USING MIXTURES OF STEARIC ACID AND β-SITOSTEROL TO SOLIDIFY VEGETABLE OILS BY INTRODUCING OLEOGELS

By

TZU-MIN WANG

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ABSTRACT OF THE DISSERTATION

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Recently, molecular gels have been studied with edible low molecular gelators and vegetable oils to respond to the increasing pressure to reduce the amount of saturated fat and *trans* fat in food products[1]. Molecular gels are a group of relatively new soft materials capable of numerous possible applications such as being an oil-spill recovery, drug carrier, cosmetic matrix, and fat substitute[2]. The gelators for forming molecular gel are low molecular weight molecules self-assembling into a three-dimensional network by means of supersaturation. The well-known 3-D network is self-assembly fibrillar networks (SAFiNs) capable of the entrapping high amount of liquid solvent (above 90 wt%) to provide the framework of molecular gels. It is widely accepted that supersaturation is the dynamic driving force for gelation kinetics; however, how the supersaturation affects the formation of a fiber network, particularly the creation of the junctions, remains poorly understood. But for a given gelling system, gelator concentration and gelation temperature can be described by supersaturation in solution. In addition, it has been studied that the force balance between gelator-gelator force and gelator-solvent force plays an important role in molecular gel formation even the mechanism behind it is still not clear. Thus, the aim of this

study is to develop an alternative to fat by using a mixture of health promotional small lipid molecules to self-assemble into the three-dimensional network for entrapping several vegetable oils. Furthermore, the physical property of molecular gels is investigated by changing the mixing ratio of small molecules and changing the liquid solvent with 27 different vegetable oils.

While choosing the edible gelators, β-sitosterol got our attention because not only plant sterols are free of saturated fatty acids but also they have been found to lower blood cholesterol by interfering with cholesterol absorption in the intestine[3]. Thus, a two-component system, stearic acid + β-sitosterol, with different concentrations and mass ratios was prepared with respect to their gelation ability, network structure, thermal property, and rheological properties in order to determine the optimum conditions under which canola oil gels. Results showed that stearic acid + β-sitosterol was a promising structuring agent to entrap canola oil by means of forming a network consisting of needle- and platelet-like crystals through supersaturation. In addition, the ability of gel formation was tailored by manipulating the concentration and ratio of the two-component system.

Based on the first stage of the study, stearic acid + β-sitosterol was a successful system to structure canola oil. Thus this system was chosen to apply on various oils to test the effect of liquid environment on gelation. Twenty-five edible oils and two non-edible oils were tested and distinguished into three groups based on the gelation result, which were oleogels, partial oleogels, and non-oleogels. It was found that oil having more portions of unsaturated fatty acids than saturated fatty acids introduces the high chance of oleogel formation. It meant that an oil having more in linolenic acid with a low percentage of saturated fatty acids (<10%) was likely to form an oleogel according to the results of hierarchal clustering analysis. More potential oils enabling

to be structured should be introduced by mixing the existed oils to match this high linolenic acid and low saturated fatty acid condition.

In order to maximize the benefits of oleogels being fat substitutes, oil-soluble compounds such as carotenoids were considered to be added when preparing the oleogels due to the antioxidative ability and health benefits of carotenoids. Mango (*Mangifera indica* L.) is the second most important tropical fruit and has high amount of carotenoids, especially *β*–carotene and zeaxanthin, which provides the color of yellow-orange of mango. Taking an advantage of oil solubility of carotenoids, canola oil was used to extract carotenoids out from fresh mango pulp via blending using an IKA ultra turrax Tube Drive control equipped with 30 stainless balls. Clear golden yellow canola oils were obtained after blending as a result of containing *β*-carotene and zeaxanthin according to the results of visible spectrum. The specific λ_{max} of 428, 450, and 480 nm were found as same as those from literature. Even though the changes made in solvents may break the force balance between gelator-gelator and gelator-solvent, the resulting oleogels were obtained using stearic acid + β-sitosterol as structuring agent. Thus, it is a promising approach to include carotenoids in canola oil and to form an oleogel. However, carotenoids are susceptibility to heat, the effects of processing temperatures on carotenoids were investigated. As the result of the visible spectrum, hypsochromic shift showed in the visible spectrum of the sample under long time processing at the high temperature of 90°C or above. According to the numbers of λ_{max} before and after long time heating, β-carotene and zeaxanthin converted to cis-violaxanthin. Thus, the processing temperature and time have definitely to be considered when the system is containing heat sensitive compounds. Overall, this final stage of study has illustrated even canola oil is modified by having carotenoid compounds, and it still can be structured, resulting in an oleogel.

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Table of Content

List of Tables

List of Figures

Abbreviation

12HSA	12-Hydroxystearic acid
AVO-B	Avocado oil in B brand
AVO-C	Avocado oil in C brand
β-Ss	β-sitosterol
Castor-A	Castor oil in A brand
Castor-M	Castor oil in M brand
CGC	Critical gel concentration
Chol	Cholesterol
Cottonseed	Cottonseed oil
DAG(s)	Diacylglycerol(s)
DSC	Differential scanning calorimetry
EVOO	Extra virgin olive oil
FSO	Flaxseed oil
FTIR	Fourier transformer infrared
LMOG(s)	Low molecular weight organogelator(s)
MAG(s)	Monoacylglyceride(s) or monoglyceride(s)
MNO	macadamia nut oil
MUFA(s)	Monounsaturated fatty acid(s)
PLM	Polarized light microscopy
PO	Peanut oil
PSO	Pumpkin seed oil
PUFA(s)	Polyunsaturated fatty acid(s)
RBO-C	Rice bran oil in C brand
RBO-K	Rice bran oil in K brand
S#B#	ex. S1B7 represents 1 wt% of stearic acid and 7 wt% of β-sitosterol
S#C#	ex. S1B7 represents 1 wt% of stearic acid and 7 wt% of β-sitosterol
Sa	Stearic acid
Sa-β-Ss	A two-component system consisting of stearic acid and β-sitosterol
Sa-Chol	A two-component system consisting of stearic acid and cholesterol
SAFiNs	Self-assembled fibrillar networks
SAXS	Small-angle x-ray diffraction
SFA(s)	Saturated fatty acid(s)
TAG(s)	Triacylglerol(s) or triacylglyceride(s)
WAXS	Wide-angle x-ray diffraction
XRD	X-ray diffraction

1. Research Background

Fats not only provide significant calories but also serve importantly many chemical, physical, and nutritional functions. They provide flavor, aroma, hardness, smoothness, creamy texture, and a lubricating mouth-feel that are desirable and required in many food products. Although fats are necessary for us, diets high in fats is related to numerous deleterious health implications ranging from obesity, diabetes, hypertension, cardiovascular disease, metabolic syndrome, high level of blood LDL-cholesterol (low-density lipoprotein-cholesterol), and cancer[1–3]. Hu et al. published a well-known long-term study on the risk of fat diets of 80,082 women aged 34–59 with no known diabetes, coronary disease, cancer, stroke, or hypercholesterolemia history.[4] During 14 years of tracking, each five percent increase in energy intake from saturated fat rather than carbohydrate increased the risk of coronary disease with relative risk of 1.17 (95% confidence interval). Each two percent increase in energy intake from trans fat had a relative risk of 1.93 compared to carbohydrate, which was even worse than the case of having saturated fat in the diet. To reverse the negative health implications of consuming fats, replacing saturated fats and trans fats with polyunsaturated fats may provide a plausible alternative. At present, it is still a challenge to reduce or eliminate saturated and trans fats without sacrificing some of their characteristic properties because these types of fats are responsible for the structure of colloidal fat crystal networks providing incredible mouthfeel, especially for those food products containing mainly fats.

Fat crystal networks are complex structural hierarchies (see Fig. 1-1) formed by triacylglycerols (TAGs) mainly. TAGs are composed of a glycerol backbone with three fatty acid moieties esterified to the three alcohol groups. Different types of fatty acids can be present in one TAG, and this involved mix of fatty acids in TAG constitutes the various crystal structures. These

different crystal structures can be recognized and characterized by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). At the beginning of the formation of fat crystal networks, TAGs align within each other spontaneously under thermodynamic driving forces, which results in the formation of distinct lamellar domains. Next, domains form single crystallites using non-covalent interactions, such as Van der Waals forces. Then, the single crystallites aggregate together to form nano-platelet clusters merging to form spherulites (flocs). Then, flocs further aggregate to produce three-dimensional colloidal networks. The final colloidal networks that constitute fats are so-called lipid hardstocks, providing the desired elastic properties, such as a snap, mouth-feel, and hardness of fats[5–7]. The physical properties, such as melting and crystallization behavior, solid fat content, nano and microstructure, and mechanical properties of fats and oils[8,9] are determined by the structural characteristics of triacylglycerides including their fatty acids' chain length, and the number, position, and configuration of double bonds on fatty acid moieties. The fatty acids on TAGs in fats are mostly composed of saturated fatty acids with a range of chain length usually from 4–22 carbon atoms with no double bonds, and in oils, they are mainly unsaturated fatty acids with 1–6 double bonds[10]. To maintain the unique physical property (i.e., solid-state property at room temperature) of fats, completely removing saturated fatty acids from fats is not possible. Thus, the study of solidifying or structuring oils has been a focus for developing alternatives to fat hardstocks. However, not all the approaches for hardening vegetable oils toward the direction of reducing saturated and trans fatty acids.

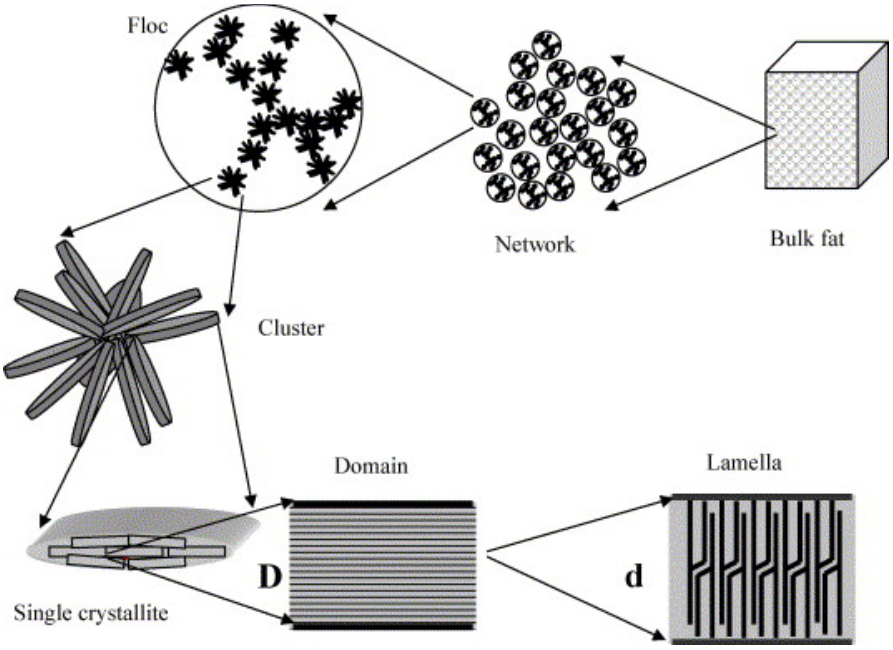

Fig. 1-1 Structural hierarchy in colloidal fat crystal networks (Taken from Tang and Marangoni, 2006[11]).

