that the low viscosity of the organogels in the temperature of the cookies supply that contributed positively to their physical characteristics like snapping, that is of breaking under a force applied

in the biscuit, one of the important physical attributes of the Cookies. According to the authors, the organogels showed promising results in replacing fats for application in cookies. However, they point out that a sensory evaluation will be necessary to investigate consumer preferences (Jang, 2015).

In 2016, Mert and colaboradores also studied organogéis (candelilla wax in canola oil) and pure canola oil as substitute of shortenings for application in cookies. With the objective of reducing saturated fat, the authors studied both the total substitution of shortening as well as partial substitution with organogel making mixtures of organogel and shortenings. According to the results presented by the author, in an undisturbed state, both organogels and organogel/shortening blend formed structures with rheological properties comparable to pure shortening. However, the shear application rapidly transformed organogels and organogel/shortening blends into viscous liquids losing their solid structure. They pointed out that organogel/shortening mixtures with a greater amount of shortening improved the shear sensitivity of the samples proportionally. The experiments with mass samples showed that the samples containing organogel presented greater extensibility and lower hardness than the samples prepared with shortening, very important characteristics that will define the final texture of the product and its yield. The mixture of shortening organogels resulted in mass samples having texture properties closer to the properties of the standard mass. When the liquid oil was used in the formulation, the cookies had higher scattering proportions and hardness values. Although the incorporation of organogels has improved the physical properties of cookies compared to liquid oil, the functionality of the organogels was not sufficient to obtain quality parameters similar to shortening. On the other hand, blends of shortening organogels have significantly improved physical properties of cookies with up to 60% shortening fat reduction in the fat phase of the cookie. The results of this study indicated that the use of organogels improved the quality of cookies compared to cookies containing pure oil. A more conservative approach can be employed to reduce saturated fat in baked goods. The partial substitution of shortening by organogels can provide much more acceptable properties of mass and cookie than total substitution (Mert, 2016).

4.4 – Cakes

In 2017, Kim and colleagues studied the application of organogels in cakes (aerated bakery product). According to them specifically in aerated cakes and products, solid fat plays an important role in generating the mass structure by trapping the air bubbles to the fatty phase of the cake dough. Therefore, the use of solid fat is important for the aeration of cooked cakes interfering directly in its maximum volume (yield) and soft texture. However, the solid fat used mainly in industrial cakes is generally high in saturated fatty acids and may also contain trans fatty acids. The authors reported that there are only a few studies on the application of organogels in deliverable products. Thus, they proposed this study to investigate the influence of organogels as a substitute for solid fat on the qualities of aerated products like cake. Different levels of shortening in cakes were replaced by canola organogels with carnauba wax and their effects on cake quality were examined in terms of physico-chemical, rheological and structural characteristics. They reported the existence of many studies looking at various types of oil such as soybeans, canola, rapeseed, cartamo among others, in combinations with various waxes such as carnauba, candelilla, shellac and rice bran. However, they did not observe many studies with high oleic oils. According to the American Heart Association, the consumption of oils with high levels of oleic acid can reduce LDL cholesterol and consequently the risk of heart disease and stroke, in addition, foods prepared with oleic acid remain stable for ingestion for longer periods, when compared to polyunsaturated oils, because of its greater oxidative stability, it has greater durability and also serves as a natural preservative. This motivated the authors to use canola oil and carnauba wax as a structuring agent for use as a substitute for fat in cakes. Physical, rheological, and structural characteristics were used as comparative features. The total shortening substitution by organogels negatively affected the rheological properties and density of the cake masses. However, no apparent differences were observed between the standard and the cakes by replacing the fat phase in the proportion of 25% organogel and 75% shortening, suggesting that the fatty phase in the cake formulation in these proportions could be used without loss of quality in cake. In addition, replacing shortening with organogels could provide health benefits by decreasing the proportion of saturated fatty acids. For this mixture considered to be more promising (25% organogel and 75% shortening) the reduction

of saturated cake mass reached 10% in addition to the increase of 10% unsaturated. Therefore, the use of organogels as partial substitutes for cakes presents possible opportunities for producing healthier products with significant reduction of saturated and trans-fat.

4.5 - Confectionery fillings

Doan and colleagues in 2016 studied organogel with beeswax in rice bran oil as an alternative to the use of palm oil in order to control the consumption of saturated fats in confectionery products, especially hazelnut fillings. The use of palm oil contributes to the taste and texture of the confectionery products due to its high content of saturated fatty acids (approximately 50%). Mixtures of palm oil with organogel were obtained as an oil phase for application to the fillings, the palm was replaced with organogel in the proportions of 17, 33 and 50% by weight. By replacing palm oil, the equivalence of the physicochemical properties of the new fat-based products, for example the solid state at room temperature and the melting behavior, are crucial for obtaining similar sensory and texture attributes of the derived products. In addition, the additional fat phase should not severely alter the crystallization and melting behavior of the palm oil, in cases where it is partially replaced. At body temperature, the solid mass fractions of palm and wax mixtures were less than 2.0%, suggesting a non-waxy mouthfeel. The crystallization and melting temperatures of the palm blends and waxes were changed to lower temperatures due to the dilution effect when the palm was partially replaced with organogel. Interestingly, in spite of a lower melting enthalpy, the palm and organogel mixtures presented higher crystalline density and resistance when compared to reference samples (100% palm). The authors indicate the efficiency of organogels from beeswax to rice bran oil as partial substitutes for palm oil. However, they were more conservative, suggesting a substitution of at most 17% of the palm, since in this way it was possible to maintain a good property of palatability and physical characteristics more similar to the reference sample (DOAN, 2016).

5 - CONCLUSIONS

The organogels came as an alternative to the replacement of saturate and trans-fats for the purpose of producing healthier foods. The biggest challenge for researchers is to find an organogel that provides the same level of technological performance and versatility of solid fats for their applications, both in process and sensory requirements. The waxes are among the most promising edible structuring agents for food organogels and their efficiency is sensitive and related to complex factors such as chemical composition (origin) of both the wax and oil to be applied, as well as interactions with other structuring agents or ingredients where it is applied. These characteristics influence the structuring mechanisms, the shapes and sizes of the wax crystals, and consequently the texture and quality of the products where they are applied.

Among the most studied waxes are the waxes of rice bran, sunflower, bees, candelilla, carnaúba and berry wax, they are the ones that result in the most stable and consistent organogels. Among the most promising applications are margarines, ice cream and cookies, with great potential for total replacement of conventional fat by organogéis. Other applications such as cakes and confectionery have studies with more conservative answers where partial substitution is recommended.

Many studies where the application of these organogels of wax are presented, prove their ability to replace totally or partially solid fats to obtain healthier products. Many challenges of application, production process, commercial viability and availability of these structuring agents need to be investigated, but the studies are in constantly advance, this type of material is coming closer to arriving in the form of healthier foods to its consumers.

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CAPÍTULO 3 – RESULTADOS EXPERIMENTAIS

EFFECT OF FOOD EMULSIFIERS IN EDIBLE SOYBEAN OIL ORGANOGELS WITH CANDELILLA WAX

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ABSTRACT

Organogels arise as a promising alternative to replace trans and saturated fat in processed foods. Among the main challenges of preparing an organogel is the difficulty to find compatible and viable structuring agents available and allowed in the food industry. Waxes have been studied for this purpose and are among the most promising structuring agents for food organogels. Candelilla wax is well known due to its structuring power for vegetable oils. However, when organogels are applied in food formulations the presence of other ingredients can interfere on the organogel characteristic. The literature points out the possibility of synergistic effects obtained by the combination of Candelilla wax and emulsifiers or stabilizers, reducing the wax amount and improving the required characteristics of food products. structuring agent Due to this, the main objective of this study was to evaluate the effect of the total and partial substitution of candelilla wax as structuring for soybean oil by industrial emulsifiers basically composed of mono, di and triacylglycerols. Depending on the type and the combination of structurers employed, they may have a synergistic or antagonistic effect, one of which may impair or stimulate the effect of the other. The solids content conventionally applied by the oils and fats industry is not directly related to the hardness and consistency of the organogel, and even though a gel having a high solids content is not necessarily the most structured and resistant. On the other hand, the shape and quantity of the solid particles and crystals formed in the organogel are among the most determinant factors with respect to the hardness and consistency of the organogel, and therefore should be carefully studied and evaluated before application in each formulation.

1 – INTRODUCTION

In the food industry lipids play an important role in the structure, stability, quality, sensory characteristics and shelf life of products. For food technology, lipids are considered as oils (liquid at room temperature) or fats (solid at room temperature), whose main macroscopic difference is the melting point. This physical property is a reflection of the fatty acids composition and consequently of the triacylglycerols composition of each lipid source (CHAVES; BARRERA-ARELLANO; RIBEIRO, 2018). The most common fatty acids for the food industry are those with a carbon chain from 4 to 22 carbons. According to the chemical configuration, the carbon chain of fatty acids can be saturated (with no double bonds) or unsaturated (with one or more double bonds). In unsaturated fatty acids each double bond can be found as cis or trans configuration, with different physicochemical properties. Saturated fatty acids have a higher melting point than the corresponding fatty acid of the same chain size with one or more double bonds. (MOURA, 2007).