The most common and effective alternative to natural fat so far is hydrogenated oil. However, its byproduct, trans fatty acid, has been questioned due to its negative associations with health. Hydrogenation is a chemical reaction to solidify liquid oils, converting unsaturated double bonds on fatty acids to saturated single bonds by addition of hydrogen (H_2) with the aid of a metal catalyst, such as nickel. This technique was first patented in 1906 and was introduced into the U.S. in 1911 to extend shelf-life of oils. It was used extensively in manufacturing margarine starting in 1950 because it gives not only longer shelf-life but also better organoleptic properties than liquid oils and natural butter. By the 1970s, margarine sales were twofold those of butter due to its stable property and its similarity to butter concerning being able to maintain the texture of food products. Since then, hydrogenated oils have been used widely in food products such as cookies, ice creams, chocolate, bread, etc.[12–14]. However, during the addition reaction, *cis* form double bonds can open

up and reform into *trans* form, as well as shift positions of double bonds along the fatty acid carbon chain; these non-natural trans fatty acids are concerning for their adverse health impacts[15]. Many studies have shown that *trans* fatty acids are even more of a burden to the human body than saturated fatty acids; this conclusion has been achieved by measuring the increment of the number of biomarkers (such as blood low-density lipoprotein-cholesterol) of coronary heart disease[13,14]. Moreover, the ability of *trans* fatty acids to inhibit the synthesis of polyunsaturated fatty acids in the phospholipid of arterial cells[13]. With growing scientific evidence, in 2003 the Food and Drug Administration (FDA) issued a directive that trans fatty acids must be shown within the nutrition facts label if they are present in foods. In 2015, the FDA announced that trans fat is no longer considered a GRAS (Generally Recognized as Safe), and is not allowed in food products. The FDA asked food manufacturers to remove trans fat from existing products in three years[16]. It is imperative to find a new way to produce fat replacers, such as producing hydrogenated oil with low trans fatty acid content, establishing oilseeds with modified fatty acid profile through plant breeding or genetic engineering techniques, fatty acid interesterified triacylglycerols[15], and structuring vegetable oils. Among these alternative ways, molecular gels have been seen as a potential choice because they are capable of retaining the desirable fatty acid profiles of vegetable oils without chemical rearrangement of fatty acid chains, and have a solid-like property at room temperature. That is, molecular gels have been studied for their ability to form novel oil-based semi-solid materials to incorporate natural oil components without chemical modification. With the presence of self-assembled network formed by molecular gelators, it is believed that the mobility of oil is limited resulting in less fluidity to provide the similar rheological properties as those of fats.[17]

Molecular gels are relatively new “soft materials” having thermo-reversible and solid-like

properties. Molecular gels are prepared by heating a mixture of gelators and solvent until the solid of low molecular weight organogelators (LMOG(s)) dissolves and subsequently cooling it below its gelation temperature. During the cooling process, the mixture becomes a supersaturated solution, giving the system thermodynamic driving forces for LMOG(s) to overcome the chemical potential to crystallize into certain crystallites, as shown in Fig. 1-2. It has been found that only relative high-aspect-ratio crystallites such as rod-, platelet-, needle-, and fiber-like crystals[18,19] are the potential building blocks for constructing three-dimensional networks. No matter the shape of crystals, additional non-covalent interactions are needed to make them into volume-filling three-dimensional networks. Non-covalent interactions involve H-bonding, dipolar interactions, London dispersion forces, and pi-pi interactions[20]. The network formation includes fibrous/crystal network formation and domain network formation. Thus, a high amount of liquid solvent (above 90% generally) is immobilized in the three-dimensional network, resulting in the formation of a molecular gel.

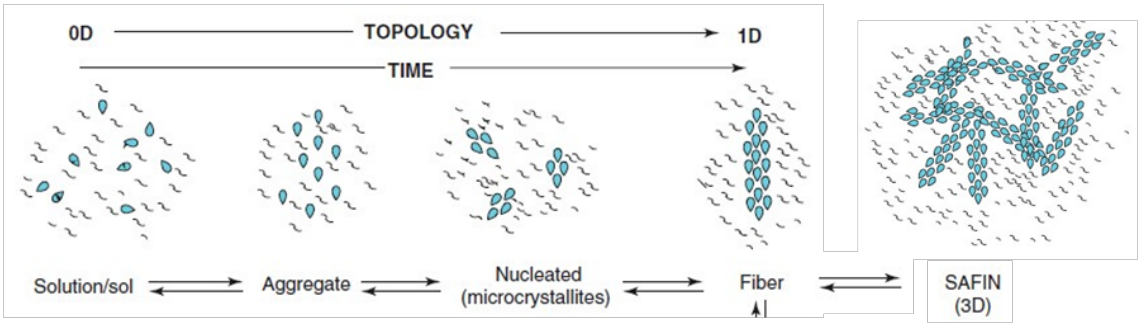

Fig. 1-2 Cartoon representation of how three-dimensional SAFiNs are formed from LMOGs[18].

The mechanisms of how a crystal network forms, how crystallites are linked with each other, and how the crystal networks are associated with each other determine the hierarchical structures of crystal networks and domain networks, and finally the rheological/mechanical properties of the

molecular gels[21,22]. Formation of specific crystallites and crystal networks can be controlled by manipulating both the choice of solvent and LMOG(s), and the cooling process, which relate to the concentration of solute, cooling rate, cooling temperature, and external force (see Fig. 1-3). Thus, altering the materials and the conditions of supersaturation gives the ability to design a fat replacer with the desired physical properties (such as elasticity and hardness).

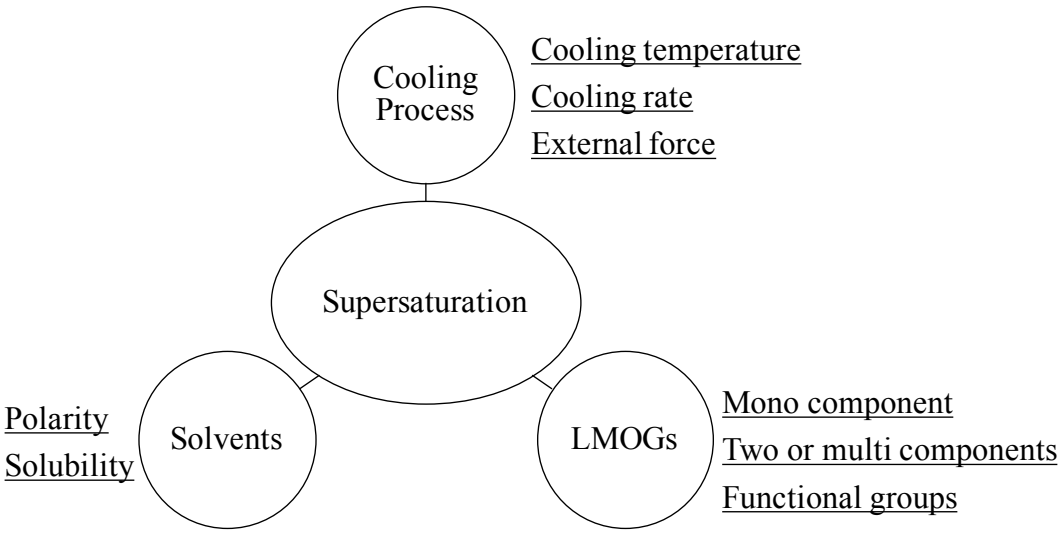

Fig. 1-3 Factors affecting supersaturation in molecular gels

An ordered self-assembled network, which is associated with the physical property of a molecular gel can be modified by types of gelators and solvents, and the process of cooling. Due to this modifiable network, molecular gels have a wide range of applications ranging from as a material for drug delivery[23,24], oil spill recovery[25], improver of chocolate and butter spread[26], and spider silk[18] to as a material in tissue engineering[18,27]. Thus, knowing how to tailor desired self-assembled crystals from LMOG-solvent mixtures and how these formed crystals further self-assembled into

crystal networks with non-covalent interactions are the keys to fabricating the desired soft materials.

Over the last decade, molecular gels have been introduced as a potent alternative to fat hardstocks due to their solid-like property at room temperature and similarity to the fatty acid profiles of vegetable oils. Numerous low molecular weight molecules with different functional groups were used to study their ability to structure vegetable oil by a trial and error method. Among those molecules that can structure oil, two types of crystallites have been found. One is a group of non-fiber-like crystallites, which have structures similar to a stack of triacylglycerols[28]. Monoacylglyceride[29] (MAG), diacylglyceride[30] (DAG), fatty acid[30,31], fatty alcohol[20,30,31], and wax[32–34] belong to this group. The other one is a group of fiber-like crystallites, which form self-assembled fibrillar networks (SAFiNs). SAFiNs are currently considered the more effective networks to immobilize liquid solvent because they have a higher surface-to-volume ratio that contributes to increased likelihood of entanglement within crystallites. More entanglements may enhance the strength of crystal networks, resulting in a harder molecular gel. So far, a few LMOGs (such as 12-hydroxystearic acid[35–37] and β-sitosterol + γ-oryzanol[38–40]) that form effective fibrillar crystals or tubules in order to construct SAFiNs have been found. This is reportedly the most complicated level to modify but highly associated with the macroscopic properties of molecular gel such as hardness, stability, and oil mobility[18]. Also, there are many types of fiber branching (i.e., fiber tip branching, type 1 and type 2 fiber side branching) found in the process of fiber formation. Thus, there are many unknown possibilities in network formation.

In this increasingly researched area, scientists are endeavoring to find the more effective edible system and to understand the mechanism of gelation by manipulating the LMOG compositions and conditions of supersaturation. Among all the factors that can be controlled, mixed gelator

systems or co-gelators have been studied very little, but the ability to modify the network structure using multi-gelators could be a promising avenue to manipulate the physical properties of molecular gels[8]. For instance, a sorbitan tristearate in sunflower oil becomes a molecular gel from a pourable solution with the addition of lecithin due to modification of the shape of crystals from a platelet- to a needle-like. Thus, the aim of this study is to develop an alternative to fat by using mixtures of health-promoting small lipid molecules (β-sitosterol and stearic acid) to self-assemble into a three-dimensional network for entrapping 27 vegetable oils. The influences of a variety of vegetable oils on the formation of crystals and networks are investigated in this study as well. Moreover, to produce healthier fat alternatives, oil containing carotenoids from fresh mango fruit are made to be entrapped by an established two-component system.

2. Literature review

2.1. Gels

2.1.1. What is a gel?

In 1861, Thomas Graham loosely defined the term "gel," relying on qualitative macroscopic observations that were available at the time[21]. His description of a gel includes "while the rigidity of the crystalline structure determines external expressions, the softness of the gelatinous colloid shares of fluidity, and enables the colloid to become a medium for liquid diffusion" [41]. In 1926, Dorothy Jordan-Lloyd provided another definition: "the colloidal condition, the gel, is easier to recognize than to define"[42]. She proposed a more thorough definition of a gel – that is, a substance is a gel if it (1) has a continuous structure with macroscopic dimensions that is permanent on the time scale of an analytical experiment and (2) is solid-like in its rheological behavior[21]. Through

over a hundred year of studying on gels by scientists from physics, chemistry, chemical engineering to biology, it makes it impossible to define what a gel is with consistency. However, they all seem to emphasize that at least two components are required to be considered a gel. Finally, a milestone was established by Flory and Stockmayer. They defined a gel with a perspective of structural criteria, such as the concept of three-dimensional network, coherence connectedness.[42] After that, Burchard, and Ross-Murphy defined it more clearly saying that a gel has a characteristic of viscoelastic, which can be measured by a rheometer, indicating storage modulus is higher than loss modulus. Later, Ferry[43], as well as Almdal and coworkers[44] stated fairly that a gel is a soft, solid or solid-like material, which consists of two or more components, one of which is a liquid, present in a substantial quantity[45].

2.1.2. Classification of gels

Since gels are mostly liquid, they can be classified based on the nature of the liquid they carry (i.e., hydrogel, emulgel, and organogel). A hydrogel is a gel that has a liquid phase of water, which is existed commonly in many food products. Agar, starch, pectin gels, and even cheese curd composed of casein proteins all belong to this group[45]. Relative less commonly noticed but equally important is an organogel. The "organ-" in organogels implies substantial liquids in organogels are organic solvents including oils[45,46]. In the same manner, an emulgel, such as lecithin gel[24], is a gel having an emulsion in it instead of the aqueous phase or an organic solvent.

So far, an accepted classification of gels was proposed by Flory[41,42], stating four type of gels based on structural criteria. A well-ordered lamellar crystalline, including gel mesophases, belongs to the first type. The disordered covalent polymeric networks dispersed in a liquid phase is considered as the second type. The third type of gels has mainly disordered noncovalent

aggregated polymer networks with few regions of ordered physical aggregation of polymers. The final one is made of particulate disordered structures. It is clear that molecular gels of focus topic is this thesis belong to particulate disordered structures, the last type of gels[47].

2.2. Molecular gels

2.2.1. Definition

A molecular gel is a gel that has a low concentration (commonly below 10%, w/w) of LMOGs, which immobilize various organic solvents as a result of the formation of a thermal reversible three-dimensional supramolecular network. In a molecular gel, a supramolecular network usually occurs when a hot solution containing dissolved LMOG in an appropriate liquid becomes supersaturated. It means that the solution cools below to specific temperature which is known as gelation temperature. In this supersaturated condition, microscopic phase separation occurs, rather than the macroscopic phase separation where precipitation occurs. Microscopic phase separation refers to crystallites formed by LMOG(s). The LMOG self-assembles in stochastic nucleation events involving highly specific non-covalent interactions (e.g., hydrogen bonding, π-π stacking, electrostatic interaction, van der Waals forces, and steric entanglement[20]). Literature shows that these forces may allow molecules to grow along a one-dimensional direction, which results in the formation of fibers and their likelihood[48]. The one-dimensional objects play a role as polymer chains in polymer gels. Subsequently, entanglement of the one-dimensional or even two-dimensional crystals produces a three-dimensional network capable of trapping the solvent and yielding the gel which is a class of viscoelastic materials.

2.2.2. Mechanism of molecular gel formation

Molecular gels have been studied to structure organic solvents with a variety of molecules for figuring out the answers to unsolved questions. The top one question is if there any structural requirements for a small molecule being a low molecular weight organic gelator. The following up question is that how these LMOGs rearrange into the different packing structures in the given solvent rather than the packing arrangements in their solid crystalline states. Also, the effects of these packing structures on the physicochemical properties of molecular gels such as optical, thermodynamic, rheological properties, remain more studies.

The gel property of molecular gels arises due to the high-aspect ratio of crystals and self-assembled network formation. The most common way to induce crystallization of LMOGs for gel formation is supersaturation, which is easily controlled by the temperature. It is a process with two steps, which involve increasing the solubility of LMOGs in a given solvent by heating, and triggering supersaturation by lowering the temperature below the gelling temperature. However, among all the low molecular weight molecules, only some of them are able to form self-assembled fibrillar networks (SAFiNs) as monomeric sub-units. To date, these organogelators are found mostly by serendipity rather than design. As knowledge has developed, low molecular weight organogelators have been found which self-assemble into a 3D continuous network consisting of fibers, which are as a result of LMOGs crystallizing in only one axis via non-covalent interactions[49,50], including hydrogen bonding, pi-pi stacking, and Van der Waals forces. Due to this 3D volume-filling network being composed of fibrillar crystals, the term SAFiNs [35,51,52] obtained its name. However, with more and more studies on molecular gels in recent years, it has been found that not only fibers (a type of crystal morphology) can form networks, but also two-dimensional crystals such as platelet-like[31,53–56] and needle-like crystals can form networks

resulting in molecular gels. Owing to a variety of crystal morphologies, this brings even more complexity to the field of molecular gels and this complexity could perhaps be described as the most exciting part of this research area. Increased combination of low molecular weight molecules, organic solvents and conditions of processing can be played with to explore the mechanisms of formation of molecular gels, which are relatively new soft materials.

2.2.3. An important factor in molecular gel formation - Solvents

Since solvents and gelators are the only components comprising molecular gels, they play the main roles regardless of the condition of processing. In particular, solvents, which are the major component (above 90 wt%) of molecular gels, have formed a particular research focus in order to increase understanding of their role in molecular gel formation. In this section, the studies of the influences of solvents on the supramolecular architectures in molecular gels are summarized and elucidated.

Investigating the precise role of organic solvents and their effects on stabilization of formed molecular gels is believed to be an essential topic for the development of LMOGs. It has been found that the chemical nature of the solvent and solvent-gelator interactions are equally crucial for influencing polymorphic forms of self-assembled crystals in molecular gels. Zhu et al.[57] reported that the changes in the structural transition from a single dipeptide building block, diphenylalanine, into a molecular gel or a flower-like microcrystal precipitants were due to the changes in a solvent. A molecular gel obtained was made of diphenylalanine and toluene. The change in the solvent was made by having a mixture of ethanol and toluene as a function of mixing ratio, instead of toluene itself. The heated solution was turning into a macrophase separation liquid with the precipitants in the microstructures of flower-like crystals rather than forming a molecular

gel. Authors proposed a credible explanation of involving ethanol in interacting with the gelator, diphenylalanine, during the cooling process due to the presence of hydrogen bonds of ethanol, which can be a hydrogen bond donor. It also means that the gelator-solvent interaction is stronger with the increment of ethanol involved. Compared with ethanol, toluene, a less polar solvent, interacts with diphenylalanine using pi-pi stacking. Though the addition of ethanol increases the gelator-solvent interaction which may interfere the gel formation, a small amount of ethanol (less than 10 wt%) does not interfere the formation of a molecular gel. It refers that the gelator-solvent attractive force due to an addition of ethanol is still not strong enough to destroy the balance between gelator-gelator and gelator-solvent forces. It is believed that a molecular gel can be formed by controlling the amount of addition ethanol precisely because the interference of hydrogen bonding provided from a solvent influences the self-assembly process.

A molecular gel made of 1-octanol and a cholesterol-derived gelator was analyzed for revealing the amount of a solvent interacted with the gelator by Wang et al.[58] Only 30% of 1-octanol was inside the fiber structures with the remaining 70% of solvent molecules in the space between fibers. It is consistent with the observation that a particular gelator can only gel selected solvents. Since the other 70% of solvent molecules are simply trapped between the fibers, it seems possible that another solvent might replace this part of the mixture, provided that it does not dissolve the gelator molecules. The successful gelation of a mixture containing 30% 1-octanol and 70% hexane (which alone is not gelled) by as low as 1% gelator supports this assumption.

Another study has provided some clarification regarding the effect of solvents on crystal morphology as well. Wang and Hao[59] found that a surfactant molecule, sodium laurate, can self-assemble into a fibrillar network; however, it only happens in certain solvents (i.e., alcohols). It is related to the solubility of sodium laurate in solvents. Compared to hexane and benzene, alcohols

have hydrogen bonding which can attract the carboxylic acid part of sodium laurate. However, the chain length of carbons in alcohols affects the affinity of sodium laurate to alcohols leading the decrement of solubility. It again indicated that the low molecular weight molecule becomes a gelator only in a matching solvent. Moreover, solvent plays a role in influencing the crystal morphology. Through the images taken using scanning electron microscopy, the shorter and denser fibers were captured in the sample with long chain length of alcohols (i.e., 1-heptanol and 1-nonanol) than those in a sample with short chain length of alcohol (*i.e.*, 1-propanol). In order to quantify the influence of solvents on molecular gel formation, Hydrogen-bonding Hansen Solubility Parameters (HSP) are used to quantify the properties of organic solvents to determine whether there is any relationship between HSP and gelation capability, which can allow prediction of gelation formation.

It is not only organic solvents which have been focused on in studies of the mechanism of forming a stable molecular gel, but organic-aqueous interfaces also have gained significant interest regarding widening its applications in many aqueous systems. For instance, fluorescence molecular gels have potential application for the preparation of optoelectronic devices and sensors for sensing water-soluble analytes such as ions or biomolecules in aqueous interfaces employing an adequate sensitive fluorophore dopant. Bonifazi et al.[60] reported a synthesized cholesterol-derived LMOG used to immobilize fluorescence-containing organic solvents as a result of a stable molecular gel, the stability of which is even enhanced with 10% additional water. The critical gelation concentration in the 90/10 methanol/water mixture decreased to 0.44% w/v compared to 1.6% w/v of original without water, in agreement with a stronger fibrillar network in the presence of water. Another piece of evidence is that the FT-IR analysis of the gelling process also revealed that two hydrogen bond interactions are involved in the supramolecular self-assembly. Therefore,

changes in solvent affecting the gelation make the area of molecular gels more possibilities along with bringing more puzzles.

2.2.4. Another key factor in molecular gel formation - Gelators

Compared to solvents, the development of gelators has been a focus all the time since the choice of solvent is limited. Especially for the food products, aqueous is the primary medium followed by a mix of oil and water, and then oil phase. Therefore, over the past twenty years, the finding of low molecular weight gelators and understanding the mechanism of gel formation have been studied numerously. So far, there is no principle found yet to obtain a matching gelator in given solvent. However, the small molecules with specific chemical structures, such as H-bonding, a ring structure with double bonds, have been recognized as potential candidates, which may increase the non-covalent interactions.

For instance, cholesterol derivatives have been found to be efficient gelators, presumably due to the tendency to form one-dimensional stacks of the steroid units, and it has been suggested that this is the primary driving force for gelation. Compounds with much simpler chemical structures, such as alkanes, fatty acids, waxes, peptides, and surfactants have also been investigated to determine their gelation capability. In this section, those studies involving vegetable oils as solvent are not dealt with, but these oils are summarized and discussed in section 1.3. Only cases involving organic chemical solvents are included here.

Wang and Hao[59] proposed that sodium laurate is capable of forming molecular gels in alcohols because Na^+ ions can induce the transition from spherical micelles to crystalline cylindrical micelles, whereas other ions such as Li^+, K^+, Ca^{2+}, and Mg^{2+} do not have this capability. They

would destroy the formation of nanofibers in the same given environment of solvent. According to Shirakawa et al.[61], porphyrin acts as a potential central core to form a one-dimensional molecular stack for organogel fibers composed of synthesized designed gelators (i.e., the amide groups at the 4-position of the *meso*-phenyl groups). Besides, compared to the amide groups at 3, 5-position or 3-position, the one with an amide group at 4-position was a versatile LMOG which was capable of gelling 10–14 of 23 solvents.

2.2.5. Applications

There are several specific characteristics and applications of molecular gels making them valuable for studying and developing. First of all, a molecular gel has solid-like behavior[55,62], with spreadability, which can be a substitute of many products from fat hardstock, lubricant, to oil paint. Secondly, the supramolecular structure of a molecular gel is thermo-reversible. Its supramolecular structure, a self-assembled three-dimensional network, restrict the mobility of solvent giving the molecular gel a capability of restriction of oil migration[25]. It is a very beneficial characteristic of some food and paint products. For example, the phase separation of peanut butter can be retarded by applying the molecular gel[63,64]. Thirdly, the molecular gel has the high amount of organic solvent, which can carry many insoluble nutraceuticals in it as a carrier or a delivery holder. Finally, only small amount of gelators is needed for producing a molecular gel to lower the cost if gelators are expensive.

2.2.5.1. Delivery vehicle with controlled release

The use of organogels (one type of molecular gels) as delivery vehicles (particularly in transdermal drug delivery) in pharmaceuticals and cosmetics is an active and well-developed field

of research, due to characteristics of solid-like, spreadability, and non-aqueous system. Lecithin has been used commonly in making organogels for delivery vehicles because of its amphiphilic property[24,65]. As an amphiphilic compound, it can dissolve water-soluble and insoluble compounds, which is suitable for the pharmaceutical and cosmetic products with complex formulas. For instance, lecithin organogel has been applied in making a lip balm. It provides the desirable solid-like property with spreadable property by applying low force on it, since it is examined as a low yield stress material. The restriction of oil migration works ideally on lip balm products preventing oil leaking. Besides, moisture, flavor, and coloring agent can be either entrapped or dissolved in organogels.

As a delivery vehicle, it has been required to have controlled release technology, especially in pharmaceutical industry[66,67] Controlled release technology is delaying or controlling the releasing rate of drugs from "vehicles" to the bloodstream to provide the most effective medication[68]. Nutraceuticals form another key research area attracted by controlled release. The most common vehicle is the hydrogel which has polymers as gelators to form three-dimensional polymeric networks via cross-linking to entrap water and a bioactive compound. A bioactive compound is protected by the hydrogel from the acidic environment of the stomach[68,69]. However, many valuable bioactive compounds and functional ingredients are water insoluble, such as carotenoids, flavoring compounds, and essential unsaturated fatty acids[25,26,55,70]. Mun et al[71]., reported a bioaccessibility of β-carotene sourced from a starch-based filled hydrogel with methylcellulose as a gelator. Through the result of the analysis of an in vitro human stomach model, they found that increasing the usage of methylcellulose does not help increase the bioaccessibility with β-carotene due to interfering the extensive lipid droplet flocculation. Besides, they had to form lipid droplets first to include β-carotene and further mixing in the aqueous system. It seems that the organogel

is a better approach than the hydrogel for encapsulating those hydrophobic bioactive compounds in a gel system.

2.2.5.2. Fat/hardstock replacer

Molecular gels, which contain edible oil as a liquid solvent and have a solid-like property, can be potential candidates of solid fat replacers. Solid fats are principally composed of saturated fatty acids and *trans* fatty acids. Numerous studies have shown that a high amount of saturated and *trans* fatty acids leads to increased cardiovascular disease risk. So the reduction or elimination of saturated and *trans* fatty acids from the diet which especially sources from high fat, is an approach to reduce the consumption of saturated fatty acids and *trans* fat. However, it causes the severe problem in the food manufactures, like making a change in formulas without influencing the texture including solidity, and hardness and mouthfeel[20,46,62,72,73]. It is challenging to replace solid fat with healthy vegetable without changing physicochemical properties of final products. Moreover, it brings the difficulties during the process. Because vegetable oils are liquid forms at room temperature in general, they do not have elasticity and oil-binding capacity

For instance, the addition of vegetable oils in a bread formula causes the oil leaking out of dough during the dough kneading process. Moreover, without the solidity of the fat, vegetable oils cannot form a barrier between dough sheets in order to produce pastry products, such as croissants and danish, which provides desirable multi-layer and crispy mouthfeel[74–76].

In contrast, molecular gels with vegetable oil as solvent provide solid-like property even though with a large number of vegetable oils. Having mainly polyunsaturated fatty acids rather than saturated fatty acids and *trans* fatty acids can lower the risk of cardiovascular diseases[77–79]. \

Besides, molecular gels can be tailored to desirable physical properties by controlling the concentration of gelators, modifying on the solvents, and condition of a cooling process, which is similar to fats[26,80–82]. For example, tempering process turns an α-crystal to a β'-crystal by controlling temperature for providing better mouthfeel to consumers. It is a critical requirement for making a top-grade chocolate. Several molecular gels have this similar favored crystal form by selecting the gelator and controlling the concentration of gelators. For instance, 12-hydroxystearic acid[35,35,83], ricinelaidic acid[36,84], candelilla wax[32,85,86], mixtures of β-sitosterol and γ-oryzanol[39,87–91], mixtures of lecithin and sorbitan tri-stearate[92–94], and mixtures of stearic acid and stearyl alcohol have been reported for their ability of structuring either solvents or oils.

2.2.5.3. Inhibition of oil migration

In complex food systems, oil migration is a serious problem associated with shelf-life of food products due to oxidation of leaking oil or texture defects such as fat bloom in chocolate[25,26]. Three-dimensional networks formed in molecular gels can entrap high amounts of liquid oil in preventing or delaying oil migration.