These molecular characteristics directly interfere with the sensory properties of fat-based foods. Therefore, the presence of saturated and trans fatty were considered a technological advantage because they result in a better texturing and sensorial characteristics. Studies have shown that diets with high intakes of this type of fat have negative consequences for health, contributing to several human diseases , mainly cardiovascular diseases that strongly reduce the quality of life of the individuals and can lead to death (HUNTER ZHANG, KRIS-ETHERTON, 2009). Because of this, regulatory agencies around the world and the even the consumers are engaged to remove and control these ingredients in the consumption.

The organogel technology has emerged as an alternative to replace saturated and trans fats has been intensively investigated with different edible oil systems in recent years. Studies have shown that organogels can have their technological performance compared to a solid fat, making their use possible in some applications. An organogel is characterized by a liquid oil trapped by a structuring agent network, with a macroscopic behavior similar to a gel. These systems are usually prepared by heating and solubilizing one or more structuring agents into the oil, followed by a cooling step enabling the network formation (MANDU, 2018).

One of the most interesting structuring agent for edible organogels is Candelilla wax, a well-known food additive, notable for its recognition as a safe additive for use in foods by the FDA (Food and Drug Administration) - Generally Recognized as Safe (GRAS) - FDA and used without any limitations other than already known good manufacturing practice (GMP). The waxes naturally form through their components, crystals with stem or platelet morphology, resulting in gels of stronger structure (TAVERNIER et al., 2017).

Organogels can be monocomponent, when a single structuring agent is used, and mixed gels where more than one structuring agent is applied. In mixed systems the structuring process is influenced by heterogeneous nucleation, increased nucleation and strengthening of the crystalline network through its structuring agents. In addition, to improve sensory characteristics, mixed systems have advantages when, for example, one of their structuring agents presents a marked sensorial characteristic or a high cost, as is the case of Candelilla wax. In this context, the development of mixed systems has gained interest from researchers (Patel & Dewettinck, 2016).

Mono and diacylglycerols used as commercial food additives are molecules that have one or two fatty acids esterified into one glycerol molecule, respectively. Its fatty acids composition is quite varied, you can find from the rich in saturated fatty acids, to the rich in unsaturated fatty acids. In sufficient concentration the organogels structured with this group of additives have relatively high firmness, however, their dosage and combination must be very cautious since they can influence the crystallization efficiency of each other and even disrupt the structuring (Pernetti et al. 2007).

This study aims to study the possible synergy between candelilla wax and industrial food grade emulsifiers composed basically of mono-, di- and triacylglycerols as structuring agents for soybean oil organogels.

2 – MATERIALS AND METHODS

2.1 - Raw Materials and Reagents

Soybean oil from the Soya - Bunge (São Paulo, BR) in 900 mL PET bottles was locally purchased. Candelilla wax in lentil forms (Light Special Candelilla REAL®) was supplied by Multiceras S.A., Monterrey, Mexico. The GRINDSTED PS 233 F-B, DIMODAN US / C, DIMODAN UP / C, GRINDSTED SIMPLIFIER 100, GRINSTED PGPR SUPER and GRINDSTED CRISTALLIZER were supplied by DuPont™ Danisco®, Cotia / SP. Dupont emulsifiers are produced from vegetable-based fatty acids. They may be a mixture or a pure compound with saturated and unsaturated fatty acids. Esterified as mono, di and or triacylglycerol.

2.2 – Characterization of raw materials

2.2.1 –Fat Acid Composition of Emulsifiers

The fatty acid composition of the commercial emulsifiers was determined by CGC Agilent 6850 Series GC System (Santa Clara, CA, USA) after esterification by the method of Hartman and Lago (1973). The fatty acid methyl esters were separated according to AOCS method Ce 2-66 using an Agilent J & C DB-23 (Santa Clara, CA, USA) (50% cyanopropylmethylpolysiloxane) column length: 60 m, internal diameter: 0.25 mm and film thickness: 0.25 µm. 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; 1:50 split ratio; volume injected: 1.0 µL. The qualitative composition was determined by comparing the peak retention times with those of the respective fatty acid standards. The amount of each fatty acid was determined by peak area normalization. Due to analytical limitations, candelilla wax had its composition obtained in the literature.

2.2.2 - Mono, di and triacylglycerol composition of emulsifier

The mono, di and triacylglycerol composition of the commercial emulsifiers was determined in HPSEC (High Performance Size-Exclusion Chromatography). The high efficiency liquid chromatograph consisted of a Waters 510 pump with an injection volume of 10 μ L, two columns 300mm long and 7.5mm internal diameter, packed with styrene / divinylbenzene with pore sizes of 100 to 500Å, connected in series, and a Merck refractive index detector L-7490. Hewlett-Packard HP3390A Integrator. Two drops of each structuring agent were added in 1.5 mL tetrahydrofuran solvent (THF) and injected into the system. Peaks were identified according to each mono, di and triacylglycerol standards, PGP (polyglycerol polyricinoleate) peak was identified as the last one peak in the chromatogram. The proportion of each component was obtained by normalizing the peak areas.

2.2.3 - Preparation of organogels

For the general formulation of the organogels a total of 5% of structuring agents and 95% of soybean oil were used. The 5% candelilla wax was replaced by each of the commercial emulsifiers in the following proportion structuring agent: 90% candelilla wax / 10% emulsifier, and respectively 70/30, 50/50, 30/70, 10/90 (Figure 1).

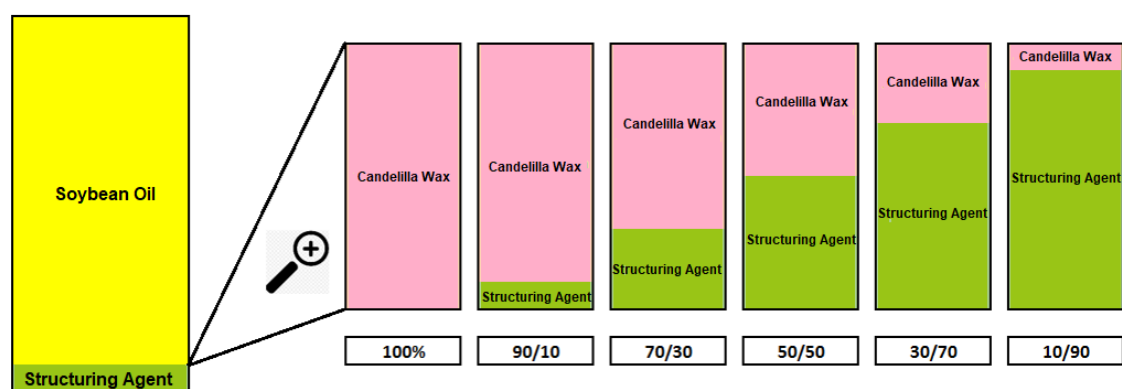


Figure 1: Schematic proportion of structuring agents and candelilla wax

In addition, control, organogels were prepared only with candelilla wax in the corerespondent proportions, 90, 70, 50, 30 and 10%. The oil and structuring agent mixture was heated under intense magnetic stirring (~3000 rpm). After reaching 80°C, the mixture was kept under stirring for 3 minutes with temperature control +/- 3

° C. The samples were then placed in 50 mL beakers for viscosity, texture measurement and differential scanning calorimetry (DSC), and in solid measurement tubes for the solid content determination (SC). All samples were kept in a temperature chamber under 25 ° C for 48 hours. Each emulsifier was coded according to Table 1.

Table 1: Structuring agents encoding

Structuring Agent 1	Candelilla Wax
Structuring Agent 2	Grindsted OS 233 F-B
Structuring Agent 3	Dimodan US/C
Structuring Agent 4	Dimodan UP/C
Structuring Agent 5	Grindsted Simplifier 100

2.3 - Characterization of organogels

2.3.1 – Mechanical Behavior of Organogels (Hardness Analysis)

The hardness was determined by the compression/extrusion methodology using a texturometer (TAXTi2, StableMicrosystems, England), controlled by computer software. An acrylic cylindrical probe 25 mm in diameter and 35 mm long with a velocity of 1.0 mm/s and a fixed distance for penetration of the probe of 15 mm was used. The analysis was performed in triplicate and the value considered for evaluation was the average of the maximum force obtained in kg.

2.3.2 – Apparent Viscosity of Organogels

The apparent viscosity of the samples was determined in triplicate at 25°C in Vibro Viscometer model SV-10 from A & D Company - Japan. Organogel samples were transferred to 25 ml cuvette at room temperature and the viscosity was immediately determined.

2.3.3 – Solids Content in Organogels (SC)

The solids content of the gels was determined in triplicate at 25 ° C, in a Bruker pc 20Minispec nuclear magnetic resonance (NMR) spectrometer. for this determination, tubes were packed as described above.