Adding 12HSA (12-hydroxystearic acid) into peanut butter prevented phase separation by thickening/gelling the peanut oil[95] . Applying molecular gel into chocolates or cream-filled chocolates can prolong the time of forming fat bloom and can also reduce the need for saturated and *trans* fatty acids. Further, in cream-filled cookies this oil migration can lead to softening of the cookies. It was hypothesized that by gelling the liquid oil portion of the cream, the oil would be prevented or slowed from migrating, thereby eliminating or delaying the aforementioned defects.

Though many versatile applications have been proposed, the mechanism of molecular gels is still not clear at all. Especially there are only a few studies working on edible oils as solvents. Those LMOGs that form a crystal close to β′-form crystals cannot be predicted at all. The gelation formation or the desired crystal form is all about luck. In recent, two- or multi-component systems have gained more interests due to more possibilities.

2.3. Oleogels (Oil as the solvent)

Oleogels included in molecular gels are semi-solid gels made of low molecular weight organic gelators and oils as the portion of solvents. Interest in oleogels has increased over the last decade because these gels provide a promising alternative to solidify vegetable oil for creating fat replacements with the requisite firmness, low saturated fats, *trans* fat free, and high amounts of healthy unsaturated fatty acids.

The first low molecular weight gelator candidate was TAGs for producing oleogels. However, the high level of saturated fatty acids of TAGs needs to be used to achieve the goal of solidifying vegetable oils. Dietary TAGs contain a high amount of saturated fatty acids are associated with the negative impact on health., such as obesity, cardiovascular diseases, and diabetes[96]. In order to determine which low molecular weight molecules can be gelators to structure oils, trial and error has been primarily used, with serendipity playing a large part in this process. However, these experiments have not been entirely unguided, with the belief that those molecules with hydroxyl groups or double bonds in their ring structure should theoretically be highly suitable candidates because oleogels are stabilized by non-covalent interactions between molecules, such as hydrogen bonding, Van der Waals, and pi-pi stacking. Besides, it has been found that not only mono-component gelator system can structure oils, but also multi-component systems create even more

possibility of enabling the structuring of oils. So far, some low molecular weight molecules such as fatty acids[31], fatty alcohols, monoacylglycerols[29,97], waxes[82,85,98], and sorbitan monostearate[93,94,99] have been reported for their reasonable success in structuring oils under certain conditions. Several two-component systems (or so-called mixed systems) have been studied, i.e., lecithin + sorbitan tristearate, fatty acids + fatty alcohols[31,95,100–102], and phytosterols + γ-oryzanol (sterol ester)[3,39,87,91,96,103]. Therefore, in the following paragraphs, selected systems will be summarized and discussed based on these two classes: mono- and multi-component systems.

2.3.1. Mono-component oleogels

The requirement of gelators for structuring vegetable oils is a severe limitation because those potential gelators are required to be used in edible food systems. In spite of that, several systems have been identified based on their history of consumption (e.g., wax esters occur naturally in many vegetable oils, and free stearic acid is present in kokum (Garcinia indica)[31] fat naturally). Thus, in this section, wax, fatty acid, and derivatives of fatty acids will be summarized and discussed showing the possible reasons why these molecules are capable of structuring vegetable oils, and physico-chemical properties of corresponding oleogels.

2.3.1.1. Stearic acid and Stearyl alcohol

A patent has reported an approach to thicken edible oils by dissolving edible solidifying agents in edible oils under heat and then subsequently cooling the oil solutions to thicken the oils. A specific edible solidifying agent mentioned in the patent is the mixture of behenic acid and stearyl alcohol. In addition, saturated fatty acids play key roles in forming colloidal fat crystalline networks when they are esterified onto the glycerols as the major part of TAGs. Thus, the capability of structuring

oils of free fatty acids has gained significant interest in addition to that shown for fatty alcohols. The abbreviation Cn-acid and Cn-OH, with n = carbon numbers on the backbone chain, will be used for the n-fatty acids, and n-fatty alcohols respectively in the following paragraphs.

Concentration is an inevitable variable when investigating the gelation capability of potential structurants because in general there should be a critical gel concentration for each gelator's ability to structure oils. Both fatty alcohols and fatty acids were reported to structure sunflower oil or lavender oil at concentration as low as 2% (w/w)[31,100]. The structuring observed was attributed to particles forming a network via hydrogen bonding. Furthermore, Gandolfo et al. observed that the hardness of oleogels was increased with the increasing concentration of either fatty acids or fatty alcohols due to more solids being formed at the same controlled temperature. One possible reason for this observation is that solubility in the same oil is constant at a fixed temperature.[100] In addition, at the same concentration, it was found that the gels made of fatty alcohols were harder than those made of fatty acids, with the exception of palmitic acid. It seems that fatty alcohols are the better structuring agents for oil than fatty acids. However, Daniel and Rajasekharan reported that fatty alcohols had lower efficiencies compared to fatty acids. After cross comparing, the efficiencies were found to be affected by oils. Same chain length fatty acids or fatty alcohols showed different gelling efficiencies when they were investigated in sunflower oil, soybean oil, lavender oil, and so on.[31,55,100]

The microstructures of both fatty acid-gels and fatty alcohol-gels were captured by Gandolfo et al. using polarized light microscopy. The stearic acid gel shows well-defined lozenge-shaped crystals in the size of 200 um and the stearyl alcohol gel shows large platelet-like crystals of about 1800 um in size (Fig. 2-1). In this case, even though the crystal size found in stearic acid-gel was smaller than those in stearyl alcohol gel, the hardness of stearic acid gel was subtly lower than

stearyl alcohol gel. More research on the relationship between structurants, crystal formation, and rheological behaviors is required.

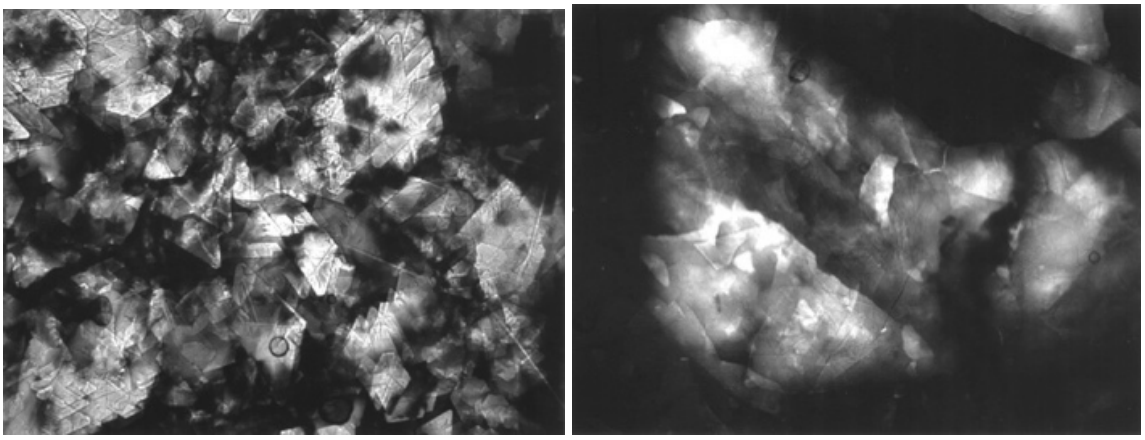

Fig. 2-1 Micrograph of stearic acid, and stearyl alcohol at 5% (w/w) in sunflower oil after 1 d at 5°C (width 1900 um)[100].

2.3.1.2. Rice Bran Wax

In recent years, rice bran wax derived from rice (*Oryza sativa*) has shown increased potential for structuring oils compared to candelilla wax. Only 0.5% of rice bran wax compared to 2-5% of candelilla wax was able to structure soybean oil[56]. However, the critical gel concentration (CGC) of rice bran wax varies from 0.5% to 5% in soybean oil under the same processing conditions depending on its sources. Because most refined wax esters principally contain esters of fatty alcohols and fatty acids with varying chain lengths, the compositions of fatty acids and fatty alcohols in rice bran wax depend on different rice sources[17,104,105]. As mentioned, rice bran wax is a natural wax derived from the bran of milled rice kernels, so the advantages of rice bran wax are their high availability and low cost due to high production of rice, particularly in eastern Asia[17].

The microstructures of rice bran wax oleogels support the network forming by rice bran wax. The long (20-50 μm), strong needle-like crystal matrixes, which are easily able to entrap large amounts

of canola oil, were captured using polarized light microscopy (Fig. 2-2). A possible reason for this observation is that rice bran wax has long chain fatty acid (16 to 24 carbons) and fatty alcohol (24 to 38 carbons) esters with a higher melting temperature (78-81°C) compared to candelilla wax. Due to this natural characteristic, rice bran wax tends to crystallize easily at room temperature, resulting in finely dispersed crystals and enabling entrapment of large amounts of oils.[104] The firmness of crystal matrixes for entrapping oils was also shown in hardness analysis. Dassanayake et al.[104] investigated the gelation ability of rice bran wax and candelilla wax in olive oil, salad oil, and liquid paraffin by analyzing their microstructure and the hardness of corresponding oleogels. Rice bran wax has the higher hardness assessed by measuring penetration depth due to its long needle-like crystals in a comparison with the spherulites found in candelilla wax oleogel.

Since rice bran wax formed long needle-like crystals, which are promising in terms of their ability to structure oils, the effect of oils on oleogel formation was of interest. The influences of oils were shown not only on the critical gel concentration but also on the crystal morphology. Only 0.5% (w/w) of rice bran wax was needed for structuring olive oil and soybean oil, while up to 4% was required for salad oil (a mixture of 50% of canola oil and 50% of soybean oil). No significant difference was observed in crystal morphology between soybean, canola, olive oil and salad oil; however, rather round crystals which were not conducive to oleogel formation were observed in liquid paraffin[104]. Thus, oils also play important roles in oleogelation.

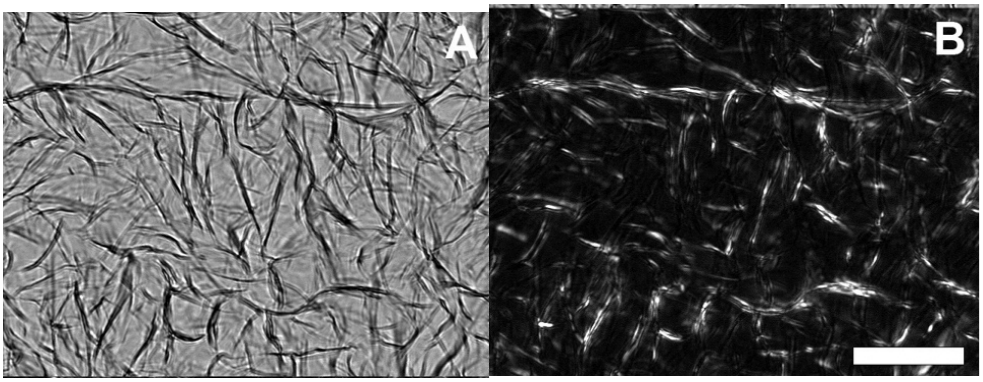

Fig. 2-2 Non-polarized (A) and polarized light (B) microscopic images of rice bran wax in canola oil at a concentration of 5% (w/w). Magnification bar = 50 μm.[106]

Although rice bran wax has been reported as a successful structurant for structuring oils and could be obtained in high production with low costs, the oily appearance, wax mouth feel and taste are rather unsuitable for use in food products. For example, when it was used in ice cream to replace milk fat, the droplet behaviors were similar to fat crystalline; however, it did not help form and stabilize the specific air bubbles to reduce the structuring collapse while ice cream was melting. In addition, rice bran wax has not been proposed to provide better or even the same health benefits as other reported structurants. Thus, more research on rice bran wax or on finding similar wax esters from natural sources is necessary.

2.3.1.3. Monoacylglycerides or monoglycerides (MAGs)

Monoglycerides are well known for their ability to structure vegetable oils by playing a role similar to that of TAGs in forming colloidal fat crystalline networks. It is quite remarkable that MAGs can structure oils at very low volume fractions of solids. While they are prone to polymorphic, or more appropriately mesomorphic, transformations in time, which causes the release of oil, many valuable lessons can be learned to help guide future endeavors. Ojijo et al.[107] investigated the changes in microstructural, thermal, and rheological properties of a MAG-olive

oil oleogel during storage at room temperature (25°C in this case). At the onset of storage, the microstructures were captured using polarized light microscope. The irregular, elongated, or rod-like crystal aggregates were observed which are similar to those seen in typical fat crystals. After three-day and eight-week storage at room temperature, significantly more crystal aggregates were observed as a function of time.[107] This phenomenon is attributed to the closer packing of hydrocarbon chains in the monoglyceride bilayers, reorganization and proliferation of the three-dimensional network. The melting temperature was observed to change from around 63°C (peak temperature) at the initial of storage to 58°C after two-week storage and then measured at 58°C thereafter by differential scanning calorimetry. However, no increment of crystallinity was observed, which may be explained by a reorganization of the crystals due to the dynamic equilibrium of the dissolved MAGs in the olive oil.[108] Thus, the melting temperature decreased as a function of time in two weeks. Increasing hardness over time was proof of reorganization of crystals in order to strengthen the non-covalent bonds between crystals.[107,108]

The effect of types of oils on microstructure in oleogels was also observed in MAG-oleogels. Kesselman and Shimoni reported that needle-like crystals of 5-15 μm in size were observed in an MAG (7%. w/w)-olive oil oleogel, while spherulites or rosette-like crystals (Fig. 2-3) were captured in corn oil in the presence of 0.5% NaCl. Significant differences in microstructures are highly associated with impurities in oil (such as salt) rather than with intrinsically different TAG profiles in the two oils.