2.3.4 – Microstructure of Organogels (Morphology of Crystals)

The morphology of the crystals was evaluated under a polarized light microscope (Olympus, model BX51, USA) coupled to a digital video camera (Media Cybernetics, USA). The temperature of the samples was maintained at 25 ° C during the analysis. A small amount of the sample was placed on the glass slide and covered by a cover slip. For each slide - duplicate - 3 visual fields were focused, totaling 6 fields analyzed per sample, of which three were selected for visual evaluation of the crystalline dimensions. The analyzes were performed in 3 magnifications of lenses 100x, 200x and 400x, however, only the images of 200x will be presented in the results.

2.3.5 - Melting Thermal Behavior of Organogels

All gels were evaluated for their melting thermal behavior. The behavior of the samples was determined by differential scanning calorimetry (DSC) (Model Q2000; TA Instruments, New Castle, DE, USA). Approximately 12 mg of each sample were placed in aluminum pans and closed with a hermetic aluminum lid at 25°C. The pans were placed in the apparatus at 25°C and the temperature raised to 100 ° C at a heating rate of 2°C/min. The parameters used as a comparison between the samples were the melt initiation temperature (T_{if}), the melt peak temperature (T_{pf}) and the final melt temperature (T_{ff}) in order to establish a pseudo phase diagram of the systems.

3.3 – RESULTS AND DISCUSSION

3.1 – Chemical composition of Structuring Agents: Glycerid Classes and Fatty Acid Compositions Structuring agent

As previously mentioned, due to analytical difficulties, the composition of candelilla wax was withdrawn from the literature (DOAN, 2017). The other emulsifiers were analyzed for their glyceride classes and fatty acid compositions (Table 2).

Candelilla wax has a majority hydrocarbon composition, approximately 70% of its composition, followed by approximately 15% wax esters, 9% free fatty acids and 2% free fatty alcohols. Regarding the composition of hydrocarbons, gas chromatographic analyzes described in the literature show that these hydrocarbons are composed mostly of long chains and odd number of fatty acids, mainly (C31H64) and (C33H68), that together represent around of 90% of the hydrocarbon fraction of candelilla wax. For the fatty acid composition within the wax esters family, the C16:0, C18:0 and C22:0 chains are predominant (62%) while C18:0, C28 and C30 were predominant in fatty alcohols fraction (65%). The composition of the free fatty acids fraction is also mostly of long chain fatty acids (C30) (32%) and considerable amounts of palmitic acid (C16:0) 18%. Regarding the composition of the free fatty alcohols, again the long chains molecules of C30 and C32 appear as majority (57%). (DOAN, 2015).

For the other structuring agents it was possible to identify that the composition in fatty acids is mainly palmitic, stearic and linoleic fatty acids and small differences of composition in other fatty acids, as well as the presence of the C18:0:1-OH (ricinoleic) fatty acid, referring to the presence of polyglycerol polyricinoleate (PGPR) in the composition of structuring agent 5. Structuring agent 2 is composed almost exclusively of saturated fatty acids (97%), most of which are C16:0 and C18:0. Its commercial description is compatible with the analytical results, it is presented as an emulsifier composed of the mixture of mono, di and triacylglycerides of different vegetable oils, with typically 98% of saturated for application in low fat margarines. Structuring agents 3 and 4 are commercially presented as distilled monoglycerides (> 90%) of vegetable oil, confirmed by the analytical results of glyceride composition, suggested application for margarine and baking products. This structuring agent differ mainly in their amount of saturated fatty acids (around of 30% for emulsifier 4

and 15% for 3) being C16:0 and C18:0 the main saturated fatty acids. In the analytical results obtained, both structuring agents presented a great amount of polyunsaturated fatty acids (approximately 50%) mainly C18:2. The structuring agent 3 presented also higher amounts of C18:1 and C18:3, 26 and 4% respectively. For the structuring agent 4, around of 17% of C18:1 was detected. Structuring agent 5 has the more heterogeneous composition, around of 56% of monoacylglycerols, 22% of PGPR, 17% of triacylglycerols, and 4% of diacylglycerols. This samples presents 40% of SFA, mainly C16:0, C18:0 and C22:0, 32% of PUFA, mainly C28:2 and 16% monounsaturated, mainly C18:1. Around of 8,5% of C18:1-OH was also found in the structuring agent 5, due to the amount of PGPR.. It is commercially available as a blend of mono-, di- and triglycerides of vegetable oils with the addition of polyglycerol ester of ricinoleic acid and approximately 31% of saturates, with a suggested application for margarines. By characterization it was also possible to observe that only structure 2 and 5 present in their composition significant amounts of long chain fatty acids, 2% C20:0 for emulsifier 2 and 1% for emulsifier 5, and also 9% C22:0 for the emulsifier 2 and 3% for the emulsifier 5.

Table 2: Analytical results of fatty acid and glyceride composition of emulsifiers

Ácido Graxo		Emulsificante			
		2	3	4	5
C12:0	Láurico	0,43 ± 0,31	0,01 ± 0,01	0,05 ± 0	0,1 ± 0,01
C14:0	Mirístico	1,27 ± 0,6	0,02 ± 0,01	0,94 ± 0,01	0,36 ± 0,01
C15:0	Pentadecílico	0,1 ± 0,08	0,04 ± 0,02	0,06 ± 0,01	0,03 ± 0,03
C16:0	Palmitico	46,64 ± 1,1	11,73 ± 0,44	23,98 ± 0,23	18,25 ± 0,05
C16:1 cis	Palmitoleico	0,04 ± 0,04	0,08 ± 0,01	0,46 ± 0	0,06 ± 0,01
C17:0	Margárico	0,04 ± 0,04	0,13 ± 0	0,16 ± 0	0,12 ± 0,04
C17:1 cis	Heptadecenóico	0,56 ± 0,98	0,04 ± 0	0,04 ± 0,02	0,03 ± 0,01
C18:0	Esteárico	37,8 ± 1,44	6,96 ± 1,36	3,95 ± 0,02	15,97 ± 0,72
C18:1 cis	Oleico	1,9 ± 1,14	25,92 ± 0,87	16,68 ± 0,13	16,52 ± 0,89
C18:2 trans	Linolelaídico	0 ± 0	0,62 ± 0,02	0,55 ± 0,01	0,86 ± 0,01
C18:2 cis	Linoleico	0 ± 0	46,18 ± 1,61	51,91 ± 0,08	30,71 ± 0,08
C18:3 trans	Linolenelaídico	0 ± 0	1,72 ± 0,08	0,27 ± 0,02	1,74 ± 0,02
C18:3 cis	Linoléico	0 ± 0	4,01 ± 0,15	0,15 ± 0,01	1,97 ± 0,01
C20:0	Araquídico	2,06 ± 0,82	0,53 ± 0,16	0,27 ± 0,01	1,03 ± 0,01
C20:1 cis	Gadoleico	0 ± 0	0,21 ± 0,01	0,07 ± 0	0,19 ± 0,01
C22:0	Behênico	8,71 ± 1,71	1,23 ± 0,27	0,15 ± 0,02	3,29 ± 0,03
C24:0	Lignocérico	0,24 ± 0,21	0,18 ± 0,03	0,09 ± 0,01	0,17 ± 0,01
C18:1 -OH	Grupo do PGPR	0 ± 0	0 ± 0	0 ± 0	8,49 ± 0,5
SAFA	Saturados	97,29 ± 1,08	20,83 ± 3,29	29,65 ± 0,19	39,32 ± 0,98
MUFA	Insaturados	2,5 ± 0,4	26,25 ± 0,88	17,25 ± 0,12	16,8 ± 0,74
PUFA	Polinsaturados	0 ± 0	50,19 ± 1,76	52,06 ± 0,08	32,68 ± 0,2
TRANS	Trans	0 ± 0	2,34 ± 0,10	0,82 ± 0,03	2,6 ± 0,05
Classes Glicerídicas (%)					
Glicerídio					
	Triacilglicerol (TAG)	84,89 ± 1,51	0,12 ± 0,1	0,16 ± 0,05	16,85 ± 0,97
	Diacilglicerol (DAG)	6,16 ± 0,08	1,85 ± 0,17	1,89 ± 0,24	4,17 ± 0,42
	Monoacilglicerol + Ácidos Graxos (MAG + AG)	9,03 ± 0,19	97,98 ± 0,18	98,14 ± 0,60	56,21 ± 0,81
	Polirricinoleato de Poliglicerol (PGPR)	0 ± 0	0 ± 0	0 ± 0	22,74 ± 0,09

3.2 – Hardness Analysis of Organogels

Hardness is an important technological characteristic evaluated in the characterization of an organogel for application in foods. The function of the wax and the emulsifiers studied is precisely to give structure to the gel in order to keep it solid-like, homogeneous and with sufficiently high hardness (Tavenier, 2017). In Figure 2 it is possible to evaluate the effect of industrial emulsifiers next to candelilla wax (structuring agent 1) and to evaluate its structuring ability. As shown in Figure 2A, B, C and D for the total replacement of candelilla wax (structuring agent 1) by the industrial emulsifiers there was no solid gel formation for any emulsifier. Thus, there are no hardness values for the 0/100% column. The capacity of these products to form organogels at 5% was very small and the equipment could not detect their hardness. The same thing occurred at 10/90 concentrations for all structuring agents, with the exception of structuring agent 2, which although read on, the final organogel did not achieve an acceptable firmness. No combination and not even structuring agent 1 alone in that amount was able to structure the gel. A similar behavior was shown for the 30/70% mixture.