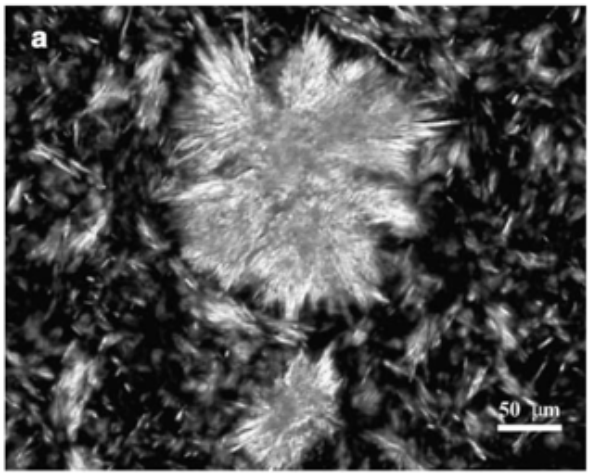

Fig. 2-3 Polarizing light microscopic graph of MAG in corn oil, containing 0.5% NaCl salt[109]. Scale bar is 50 μm).

2.3.2. Mixtures of fatty acids and fatty alcohols

In 2004, a patent outlined the concept of using edible solidifying agents to solidify edible oils through a heating-cooling cycle. A mix of stearyl alcohol and behenic acid was specifically reported as a solidifying agent in canola oil. Based on that idea, Gandofol et al. became interested in approaching an empirical method to investigate the structuring potential of a series of fatty alcohols, fatty acids, and their mixtures with varying chain lengths ranging from 16 to 22 carbons. In theory, the formation of organogels are determined by both solubility of LMOGs in solvents (thermodynamic considerations) and crystallization of LMOGs during supersaturation (kinetic aspects) because crystal size and shape will depend strongly on the kinetic properties of the crystallization process of gelator crystallization. Thus, changes in concentration, mixing ratio, and storage temperature were used to modify the solubility and crystallization kinetics.

In an individual structuring agent system, whether involving fatty acid or fatty alcohols, 2% (w/w) is needed to structure oils in general and the hardness of oleogels increases with increasing concentration. This phenomenon provides an increasing level of complexity in terms of these

mixtures. A mixture of stearic acid and stearyl alcohol is required up to 5% (w/w) to form a gel. This mixture displays phase separation with deposited crystals, forming only a white layer at the bottom of the oil phase, when it was 1.5% (w/w). At 2.5% (w/w), a viscous and flowing solution was obtained without any firmness. Solubility is influenced by the mixing ratio of a mixing system as well. It was found that at the fixed concentration, a ratio of 7:3 (alcohol:acid) resulted in the highest hardness, and it was believed that this resulted in the best synergistic effect at this ratio. The type of oil employed was not particularly influential on this synergistic effect, but did affect the hardness of oleogels. Among three oils used in the fatty acid + fatty alcohol system, the oleogel made of soybean oil was the harder gel then those made of sunflower oil and rapeseed oil. Gandolfo et al. inferred that this was due to intrinsic differences in these oils. Soybean oil has a higher amount of 11% saturated fatty acid (comprising palmitic acid) compared to sunflower oil and rapeseed oil. In addition, this gel was formed and stored at 5°C. At as low as 5°C, a part of soybean oil is crystallized naturally, which contributes to the structure providing some hardness as it would when being refrigerated by a consumer.

2.3.3. β-sitosterol and γ-oryzanol

Mixtures of β-sitosterol and γ--oryzanol have been proposed as the most promising structuring agents for solidifying vegetable oils with a specific crystal morphology (i.e., tubules). The specific self-assembled hollow tubules with a diameter of about 10 nm aggregated to form a transparent, and thermo-reversible gel in sunflower oil even when a high structurant concentration of 16%, w/w was used[110]. This is very different to other structurants which form non-transparent gels such as fatty acid + fatty alcohol, monoglyceride, fatty acids, and waxes. Besides, β-sitosterol and γ-oryzanol are both derived from dietary sources, are completely free of saturated fatty acids, and

have a blood cholesterol-lowering effect. Their positive impact on blood cholesterol-lowering due to the ability of interfering the absorption of cholesterol in the intestine has gained the attention. However, the principle behind this system is still not fully understood. It has been proposed that the transparency of this gel be dependent on the mass fraction (or ratio) of two low molecular weight molecules[89,111]. The haziness of the oleogel decreased with an increase in γ-oryzanol, but the maximum hardness always occurred at a specific ratio of 60:40 (γ-oryzanol: β-sitosterol), not changing as a function of mixing ratio[96].

Numerous studies have shown that a similar protein fibril formation in an aqueous system is an entropy-driven process; however, there are very few studies have been done using LMOGs in vegetable oils. Since the formation of the tubules of mixtures of γ-oryzanol and β-sitosterol is thermally reversible, the process is supposed to be close to thermodynamic equilibrium. Thus, the thermodynamic theories of self-assembly were applied to the process of oleogelation for calculating the corresponding binding energies of self-assembled units and networks in order to show whether the oleogelation is an entropy- or enthalpy-driven process.

Sawalha et al. [96] proposed an empirical study investigating the effect of cooling temperature on critical aggregation concentration using light scattering, thermometry, and micro-scanning calorimetry. Results showed that the formation of the tubules was correlated to a negative enthalpy change (i.e., exothermic enthalpy), indicating that the aggregation into tubules is an enthalpy-driven process. According to this, it is believed that oleogelation can be modified or promoted by increasing the differences of Gibb's free energy between a solution and a gel phase.

Oleogel formation is not only dependent on the nature of the structurants (gelators), but is also associated with the concentration and mixing ratio of mixture systems. When the concentration

of structurants increases, a decrease in gelling induction time occurs. In other words, crystallization or aggregation occurs earlier when the concentration of structurants is higher. Results also matched to the tendency of aggregation temperature (i.e., higher concentration of structurants in oil formed gels at higher temperature upon cooling). In addition to concentration, mixing ratio in multi-component systems is important as well. It was reported that the γ-oryzanol: β-sitosterol ratio affects the aggregation of tubules at a fixed concentration by measuring the different corresponding aggregation temperatures. The solution with 60:40 (γ-oryzanol: β-sitosterol) showed aggregation at 24.9°C, whereas with the other ratios, the aggregation occurred at even lower temperatures during cooling.[96,110] Moreover, the crystal sizes and morphologies were changed due to the changes in mixing ratio. Also, crystal size affects the transparency of gels.[89,96,111] Thus, more research on investigating the relationship between mixing ratio and oleogelation is encouraged in order to understand how to control the mixing ratio to manipulate the physico-chemical properties of oleogels.

A mixture of β-sitosterol and γ-oryzanol was also used to structure various oils, such as decane, limonene, sunflower oil, castor oil, and eugenol to determine the their potential for structuring different oils on one hand, and to study the effect of polarity of oil on the their self-assembly on the other hand[110]. A decreasing polarity of the oil was found to promote the self-assembly of sitosterol + oryzanol leading to the higher gelation temperatures and lower critical structurant concentrations from the results of light scattering and rheology. This shows the dependency of the critical gel concentration on the type of oil used. For instance, decane, which has the lowest polarity among selected solvents, showed high aggregation temperatures, whereas eugenol showed relatively low aggregation temperatures (at the same concentrations). Also, Sawalha et al. reported that the gels prepared with lower polarity solvent (decane) turn opaque rather than

transparent, due to the differences in the chemical structure of solvents, which affects the solubility of sterols and mutual interactions with sterols as well. The change in Gibbs free energy was found to decrease with the decreasing polarity of the solvent, indicating that the tubular type of crystals are more thermodynamically stable in low polarity solvent. Thus, solvent polarity plays an important role in crystal and network formation formed with the two-component system and given solvent[110].

This promising mixture system is of interest for applying to aqueous systems because most food systems are in the aqueous phase. Bot et al. published studies in succession regarding the application of oleogels in water systems. Ninety to 40% of oleogels were used in preparing with water under the stirring process to form emulsions. A stable emulsion was formed but this only occurred successfully in some critical conditions, such as low temperatures only (5°C) and high levels of structurants (i.e., 16% or even 32%, w/w). The high amount of structurants needed seems to contradict the advantage of molecular gels (i.e., only a low amount is needed to entrap a high amount of organic solvents). But since both γ-oryzanol and β-sitosterol are health-promoting ingredients, the high level under the limited usage in food products may turn into a strong trait.

2.3.4. Application studies

The selected current applications of oleogels in food products will be outlined in this section.

2.3.4.1. Inhibition of oil migration

Oil migration has been a serious problem associated with shelf-life of food products due to oxidation of leaking oil, which gives off-flavors to final food products. Also, leaking oil leads to

texture defects, such as high mobility of mixtures of oils and fats which causes fat blossom in the surface of chocolate, which is an undesirable texture.[26,112]

The earliest application of low molecular weight gelator in food products was the use of 12-hydroxystearic acid in peanut butter. A small amount of 12-HSA was used in mild heating and mixing with peanut butter, resulting in thickening gels to prevent the phase separation in peanut butter to extend the shelf-life of peanut butter. Furthermore, oil migration causes softening of the cookie bodies of cream-filled cookies. Thus, oleogels can be added to make a filling for reducing the mobility by structuring oils. Several natural waxes, such as rice bran wax, beeswax, and candelilla wax have been used to making confectionary fillings[113]. Beeswax-oleogel was used to replace part of palm oil in the formulation of making hazelnut filling. It was found that 17% was the maximum replacing amount to maintain a good quality of filling, and no waxy mouth-feel could be tasted.[114]

2.3.4.2.For bakery products

Oleogels have been proposed to replace fats in food products, although there have been concerns regarding whether these gels provide the same characteristics of fats such as melting temperature, firmness, aroma, and mouth feel. Among all bakery products, puff pastry is the most strict in terms of fat requirements. In pastry products (i.e., Danish, croissants, pie crust, and eastern pastry cake) fat acts as a barrier, separating dough sheets to increase the capacity for trapping steam-, and air-filling during baking between dough layers. The criteria for a fat to provide this function is higher-melting point than normal (~42°C) achieved by having a higher solid-fat content. This kind of fat is commonly prepared using hydrogenation. Most oleogels have a melting temperature in the range of 30-50°C depending on the types of gelators, concentration, mixing ratio, and types of oils.

Hwang et al. noted that replacing margarine with wax-based oleogels to make cookies may be appropriate. Further properties of cookies were evaluated. Four different waxes (sunflower, rice bran, bees, and candelilla) were used up to 8% to prepare oleogels with a variety of vegetable oils.[115] Results showed that hardness and melting profile of oleogels depended on the type of waxes and oils. For example, an oleogel made of sunflower wax and flaxseed oil had the highest firmness. In addition to that, many cookies made with wax-based oleogels showed similar properties to those made with a commercial margarine. Thus, the high feasibility of using oleogels in real foods to reduce saturated and *trans* fats is apparent.

2.4. Methodologies of structuring oils (turning liquid oils into solid state)

Unsaturated fatty acids are responsible for compounds which use them being liquid at room temperature and for having positive health implications, in contrast to saturated fatty acids, which should be consumed in limited portions due to their significant association with cardiovascular disease. However, semisolid state oily substances have many advantages with respect to longer shelf-life, desirable texture in food products, and a pleasant aroma. Thus, significant efforts are being made to structure liquid oil containing unsaturated fatty acids.[17, 116]

The concept of solidifying liquid oil was initially proposed in the early 1800s. Since then, solidifying liquid oils has been continuously required. There are various reasons for this requirement: (1) Such therapeutically beneficial oils are limited by their liquid form because they cannot be ingested by consumers in sufficient amounts to exert therapeutic effects, due to the unpleasant taste, and/or texture thereof, (2) Oil can have a role in protecting food from becoming hydrated due to contacting moisture or becoming dehydrated over time. In order to stick to the food and provide this protecting layer role, it must not run easily. Thus, there is a widely

recognized need for, and it would be highly advantageous to have, a thickened and/or thixotropic composition of an edible unsaturated oil, characterized by both semi-solid and liquid state, depending on temperature and/or the application of mechanical forces, thereby addressing both the solid state requirement of the edible oils and also a high degree of unsaturation benefits in food and medicinal/therapeutic products.