For the other concentrations, each structuring agent has a particular behavior. For the structuring agent 2 and 4, similar behaviors were noticed for the concentrations 90/10 and 50/50, in other words, no differences between the mixtures and the control samples were noticed. However, for the concentration 70/30 a synergistic effect was realized in the mixture with the structuring agent 2, resulting in a higher hardness than the control sample (only candelilla wax). This structuring agent 2 behavior can be related to the high amounts of saturated fatty acids, mainly the long chain fatty acids C20:0 and C22:0.

Structuring agent 3 shows an antagonistic effect with candelilla wax, resulting in a destructuring effect of the organogels. This behavior is very clear since the hardness value of the mixtures were lower than those of the control sample.

The structuring agent 5 presented the best results regarding the synergistic effect and structuring. Their values were significantly higher and reached a four-fold hardness value for the 50/50 concentration in relation to the control organogel preparation, with only candelilla wax, being close to the gel with 100% of candelilla wax. This behavior is a result of a very complex synergistic system with candelilla

wax, since the structuring agent 5 is composed of high amounts of mono and triacylglycerols in association with PGPR.

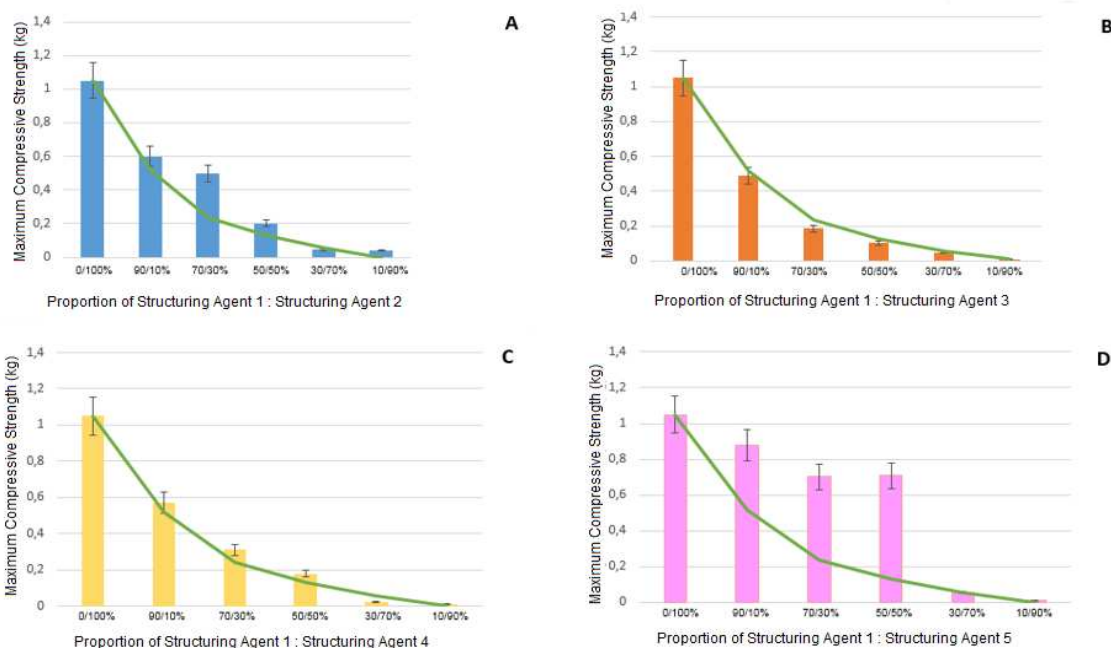


Figure 2: Results of maximum compressive force (texture). Combined structuring (bar graph) compared to pure candelilla wax (line graph). Figure A: Structural ratios 1 and 2 combined, B: Structural ratios 1 and 3 combined, C: Structural ratios 1 and 4 combined, D: Structural ratios 1 and 5 combined.

3.3 – Viscosity of Organogel Samples

It was clear that for the structuring agent combinations, the viscosity remained the same and slightly higher than the control samples. This is probably due to the fact that structuring agents also have the function of increasing viscosity because they form different structures of the wax, which does not necessarily mean that these structures will bring hardness and consistency to the organogel. Only the combination of structuring agents 1 and 3 showed a tendency to obtain lower values, probably due to the reduced amount of crystals when compared to other combinations of structuring agents as observed in the microscopy images.

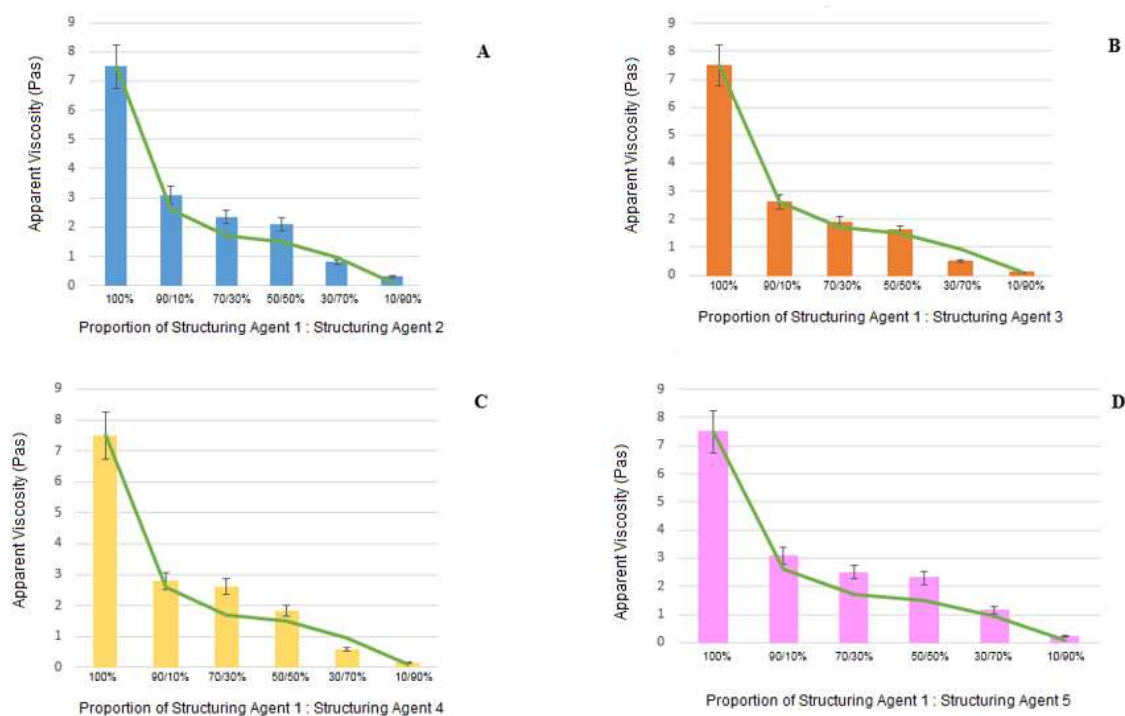


Figure 3: Apparent viscosity results. Combined structuring (bar graph) compared to pure candelilla wax (line graph). Figure A: Structural ratios 1 and 2 combined, B: Structural ratios 1 and 3 combined, C: Structural ratios 1 and 4 combined, D: Structural ratios 1 and 5 combined.

3.4 – Solid Content of Organogels

In conventional oils and fat structuring systems, where crystallization is guided by triacylglycerols, a high value of solid content is related to high values of hardness and texture (Tavenier, 2017). Probably this concept is not directly applied to organogels per solubility route. In this route there is a low increment of solid content, but high rearrangement and distribution of the crystalline structures. In Figure 4 we can see that the combination of structuring agents 1 and 2 presented the highest values of solid content but did not present the highest values of hardness. In this case, the solid content is justified by the amount of saturated fatty acids of each structuring agent. Structuring agent 2 has approximately 97% saturated fatty acids and therefore presented higher values of solid content, followed by structuring agents 5, 3 and 4. Because of this, the solids content in organogels is useful for inferring about the structuring mechanism, but it is not intended to directly indicate physical

properties such as hardness, just as it is used in conventional fat systems. This relationship is very clear when looking at the results for the mixtures with the structuring agent 5, where no increment in SC is noticed but high values of hardness were previously determined. Its also possible to deduce that for the structuring agent 1 and 2 crystals are responsible for the establishment of the self-assembled network, while for the structuring agent 5, there is more than one mechanism contributing to the network formation.

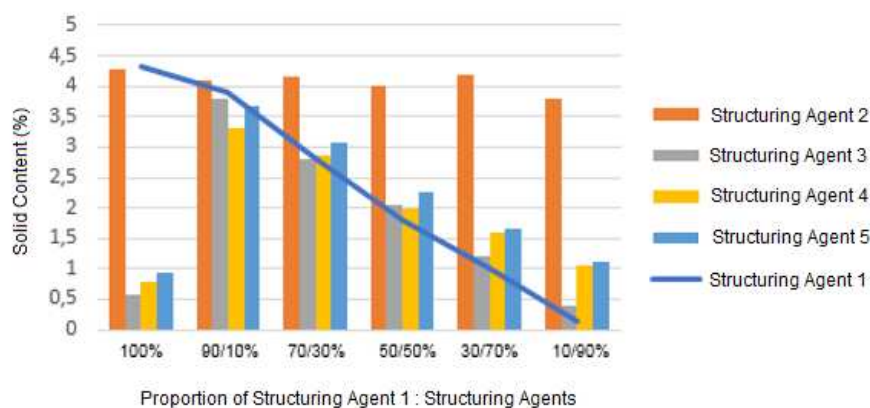


Figure 4: Results of solid content for different proportions of structuring agents. Combined structuring (bar graph) compared to pure candelilla wax (line graph). Orange bar: proportions of structuring agent 1 and 2 combined, gray bar: proportions of structuring agent 1 and 3 combined, yellow bar: structuring agent proportions 1 and 4 combined, blue bar: structuring proportions 1 and 5 combined.

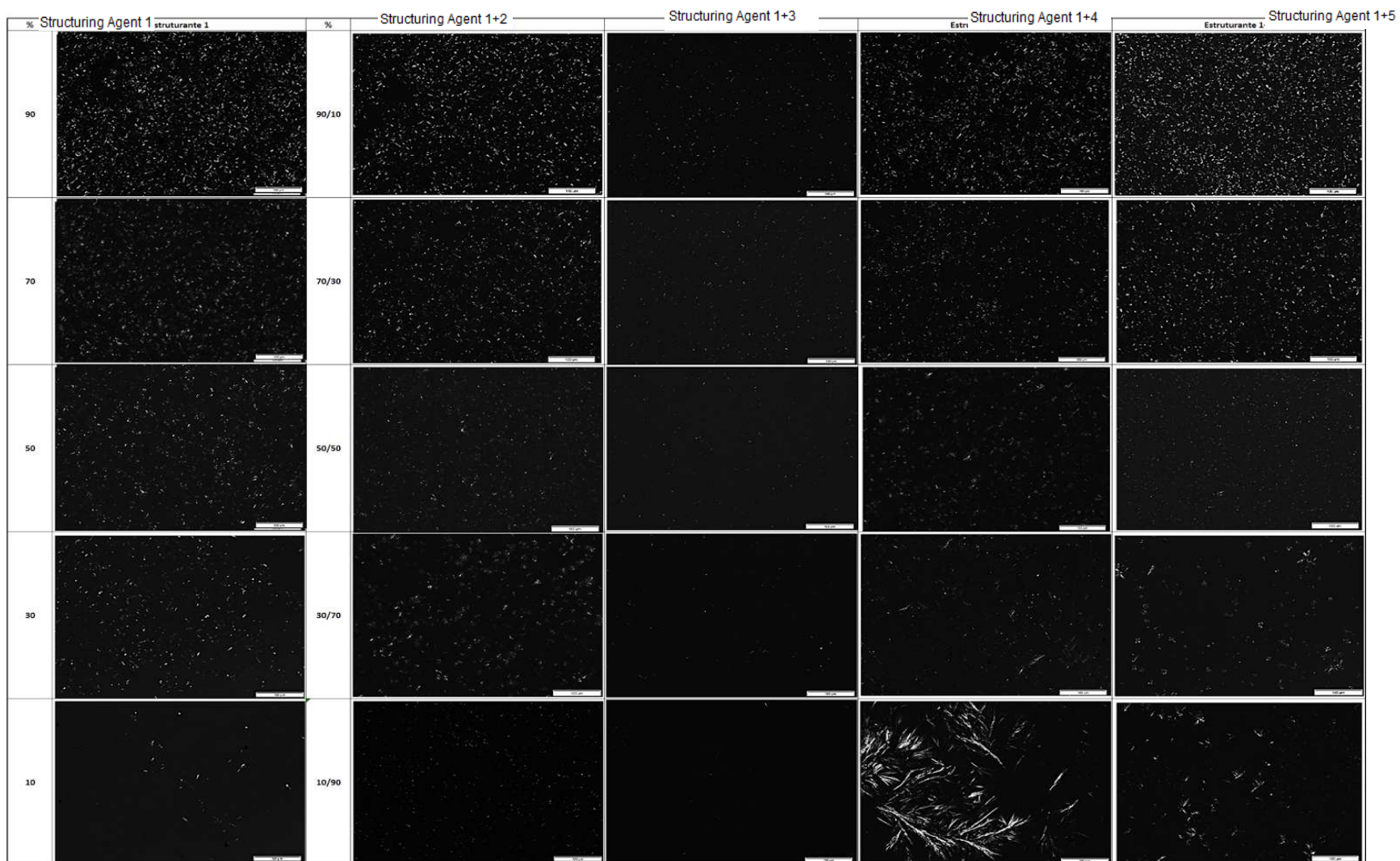
3.5 – Microscopy of the Organogel Samples

Its already described that for the wax organogels hardness and consistency have a strong influence of the average size and shape of the solid particles and their three-dimensional organization (TORO-VAZQUEZ et al., 2013). In Figure 4 it can be seen that the combination of structuring agents 1 and 3, which obtained the lowest values of hardness, also presented smaller amounts of solid particles. The antagonistic effect of this structuring agents the crystallization process in an antagonistic way, lowering the formation and/or crystallinity of its crystals, since neither the wax crystals were kept like in the control samples.. That is, with the addition of this other structuring agent (3)

there was a decrease in the amount of crystals formed, and probably for that reason were the less hard and consistent gels.

The combination of structuring agent 1 and 2, 1 and 4 generate microscopic images reasonably similar to wax images alone for most of the proportions studied, these results can also be related to the hardness results obtained for these two structuring agents, which were very similar with wax alone tending to be slightly harder.

Mixtures of candelilla wax with the structuring agent 5 generated synergistic organogels with a higher number of smaller crystals when compared to the other structuring agents. This may be directly related to its higher hardness values obtained and presented previously. Thus, the presence of the components of the structuring agent 5 modifies the organization of the self-assembled network by reducing the crystal sizes and maybe establishing a non-crystalline structuring route. In the proportions of 30:70 and 10:90 we can see dilution effect of the industrial emulsifiers when combined with wax, presented by the image with smaller amounts of crystals formed after their combinations and confirmed by the slightly lower hardness values for these proportions. Still in figure 4, ratio 10:90, it is possible to observe that there is an exception as to the quantity and uniformity of the crystals for the combination of structuring agent 1 and 2. In this condition and proportion, this structuring agent was the only one that presented images with more uniform solid particles and in larger amounts. Again this result is consistent with the result of the solid content obtained for this structuring agent, Figure 5.

Figure 5: Polarized light microscopy (PLM) images in summary for all samples and proportions.

3.6 - Thermal Behavior of Organogels in the Fusion

Figure 6 shows the melt profiles and the pseudo phase diagram constructed according to the results obtained by DSC. Phase diagrams should be considered pseudo-diagrams, because the components of organogels, especially waxes, are not pure compounds but rather multicomponent compounds of different chemical, polar and nonpolar natures, composed basically of hydrocarbons, wax esters, acids and alcohols fatty acids, as previously described (TAVERNIER et al., 2017).

The pseudo-phase diagrams were based on their respective melt profiles (DSC) and mainly on the temperatures derived from this curve (T_{on} and T_{off}). The onset and offset temperatures are obtained through an integral of the melting curve, indicating start, peak and end of the melt. For all the systems the diagrams were constructed by plotting the mean value of T_{on} and T_{off} which were made in triplicates for each test. The top line (T_{off}) is the end of the melting process or complete melting. Is the liquidus line and above all the material is liquid. The bottom line (T_{on}) indicates the beginning of the melting process. Is the solidus line which below all the material is solid. The area between the two lines is considered a pseudo - phase of the material which both solid and liquid phases coexist. Although they are multicomponent materials, the DSC curves present only one melting peak (characteristic of homogeneous materials). It may be considered that this phenomena indicates the simultaneous crystallization of two or more different types of crystals (coexisting) or cocrystallizing (only a type of crystal is formed) or even both. Even if the same structuring agent combination is done, when the ratio between them varies, different T_{on} and T_{off} can be found. This is related to the different values of hardness and microscopy obtained for different proportions of the same combination of structuring agents and consequently visible differences of organogels. From the DSC melting curves it can be deduced that the presence of commercial structuring agent emulsifiers influenced the melting peak of candelilla wax, making the peak slightly less evident. The situation was the opposite for the structuring agent 5, where the peak became more strong, it

could be related to the probable synergism between candelilla wax and structuring agent 5.