2.4.1. Chemical and enzymatic interesterification

Chemical interesterificaiton exploits alkali metals such as sodium methylate and sodium ethylate and at high temperatures to rearrange the position of fatty acyl chains within and between TAGs. Enzymatic interesterification is an approach using enzymes in order to, for example, modify the target positions. This enzymatic approach is more specific and uses milder reaction conditions in comparison to chemical methods. With respect to its mechanism, interesterification undergoes a process of rearrangement, and therefore the fatty acid profiles are not changed in the final products. Interesterification usually combines with other approaches such as fractionation and blending with other fat or oil components to achieve the goal of reducing saturated fatty acids as a potential healthy fat alternative.

3. Research purpose

The goal of this thesis is to create a semi-solid material with similar characteristics to solid fats from thermal property to rheological property but without having high saturated fatty acids and *trans* fatty acids, instead, containing vegetable oil with health beneficial polyunsaturated fatty acids. Also, incorporating some nutritionally beneficial compounds such as oil-soluble carotenoids when preparing oleogels using a two-component lipid molecule system to enhance the functionality is studied.

As discussed in the literature review, oleogels have gained increasing attention over the last decade due to their high potentiality of being alternatives to solid fats with low saturated fats and *trans* fat free. Two-component or multi-low molecular weight molecules have been suggested to study their abilities of oil structuring because more than one kind of molecule can provide more space to manipulate the performance of resulting oleogels. However, existing research on oleogels has so far led to insufficient results and knowledge regarding studying both the role of mixed low molecular weight molecules and versatile vegetable oils on oleogel formation and on their physical properties. Hence, in order to help fill this gap in our knowledge and establish a healthier fat replacement by structuring vegetable oils, this study investigated mixtures of two small lipid molecules used as agents to structure a variety of vegetable oils, as well as outline their roles with respect to oleogel formation. In addition, the role of vegetable oils was discussed. Furthermore, carotenoid-enhanced canola oil was applied to investigate the possibility of this oleogel system for carrying additional functional components.

The present study was designed to address the following research questions:

(1) Does a mixture of stearic acid (gelator) and β-sitosterol (non-gelator) self-assemble into specific crystals which subsequently form a three-dimensional network to structure/solidify canola oil, resulting in a self-standing oleogel, and how does this occur?

(2) What is the relationship between the mass fraction of mixed lipid molecules, oleogel formation, and the rheological behavior of an oleogel?

(3) Do vegetable oils influence oleogel formation in a two-component system? If yes, how?

(4) Can external health-beneficial compounds, such as carotenoids, be included in oleogels without interfering with the crystal and network formation in such oleogels?

4. Specific aims

Aim 1. Study the canola oil structuring capability of a two-component system, stearic acid + β-sitosterol

In order to test the structuring capability of stearic acid + β-sitosterol, a series of total solid concentrations were prepared from 1% (w/w) to 8% (w/w) with a variety of mixing mass fractions to determine the CGC, the minimum concentration for gelation and critical gel ratio. Generally, 8% (w/w) is sufficient for most small molecular weight gelators to structure a liquid edible oil. In the literature, stearic acid is noted for its ability to gel several solvents at concentrations as low as 0.5–1.0% (w/w); however, β-sitosterol is not able to form a gel alone in many tested solvents, but is able to structure oils in the presence of γ-oryzanol. A gelator-additive (stearic acid-β-sitosterol) system provides more possibilities (such as self-sorting, random co-crystallization or unit-repeated co-crystallization) when it crystallizes during the process of supersaturation. In addition to functional groups of molecules, mass fraction of multi-components has been proposed as a key factor for determining successful oleogel formation.

Aim 2. Study the relationship between mass fraction, crystallization, and physicochemical properties of oleogels

Aiming to investigate the crystallization phenomena induced by the mixtures of small lipid molecules, a series of mass ratio combinations at fixed 8% (w/w) of total solid content was used, because 8% is the critical gel concentration for the two-component system in canola oil. Besides, not all the mass combination of two components led to oleogel formation. It is of interest to study the effect of mass fraction on oleogel formation. The differences in crystal morphology were captured by polarized light microscopy. In addition, inducing crystallization time was suggested to be measured in order to explain the changes in crystal morphology. Polarized light microscopic graphs with time lapse were recorded as well to address the process of crystallization during the cooling process. The effect of composition ratio on the crystal and crystal network formation, and physical properties of molecular gel will be studied by differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and rheology. Through these analyses, the correlation between mass fraction of two components, crystallization, and physicochemical properties of samples was illustrated.

Aim 3. Effects of fatty acid profiles in various vegetable oils on oleogel formation with a promising two-component system at critical gel ratio

To develop an alternative to fat hardstock by entrapping vegetable oil is our goal. Thus, it is essential to have more than one kind of vegetable oil for testing the gelation capability of the stearic acid + β-sitosterol system. A critical gel ratio, S4B4 (4 wt% of stearic acid and 4 wt% of β-sitosterol) was used to prepare oleogels with 23 edible oils and two non-edible oils (castor oil) to investigate the influence of oil on oleogel formation. The ability to form a gel or not was correlated with the fatty acid profiles of oils using hierarchal clustering analysis in order to investigate whether specific types of fatty acids play a key role in oleogel formation.

Microstructures were captured as well using polarized light microscopy. Thermal behavior and rheological properties were studied to provide the physicochemical properties of oleogels. It is important to determine the role that oil plays in oleogel formation. This is required in order to widen the application of oleogels as solid fat substitutes.

Aim 4. Study oleogel formation with carotenoid-containing canola oil

With increasing knowledge regarding successful agents for structuring/solidifying liquid oils, oleogels have received an increasing amount of attention regarding their practical application and potential to substitute for the solid fats used in food products. Among the reported studies, only a few and rather regular oils have been used in forming oleogels so far; although they were highly successful in terms of their ability to replace either all or part of the solid fat, the oxidation of the oils used is of concern. In this context, and using the oil solubility and strong antioxidative potential of bioactive compounds from fresh mango pulp, crude carotenoid extract-enriched canola oil was prepared to subsequently be structured by a two-component system, which involved the combination of 5% (w/w) stearic acid and 3% (w/w) β-sitosterol. A small laboratory-scale ball miller was used to prepare crude carotenoid extracted oil, with different mass ratio of mango pulp to canola oil from 0 (w/w), 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 to 1.60 (w/w), in order to determine the optimum extraction concentration. Due to the heat sensitivity of carotenoids, four extraction temperatures (room temperature, 30℃, 60℃, and 90℃) were used to both optimize the conditions for extraction efficiency and investigate the effect of temperature on carotenoids. Microstructures, thermal behavior, and rheological properties were analyzed with the same methodologies used in the previous aims.

5. Materials and Methods

5.1. Materials (low molecular weight gelators and vegetable oils)

Low molecular weight gelators (< 3000 Dalton) has been used to make molecular gel with an advantage of low concentration and only non-covalent interactions involved. In this study, gelators or structurants refer to low molecular weight gelators. Stearic acid is an 18 carbon saturated fatty acid with and it is a white powder at room temperature without odor. Stearic acid, ≥95%, was purchased from Sigma-Aldrich (MO, USA). β-Sitosterol, white powder at room temperature, was obtained from Acros Organics (NJ, USA) and was the mixture of ~10%ofcampesterol and ~75% of β-sitosterol. Cholesterol, a white powder with purity ≥ 95% was purchased from Sigma-Aldrich (MO, USA). The chemical structures of stearic acid, cholesterol and main component in β-sitosterol are given in Fig. 5-1. Even β-sitosterol is not a pure compound, but it is noted that the minor component in ingredient is very similar to main component.

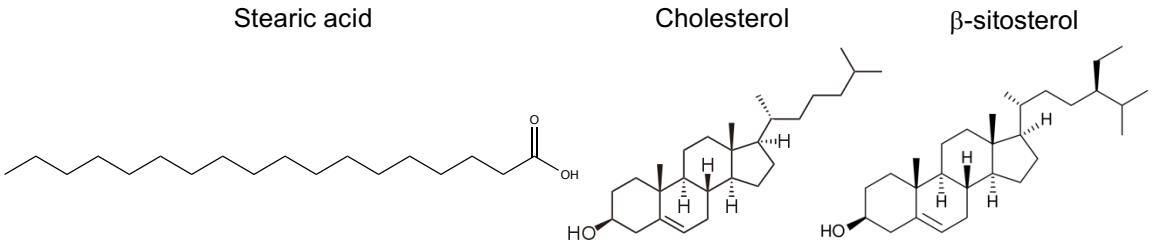

Fig. 5-1 Chemical structures of stearic acid, cholesterol, and β-sitosterol.

Samples of twenty-nine different varieties of vegetable oils as: coconut oil (2 samples), corn oil (1 sample), cottonseed fat (1 sample), flaxseed oil (2 samples), apricot kernel oil (1 sample), olive oil (1 sample), soybean oil (1 sample), sunflower seed oil (1 sample), safflower oil (1 sample), canola oil (1 sample),castor oil (2 samples), hazelnut oil (1 sample), safflower oil (1 sample), grape seed oil (2 samples), sesame oil (1 sample), vegetable oil (1 sample), rice bran oil (2 samples),

olive oil (1 sample), extra-virgin olive oil (1 sample), macadamia nut oil, avocado oil (2 samples), Blen oil (1 sample), hemp oil (1 sample), almond oil (1 sample), pumpkin seed oil (1 sample), and peanut oil (1 sample) were collected from the local market during the period between Oct 2015 to Januart 2017.

5.2.Methods for preparing oleogels

5.2.1. Oleogels preparation with the mono-component system

To test the gelation capability of stearic acid, cholesterol, and β-sitosterol as a gelator for structuring canola oil, a series of weight percentage of them in canola oil was designed. A range of weight percentage in canola oil was from 1% to 8% with 1% as interval, i.e., there were eight samples for each gelator. The rest of constituent in sample was canola oil with a range between 99 and 92%. All samples were prepared 1 g as total mass. After weighing and mixing two compositions (gelator and canola oil), a vial of suspension solution was put in the metal mode on the hot plate and heated to 155°C in order to dissolve all the particles in suspension. Heating time was around 40 min depending on the melting point of gelators. Making sure all the particles were dissolved completely, the vial was taking out to deposit on the stabilized table in the surrounding of ambient temperature (~25C). After 24 h standing with quiescent, the properties of samples either gels or non-gels were characterized by the following methods.

5.2.2. Oleogels preparation with the two-component system

In two-component systems, a 1 g of solution of the mixture of small molecules and canola oil was prepared as follows. Sa and Chol/β-Ss were weighed into a sample vial to make 8% (w/w) of total

solid content with different mass ratios. Then Canola oil (0.92 g) was added. All of the weighted samples were heated in a metallic holder using temperature controlled heat plate at 155 °C for 40 min until all the crystals melted for erasing crystals history. Subsequently, the sol was cooled at 25°C for 24 hr. After 24 hr, all the physicochemical properties were analyzed. In this dissertation, the samples were labeled as Sa + a digit + β-Ss/Chol + a digit (i.e., S1B7 represents stearic acid 1% (w/w), β-sitosterol 7% (w/w) in 92% (w/w) of canola oil).

Gel or not was determined by inversing the vial. Turned the vial up-side down for an hour to see it flew or not by against gravity. Sample named as molecular gel was determined when it did not flow after an hour.

5.2.3. Pseudo ternary system

Aim is to understand the correlation between concentration and structure formation in the pseudo ternary system comprising canola oil, stearic acid and β-sitosterol. The plot of ternary phase diagram can show us the relationship between components and their gelation capability.

5.2.4. Oleogel preparation with 27 types of vegetable oil

Based on the preliminary test, only one binary gelators system, S4B4, was used for investigating the effect of type of oil on oleogelation. As described above, a total weight of 0.5 g or 1 g of solution was prepared. Except not just canola oil but 25 different edible and two non-edible oils were used to prepare oleogels respectively. A weighted solution was heated in a metallic holder using temperature controlled heat plate at 155 °C for 40 min until all the crystals melted for erasing

crystals history. Subsequently, the sol was cooled at 25°C for 24 hr. After 24 hr, all the physicochemical properties were analyzed.

5.3. Methods for measuring gelation ability and kinetics

5.3.1. Opacity of oleogels and the induction time of crystallization

Opacity is a good indicator for the system in this study since no matter singular gelator or the mixture of two gelators crystallize in the size of big enough crystals which can scatter light and cause the opacity. Absorbance peak at 500 nm were selected and recorded using an Agilent Cary 60 UV-Vis spectrometer (Agilent Technologies, CA, USA) to determine the degree of opacity. Gel samples were prepared as above (section 5.2.2).

An oleogel sample was filled the space between two slides of customized quartz slide cuvette (Wilmad glass Inc., NJ, USA) for measuring its degree of opacity.

Spectroscopy is also a simple technique to monitor the phase transition from clear solution to opaque gel because the samples containing crystals are found to be turbid. Using this advantage, a hot clear solution transferred to pre-heat cuvette. The sample filled cuvette was put into the chamber of UV-Vis spectrometer for monitoring its absorbance at 500 nm with a function of time. A data point was recorded every 15 seconds. A plot of time (in seconds) versus intensity of light absorbance was presented. The induction time of crystallization was decided at which the time that light absorbance started to raise. The purpose of this test is to see the effect of compositions on the kinetic of crystallization which causes whether an oleogel is formed.