Figure 5 - a) Structuring agent 1: Structuring agent 2

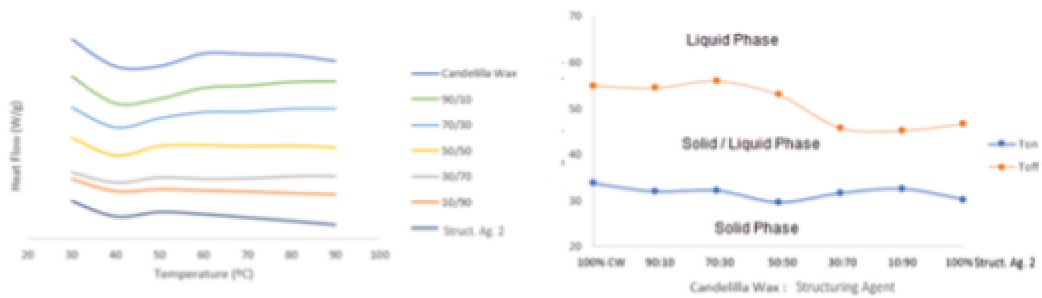


Figure 5 - b) Structuring agent 1: Structuring agent 3

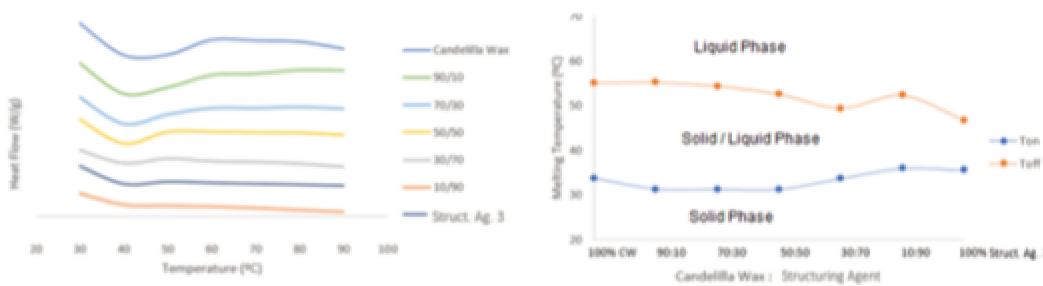


Figure 5 - c) Structuring agent 1: Structuring agent 4

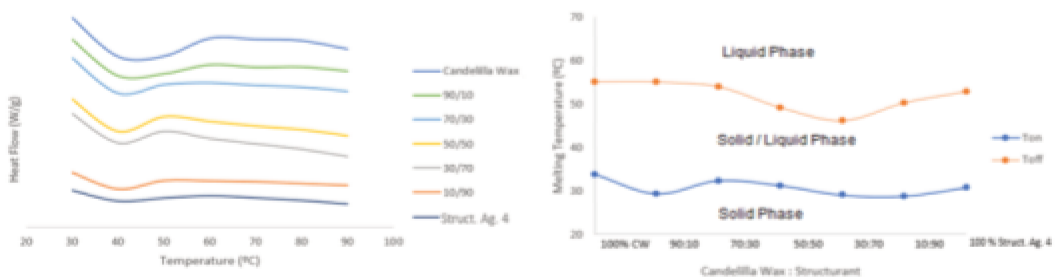
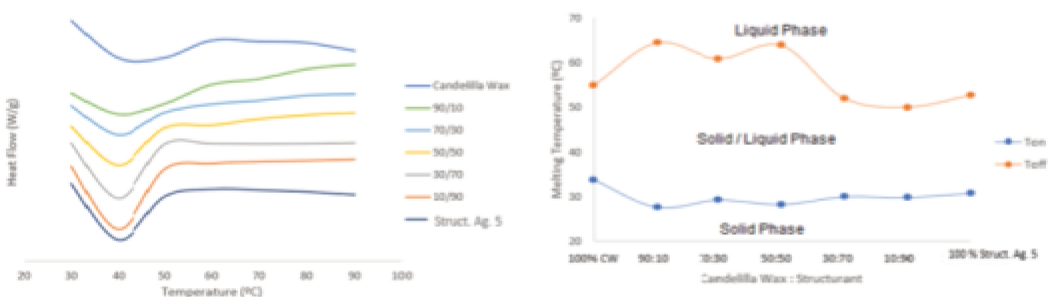


Figure 5 - d) Structuring agent 1: Structuring agent 5



4 – CONCLUSIONS

Based on the results presented, it is possible to observe that none of the commercial emulsifiers alone can structure a 5% soybean oil organogel, except for candelilla wax.

Depending on the type and the combination of structuring agents used with candelilla wax, they may have a synergistic or antagonistic effect, even one affecting the effect of the other.

Emulsifier 3 may be considered the weakest in its ability to structure organogels in combination with candelilla wax, its effect is antagonistic when used together with candelilla wax.

The structuring agent emulsifiers 2 and 4 have good structuring ability and maintain the texture and the same hardness of the candelilla wax alone, and according to the results can be considered synergistic because the structure of the gel is directed by the amount of wax.

The emulsifier 5 has synergistic effect and high structuring capacity combined with candelilla wax, the results indicated that it is possible to replace candelilla wax by up to 50% by structuring agent 5 with small losses in the hardness and viscosity of the system.

The solids content conventionally applied by the oils and fats industry is not directly related to the hardness and applicability of the organogel, and even though a gel having a high solids content is not necessarily the most structured and resistant.

The amount, homogeneity and shape of the crystals is one of the main factors that influence the hardness of an organogel and therefore for organogéis where there is more than one structuring agent the choice of them must be carefully evaluated in this sense.

5-COMPLEMENTARY FILE

Figure 7 : Polarized light microscopy (PLM) images for organogel samples in the proportions 90:10 for all structuring agents.

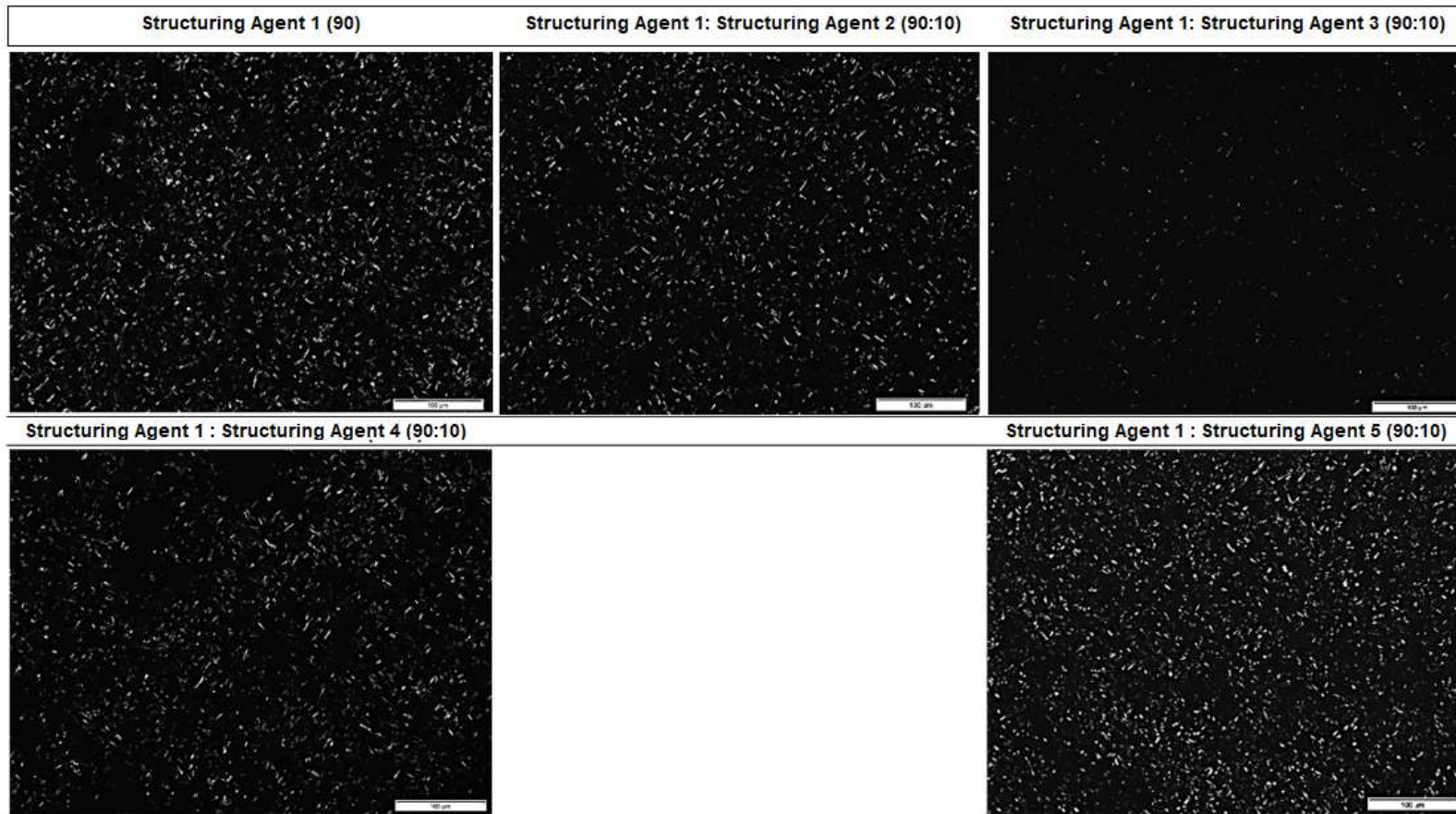


Figure 8: Polarized light microscopy images (PLM) for the organogel samples in the proportions 70:30 for all structuring agents.