5.3.2. Polarized Light Microscopy (PLM)

A polarized light microscope is a kind of optical microscopes equipped with a detector lenses and polarizing filters. Polarized light gives a better contrast that provides the better quality of the image obtained with birefringent objects than other techniques such as darkfield and brightfield illumination. Generally, when a light source pass through the first filter, only one orientation of light wave remains. This remaining one orientation light source emits to a birefringent sample which is positioned along with the light beam. Due to the characteristic of a birefringent material, the light is refracted into two waves traveling in different directions and also at different velocities. These two wave fronts, which are termed with ordinary and the extraordinary wave fronts, are recombined with constructive and destructive interference when they pass through the second polarized filter (also called as analyzer). This makes sample can be seen in higher contrast quality.

Oleogel is recognized as an anisotropic material which is birefringent, so its microstructure of crystals is observed by using polarized light microscopy commonly. Gelators for oleogels are usually the molecules form an asymmetric crystals, which means that crystals do not have the equal crystallographic axis. That is why an oleogel can be analyzed by PLM. Not only the static microstructure of crystals in oleogels can be observed, but also the process of crystallization can be recorded through polarized light microscopy. The process of crystallization can be monitored as a function of time.

5.3.2.1. Capturing the morphologies of crystals

After an oleogel or suspension being prepared, one small drop of samples were placed on a 76.2mm×25.4 mm glass slides (VWR, international, PA, USA) and covered with 22×22 mm glass coverslips (Fisher Scientific NH). Polarized light micrographs were acquired in at least six

replicates using a Linkham imaging station (Linkham, Surrey, England) equipped with a 10X Olympus lens with 0.25 N.A. (Olympus, Tokyo, Japan), and a 2560×1920 pixel CCD camera (Micropublisher, Surrey, Canada). Microscope images were captured by 2560 x 1920 pixels under 10× magnification (Lens information).

5.3.2.2. Recording the process of crystallization and network forming

There are several ways have been tried to record the whole process of crystallization during the cooling time; however, to our knowledge not a standard perfect method is described. With few trail and errors, we adopted a method to puzzle the crystallization under the scale of micrometer. A mixture of gelator and vegetable oil was heated until the particles fully dissolved. Time was counted when a hot solution was removed from hot plate. For every 5 min, an aliquot of sample was taken out and placed on the glass slide covered with a cover. We assumed that the crystal growing stopped and crystals maintained the shape they should be at that time point when a sample was stabilized between the slide and the cover. Results proved this assumption. At least 5 slides for every time points.

5.4. Methods for characterizing the properties of oleogels and oil phases

5.4.1. Melting profiles of oleogels - Differential scanning calorimetry

An oleogel can be in a status of gel because it is composed of a self-assembled network which is constructed by crystals. Therefore, a phase transition exists when a solution becomes a gel, especially crystals formed. A phase transition involves energy changes or heat capacity changes, which can be detected by Differential Scanning Calorimetry (DSC) with great sensitivity. The fundamental principle of DSC is that when a sample undergoes a physical phase transition, it

requires more or less heat to maintain its temperature as same as one of reference. More or less heat needs to flow to sample depends on whether the process is exothermic or endothermic. For instance, the process of oleogelation is exothermic because when gelators crystallize, the heat is released. Thus, the sample needs less heat than the reference to keeping its temperature as same as of reference. Since the difference in the amount of heat required to increase the temperature of sample and reference is measured as a function of temperature, the well-defined heat capacity reference should be used to receive the correct thermal properties of the sample. Generally, it is very common that using empty pan as reference sample.

A weighted amount of gelled sample (quantity between 5 and 8 mg) was placed in a hermetically sealed aluminum pan (TA Instruments, New Castle DE). Sealed sample pan was experienced heating-cooling cycle using the DSC (Q2000, TA instruments, New Castle, DE). The DSC chamber was constantly flushed with nitrogen gas (0.5 mL/min). Each sample underwent a heating and cooling cycle to see gel's melting and crystallization profiles. Heated sample to 150°C with a ramp of 5°C/minand then held isothermally at 150°C for 5 min. After 5 min balance, cooled sample to 15°C at5°C/min of cooling rate. TA Universal Analysis 2000 software (TA instruments, New Castle, DE) was used to calculate the peak crystallization temperature and enthalpy of crystallization.

5.4.2. Gel strength – Rheology

Rheology is another technique not only to observe the phase transition, but also to characterize the physical texture of viscoelastic materials. The basic principle of rheology is the study of flow or deformation of materials under applied stress or strain which is measured using a rheometer. Since oleogels is found and defined as a semisolid material having viscoelastic properties, its physical

properties are measured and shown through the test of rheology. Due to gelators in oleogel paly roles in forming a three-dimensional network which immobilizes the bulk of oil phase, it has both viscous and elastic. Depending on how strong the non-covalent interactional forces of gelator-gelator and gelator-oil phase, an oleogel shows the differences in the values of parameters which are measured by a rheometer.

Rheological measurements were performed using a discovery Hybrid Rheometer-2 (TA Instruments, New Castle, DE), equipped with an 8 mm diameter crosshatched stainless steel plate geometry. After being heated, the samples were poured to an nylon circular mold (ID: 0.453 mm x T: 0.094 mm). The samples in the mold were pressed by two glass slides to make the constant dense samples. Samples were stored at ambient temperature (~25 °C) for 24 h. After that, gels were taken out of the modes and placed on the peltier plate with the spot marked. Then the oscillation strain sweep was performed at 25 °C using an oscillation stress range from 0.1 and 20.0% of strain at 1 Hz. Through this amplitude sweep test, storage modulus (also called as elastic modulus, G′) and loss modulus (also called as loss modulus, G″) were gained to speak of the oleogels' properties. Dynamic tests were previously carried out to ensure that the applied stresses were in the linear viscoelastic region.

Besides the direct value of G′ and G″ were gained, yield stress was acquired by calculating with tangent analysis. Plot the applied stress versus modulus, a curve with a plateau region (linear region) was shown and followed by a dropped curve at certainly applied stress. In oscillatory tests, if a single tangent is applied to the linear region of the curve then the yield stress is often taken as the stress at which the curve begins to deviate from this tangent. Yield stress can provide us the

sense of strength of a gel. With a higher value of yield stress, it means that the harder you have to put on enable to breakdown the oleogel by destroying the framework consisting an oleogel.

5.4.3. Crystal polymorphisms

As the information discovered in recent ten years, oleogellation is a process of crystallization of gelators in corresponding solvent. The shape and size of crystals determine whether the hot solution becomes a gel during a cooling process. Regardless whatever the shape of crystals, crystals are formed by the arrangement of molecules themselves. How the molecules align and pack in certain way to create different visible crystals observed under microscopy in the scale of micrometer is highly related to the reason causing the state of gel. X-ray diffraction is a technique helping us to observe the things happened down to atom scale. The principle of X-ray diffraction is a given incident beam of X-ray is scattered when interact with the target material. This diffraction of scattered X-rays undergo constructive and destructive interference can be obtained. Then this diffraction of X-rays by crystalline materials is then described by Bragg's Law, n(lambda) = 2d sin(theta) in order for us to recover the pattern of atoms' arrangement in crystals. Even there is not just more single crystal in a target material which is in most of the case, number of possible interatomic planes still can be observed with a wide range of angles of incident X-ray used.

The wide-angle (WAXS) and small-angle (SAXS) x-ray scattering patterns of the 9 samples were obtained by use of a Bruker HiStar multi-wire area detector on a rotating-anode x-ray generator equipped with a 3-circle Azlan goniometer, a 0.5 mm pin-hole collimator and a Rigaku Osmic parallel-mode mirror monochromator (Cu K ; λ = 1.5418Å) operating at 40 kV and 50 mA. Data were collected at room temperature (20°C). The sample to detector distance was 9 cm for

the WAXS data. Flood-field correction and spatial calibrations were performed at these distances prior to data collection. The images collected were 512 x 512 pixels without moving the sample for 300 sec. The detector angle was fixed at 25 deg.

5.4.4. Chemical bond interaction within/ between molecules

Molecular component and structure changes are characteristics of interest in oleogels after a heating-cooling preparation. What if the new chemical bonds form or breakdown of chemical bonds is also a reason that may cause oleogelation. Fourier transformer infrared spectroscopy is a technique which is used to obtain the changes in chemical bonds in molecules by measuring the absorption of infrared spectrum of samples. The wavelengths that are absorbed by the sample are characteristic of its molecular structures. When a sample is irradiated by infrared radiation, the molecules excite into a higher energy vibrational or rotational state. Different chemical bonds in molecules have different energy difference between at-rest and excited states, so the absorbed wavelengths can speak for molecules in which molecular components and structures. The FTIR spectra are usually presented as plots of intensity versus wavenumber (in unit of cm^{-1}).

A Nicolet Fourier Transform-InfraRed spectrometer (Thermo Electron Corporation, Madison, WI, USA) was used to collect interferograms which were obtained from average of 256 scans. Samples including liquid and gel samples were stored at ambient temperature (~25°C) for 24 h before analysis. Peaks between 1000-3600 cm^{-1} were analyzed to determine the state of functional groups of structurants and oils.

5.5.Extraction carotenoids via blending canola oil with mango pulp

5.5.1. Materials

Mango was bought from a supermarket (New Brunswick, NJ) with the code number of 4959. Canola oil was collected from the local market between Oct 2016 to May 2017. Unmodified canola oil and carotenoid-enriched oils were stored in the freezer when they were not in use.

5.5.2. Extraction of oil soluble carotenoids from fresh mango pulp

Mango pulp was removed from peel and seed and was cut into small spices for the following ball blending usage. Small mango pulp pieces and canola oil were weighed in an IKA ultra turrax tube with a series of blending ratios from 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 to 1.6 (mass ratio of fresh mango pulp to oil). About 15 g of sample in total was used for each batch, and the mixture was blended by using an IKA ultra turrax Tube Drive control equipped with 30 stainless balls (250g, 6 mm). A speed of 5000 rpm/min and a time of 150s were set. After blending, the mixture was transferred into centrifugal tubes. Centrifugation was performed at 3000 rpm/min for 5 min. After centrifuging, the oil layer (upper layer) was transferred into Eppendorf tubes and centrifuged again at a higher speed of 7000 rpm/min at 20℃ for another 5 min to completely remove mango pulp residue. The golden yellow carotenoid-enriched canola oil with a 1.2 of mango pulp to canola oil ratio during extraction was chosen to apply in the following oleogel experiments because the estimated quantity of colorant compound was the highest (ratio of 1.2), according to spectrometry data (Agilent Cary 60 UV-Vis spectrometer (Agilent Technologies, CA, USA).

5.5.3. Oleogel preparation and distinguishing

5% Stearic acid and 3% β-sitosterol were both added into the carotenoid-enriched canola oil (92%, w/w) to act as a structuring agent. The sample scale was 0.5 g to 3.0 g depending on different testing. In order to use two-component structurants, 5% stearic acid was used alone as well for the lower heating (65°C) treatment. The mixture was held in capped vials and the capped vials were placed into the metallic holder which was heated on the hot plate with a setting of 155°C (actual temperature for the sample was 120°C) for 40 min. Every 15 min, the bottles was taken out and gently shaken to thoroughly dissolve the stearic acid and β-sitosterol powders. After 40 min heating, the vials were placed onto the quiescent table at room temperature (RT) for 24 hours. Then, gelation evaluation was performed by inverting vials for 1h.

5.5.4. Crystal morphology observation

After being prepared, sample drops were placed on 76.2 mm × 25.4 mm glass slides (VWR, international, PA, USA) and covered with 22×22 mm glass coverslips (Fisher Scientific NH). Polarized light micrographs were acquired in triplicate using a Linkham imaging station (Linkham, Surrey, England) equipped with a Q imaging 2560×1920 pixel CCD camera (Micropublisher, Surrey, Canada) and a 10X Olympus lens (0.25 N.A.) (Olympus, Tokyo, Japan). Microscope images were captured by 2560 x 1920 pixels under 10× magnification (Lens information).

5.5.5. Effect of blending and oleogel making temperature on carotenoids

Carotenoids have been well known for their antioxidative activity and nutritional benefits, but their susceptibility to heat has been recognized as well. In this study, there are two processes which may expose samples containing carotenoids to heating. Thus, three sets of heating treatments were

designed to investigate the effects of temperature on carotenoids either in canola oil or in oleogel condition.

Higher temperatures may lead to increased extraction efficiency due to higher mobility of molecules and softening of the cell wall structure of mango. Four temperatures were used when preparing the carotenoid-enrich canola oil. As previously mentioned, a 1.2 mass ratio of mango pulp oil was weighed and then heated to target temperatures (i.e., 30, 60, 90℃) in a water bath for 20 min. No further heating applied formed the control, which means that the blending process occurred for mango pulp oil at RT. A non-heated or heated sample was blended using an IKA ultra turrax Tube Drive control equipped with 30 stainless balls (250g, 6 mm). Clear yellow golden carotenoid-enrich canola oil was obtained after centrifuging. These carotenoid-enriched oils were analyzed with respect to their visible spectra using a spectrometer.