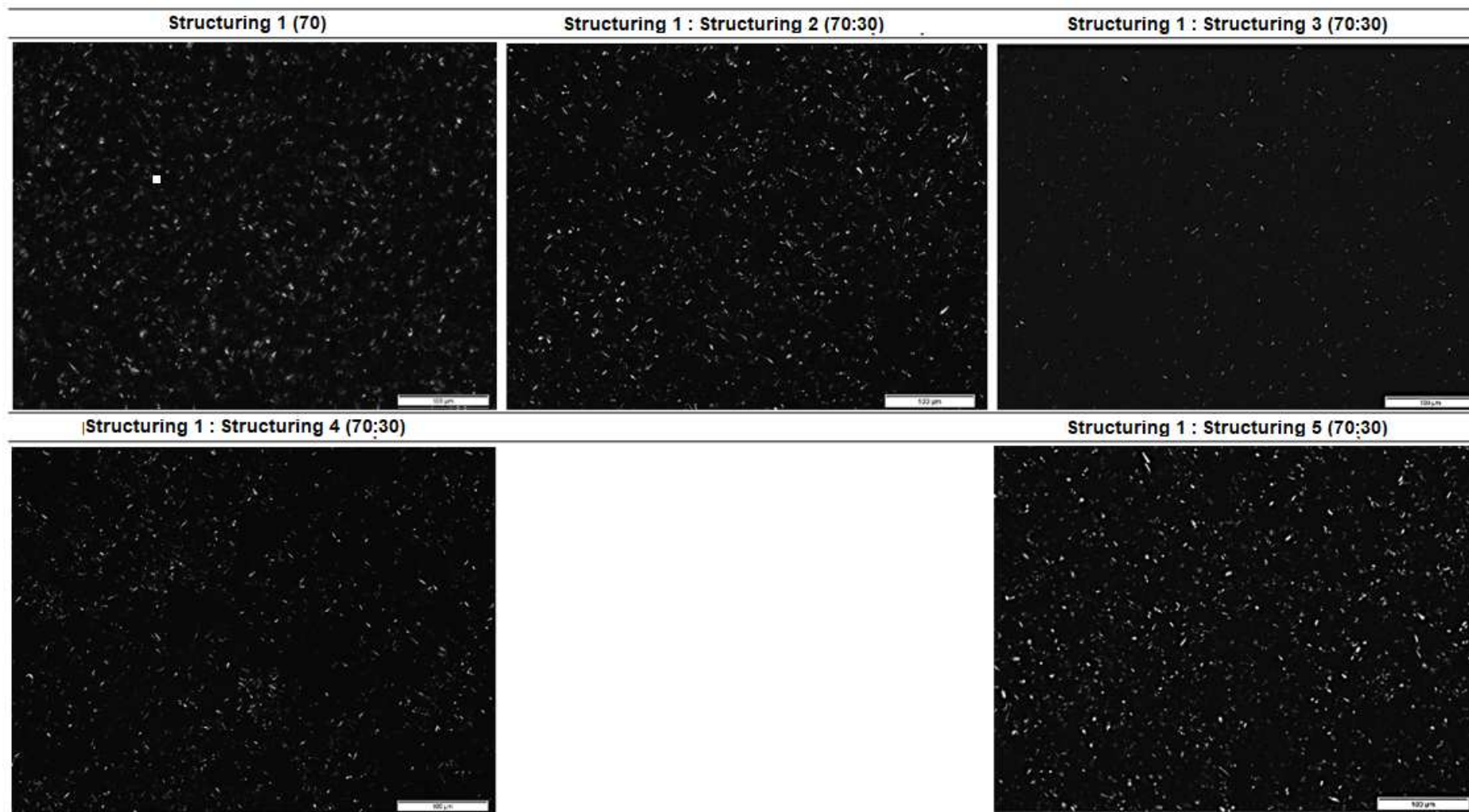


Figure 9: Polarized light microscopy (PLM) images for organogel samples in the proportions 50:50 for all structuring agents.

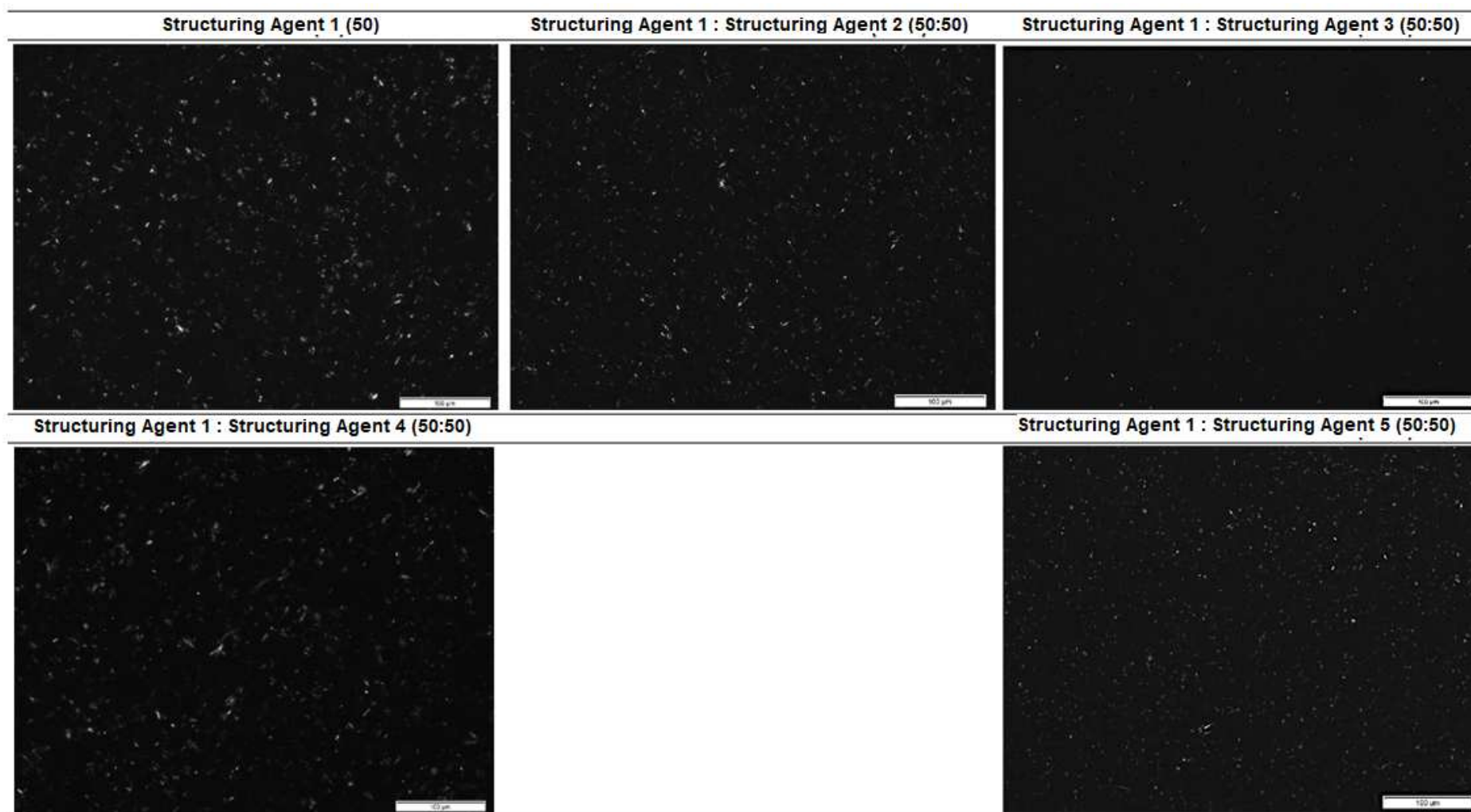


Figure 10: Polarized light microscopy (PLM) images for organogel samples in the proportions 30:70 for all structuring agents.

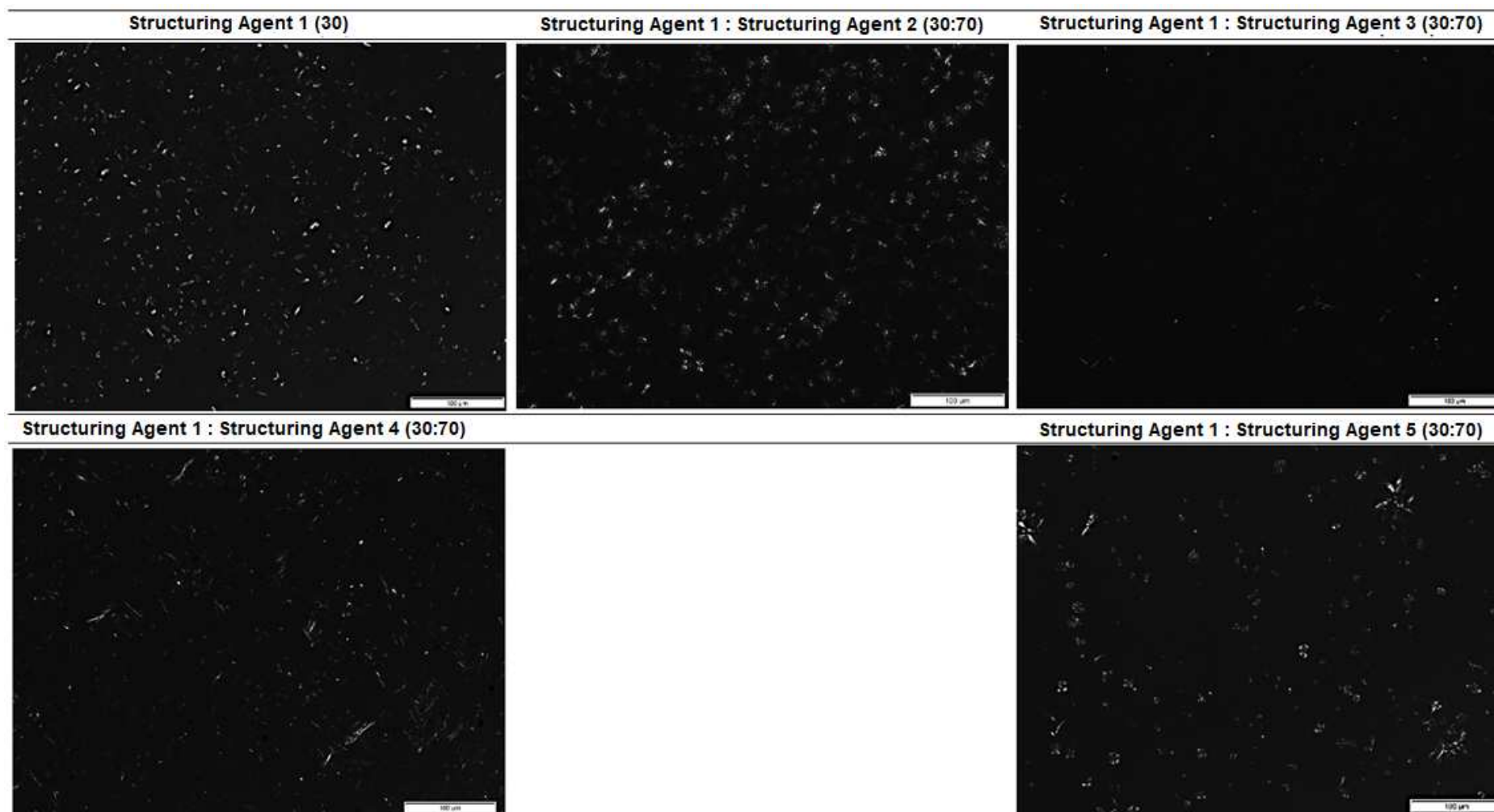
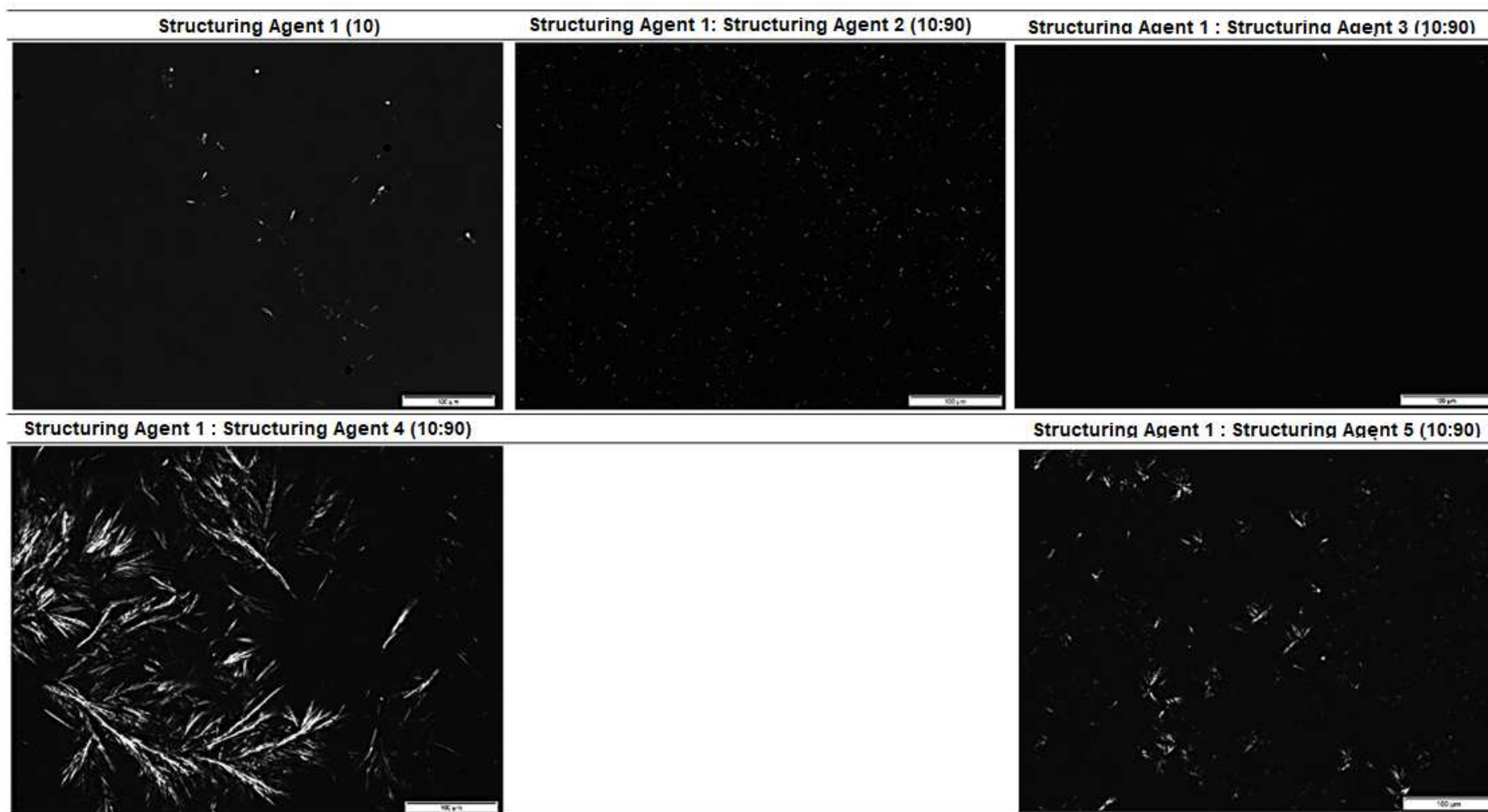


Figure 11: Polarized light microscopy (PLM) images for organogel samples in the ratio of 10:90 for all structuring agents.



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CAPÍTULO 4 – CONCLUSÕES GERAIS

Os efeitos nocivos das gorduras *trans* e saturadas na dieta humana estão claramente comprovados. Órgãos públicos em muitos países já promulgaram legislação e regras que controlam e restringem o uso dessas gorduras em alimentos processados que são comercializados em seu território. O consumo dessas gorduras contribui para mortes prematuras, aumenta o risco de doenças cardíacas, diabetes e câncer. Por outro lado, os benefícios do uso de óleos insaturados, como redução de colesterol, efeitos anti-inflamatórios e capacidade de carga e absorção de vitaminas essenciais também são bem citados. Os organogéis vieram como uma alternativa a essas gorduras sólidas com o objetivo de produzir alimentos mais saudáveis. As ceras estão entre os estruturantes comestíveis mais promissoras e sua eficiência é sensível e relacionada a fatores complexos como a composição química (origem) tanto da cera quanto do óleo a ser aplicado, presença de outros estruturantes ou ingredientes onde é aplicada influenciando os mecanismos de estruturação, processo de produção e aplicação de organogel. Estudos onde a aplicação desses organogéis de cera são apresentados, comprovam sua capacidade de substituir gorduras totalmente ou parcialmente sólidas, prejudiciais à saúde. Dependendo do tipo e da combinação dos estruturantes utilizados com a cera de candelilla, eles podem ter um efeito sinérgico ou antagônico, afetando um o efeito do outro. O teor de sólidos convencionalmente aplicado pela indústria de óleos e gorduras não está diretamente relacionado com a dureza e aplicabilidade do organogel, e mesmo que um gel com um elevado teor em sólidos não seja necessariamente o mais estruturado e resistente. A quantidade, homogeneidade e forma dos cristais é um dos principais fatores que influenciam a dureza de um organogel e, portanto, para organogéis onde há mais de um estruturador a escolha dos mesmos deve ser cuidadosamente avaliada nesse sentido. Explorando o efeito sinérgico de agentes estruturantes é possível formular sistemas de menores custos e reduzir possíveis efeitos adversos de alguns estruturantes como off flavour e cerosidade.

CAPÍTULO 6 – BIBLIOGRAFIA

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