A second set of heating treatments was designed for short-term heating to target temperatures (i.e., RT, 30, 60, 90, 120℃) after the blending process. A prepared carotenoid-enrich canola oil was prepared at RT first, then heated to the target temperature. Once the temperature was hit, the heating process was stopped. All oil samples were analyzed for their visible spectra to determine carotenoid structure changes.

A third set of heating treatments was designed for the process when making oleogels. Since a heating process is necessary for making an oleogel, temperature should be considered to investigate the effects on carotenoids. Carotenoid-enriched canola oil was prepared at RT with ratio 1.2 (fresh mango pulp: canola oil). Then, one- (5% stearic acid), and two-component systems (5% of stearic acid and 3% of β-sitosterol) were added to the oils. Mixtures were heated to 65℃

and 120°C, respectively for 40 min. After cooling to RT for 1 d, oleogels were analyzed for visible spectra using a spectrometer.

6. The influence of mass fraction of stearic acid + β-sitosterol mixtures on their capability of structuring canola oil, crystal morphology, network formation, and rheological behavior of oleogels.

Abstract

A two-component structuring system, stearic acid (Sa) + β-sitosterol (β-Ss), was used to structure canola oil with a series of concentrations and mass fractions to investigate the influences of mass fraction of two components on oleogel formation, crystal morphologies, and physicochemical properties of oleogels including thermal and rheological properties. Sa-β-Ss was able to structure canola oil, resulting in a self-standing oleogel by the formation of co-crystallization when the mass ratio was greater than 1:1 (Sa to β-Ss) at 8 wt% of total solid content. In addition, a universal rule for any condition of total solid content for this two-component system to form an oleogel is for the mass ratio of Sa to canola oil to be greater than 0.0435. This finding showed that oleogel formation can be used to predict, which has not previously been reported. Sa-β-Ss structured canola oil into a semi-solid state at room temperature by forming needle- and fiber-like crystals. In contrast, ribbons and rosettes crystals were seen in those non-oleogels. This was in agreement with previous studies finding that thin fibers, needles, rods, and even some platelet crystals were present in gels due to their high aspect ratio. With respect to crystal morphology, the long aspect ratio crystals accounted for the interaction of entanglements to construct the three-dimensional network leading to oleogel formation. This increases the opportunity for entanglement or contact with each other, resulting in formation of a three-dimensional network. Rheology results showed that gel strength was able to be manipulated by controlling the mass ratio of two components. This study illustrated the potential of two gelator systems for structuring edible oils to acquire the required texture for suitability as a solid fat alternative.

6.1. Introduction

Oleogelation is a focused approach for developing a solid fat substitute with respect to low saturated fat, no *trans* fat, and a high proportion of oils (above 90% generally), and research in this area has been particularly active during the last decade[62,75,113,117]. Over recent decades, hydrogenation has been seen as an effective chemical approach to produce solid fat by converting a liquid oil rich in unsaturated fatty acids to a saturated fatty acid-rich solid fat[118,119]. The main purpose of hydrogenation at first was to extend the shelf-life of liquid oil due to relatively easy oxidation of unsaturated fatty acids. However, hydrogenated oils have been increasingly questioned regarding their health implications, particularly with respect to their association with heart disease due to their high amount of saturated fat and relatively high amount of *trans* fat compared to natural solid fats[12,13,119]. Facing the pressure of removing hydrogenated oils and reducing saturated fats in the diet, food manufacturers and food scientists are looking for other ways to produce a solid fat replacer low in saturated fatty acids without affecting quality. Oleogel has been considered a potential fat replacer because it is present in a solid state at room temperature and contains mostly unsaturated fatty acids as components. In 2003, molecular gels first gained attention due to their ability to structure an edible oil. This attention was followed by studies into understanding its structuring principles. Even though the mechanisms behind this aspect are still unclear, more and more application studies have been carried out to test the influences of product textures and mouth feel by replacing solid fat hardstock with oleogels. Several studies have shown that it is a good fat replacer, leading to the same desired quality in food products such as chocolate[26], ice cream[82,120], spreads, and frankfurters[121,122]. The positive results on application illustrate that further studies on understanding the structuring principles are necessary, as well as for developing ways to fabricate oleogels for desired physical properties to extend its applications.

In order to develop a solid fat replacer close to the compositions of natural oils, lipid-based molecules have been suggested to test whether they are good gelators. Besides, lipid-based molecules are more easily dispersed into oils than other more polar molecules. Some lipid-based molecules (i.e., long chain fatty acids, fatty alcohols, dicarboxylic acids, wax esters, hydroxylated fatty acids, natural waxes and partial glycerides)[31,82,100,100,123,124] have been studied with respect to understanding structuring principles and/or investigating the influence on texture by using an oleogel instead of solid fats such as shortening, butter, and margarine in food products. Most of these molecules were studied as a monocomponent system. So far, to our knowledge, some low molecular weight molecules have not been found to be effective gelators and have not been able to structure an oil such as β-sitosterol, and γ-oryzanol[90,103], despite their apparent positive health implications as nutraceuticals. In contrast, some two components/gelators have been found to be beneficial in this regard, increasing the possibility of success in forming appropriate oleogels.

To understand the reason why this mixture is able to form a gel within a vegetable oil, Sawalha et al.,[103] and AlHasawi and Rogers[39] studied ternary phase diagrams of β-sitosterol, γ-oryzanol, and edible vegetable oil. They found that this binary LMOG mixture produced hollow tube crystals (and thus a stable gel) only in the presence of the oil. However, this specific hollow tube crystal only occurs at a certain molar ratio (1:1) of β-sitosterol to γ-oryzanol[38,39,89]. In other cases, the addition of lecithin to sorbitan tristearate modifies the gelation ability of sorbitan tristearate by modulating the forms of crystal from platelet- to needle-like crystals[93]. Herein, crystal morphology is modified not only by adding co-gelator but also by changing the ratio of two-component systems to structure an edible vegetable oil. However, two-component systems have been studied very little, even though the ability to modify the network structure using co-gelators could be a promising method to tailor the physical properties[125].

In this study, a two-component system (i.e., stearic acid + β-sitosterol) was used to investigate the effect of mass ratio and molecular structure on gelling behavior of two gelators in canola oil, and further to discuss the physical properties of oleogels being tailored by molecular gel fabrications using the change of mass ratio. Stearic acid is chosen to cooperate with sterols because it is an efficient gelator in several solvents due to its similar crystallization of triacylglyceride. Additionally, stearic acid is the only saturated fatty acid that has a neutral effect on blood cholesterol level [84,126] as compared to other saturated fatty acids and is a GRAS as a food additive. β-sitosterol was chosen because it lowers blood cholesterol adsorption and shows potential in terms of its ant-inflammatory and anti-cancer functionalities.[79,127,128]

6.2. Results

6.2.1. Effect of concentration of Sa, β-Ss, and Chol on appearance of oleogels

Stearic acid, β-sitosterol, and cholesterol (as a comparison molecule to sitosterol) were prepared individually with canola oil at a series of concentrations ranging from 1% (w/w) to 8% (w/w) in capped vials through a heating-cooling process to show their ability and efficiency of solidifying canola oil. After heating (to dissolve all the solids) and cooling (to allow crystal and network formation), the cap-sealed vials were inverted and the self-standing ability of the samples was assessed visually. In general, should a sample in an inverted vial resist flow by the dragging force of gravity for at least an hour resulting in a self-standing (non-flowing) sample, it is referred to as a gel. The strict definition of gel formation is determined by its rheological behavior, which will be discussed later in this chapter. As shown in Fig. 6-1, Sa and Chol are able to be low molecular weight gelators for canola oil with the critical gelation concentration at 2% and 5% (w/w), respectively, whereas β-Ss is not able to be a LMOG to structure canola oil even when used up to

8% (w/w). In a comparison of other low molecular gelators used for gelling organic solvents, 8% (w/w) is a relatively high concentration. Stearic acid has been proposed to structure sunflower oil, and lavender oil at as low as 2% (w/w)[20,31] by forming needle-like crystals. Although β-sitosterol (a phytosterol) is structurally similar to cholesterol with an extra methyl group on the alkyl group, it has more adverse properties. However, with the presence of γ-oryzanol, β-sitosterol forms hollow tubules to structure varying oils well and forms transparent gels.

The efficiency of a LMOG is revealed by its critical gel concentration. A gelator has low CGC, meaning it can gel the solvent with low concentration; thus, it is termed an efficient gelator. In the case here, Sa is more efficient than Chol when structuring canola oil. Although no gels were observed in the β-Ss group, whiteness and opaque viscous solutions were seen starting from 6% (w/w). Deposited clumps instead of a whole compacted gel were shown in these white viscous liquids. The rational explanation is that when the concentration of β-Ss is high enough (i.e. 6%), molecules of β-Ss still self-assemble into crystals, and these crystals are not favored to form a continuous three-dimensional network in order to form self-standing gels, but clumps deposited at the bottom of vials. More details will be discussed in the following section.

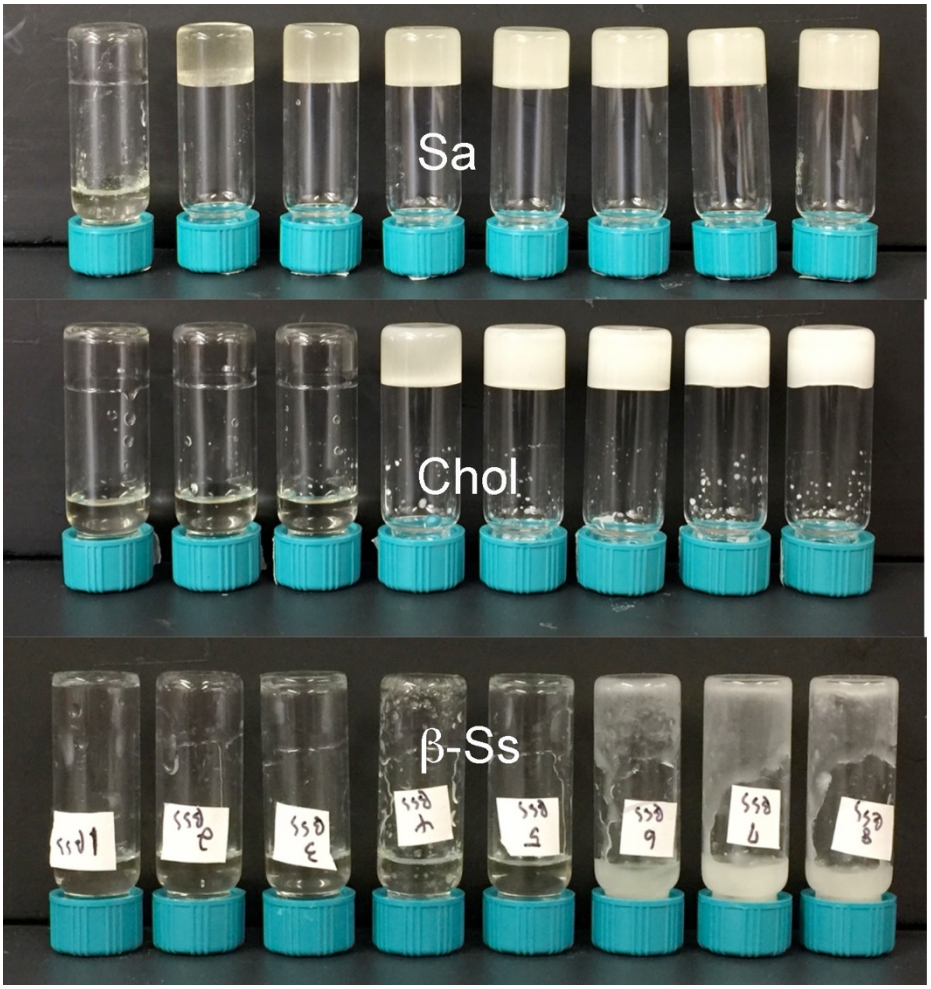

Fig. 6-1 The appearances inverted samples of mono-component oleogels, and mono-component non-oleogels with a series of concerntration ranging from 1% (w/w) to 8% (w/w). Sa represents stearic acid; Chol means cholesterol; β-Ss means β-sitosterol.

CGC was not only obtained from its appearance, but also from the degree of transparency of gels. In the case of Sa-oleogel and Chol-oleogel, the higher the concentration used, the higher degree of haziness was observed visually. Spectrometry was used to quantify the degree of non-transparency by detecting the transmittance of light passing through oleogels (Fig. 6-2). More absorption of light was measured with increasing concentration of Sa or Chol, which is consistent with visual observations. The gel is non-transparent because the crystals or the aggregation of crystals were big enough to scatter the light, resulting in the phenomenon of non-transparency.

Some molecular gels are reported to be transparent, and are often composed of thin fibers with a diameter of around 10 nm; in contrast, Sa-oleogel and Chol-oleogel should have larger crystals of a width of around 10-50 micrometers. Nevertheless, the size of crystals is not as thin as those of fibers, and they are able to entangle to form the three-dimensional network for entrapping oil as well.

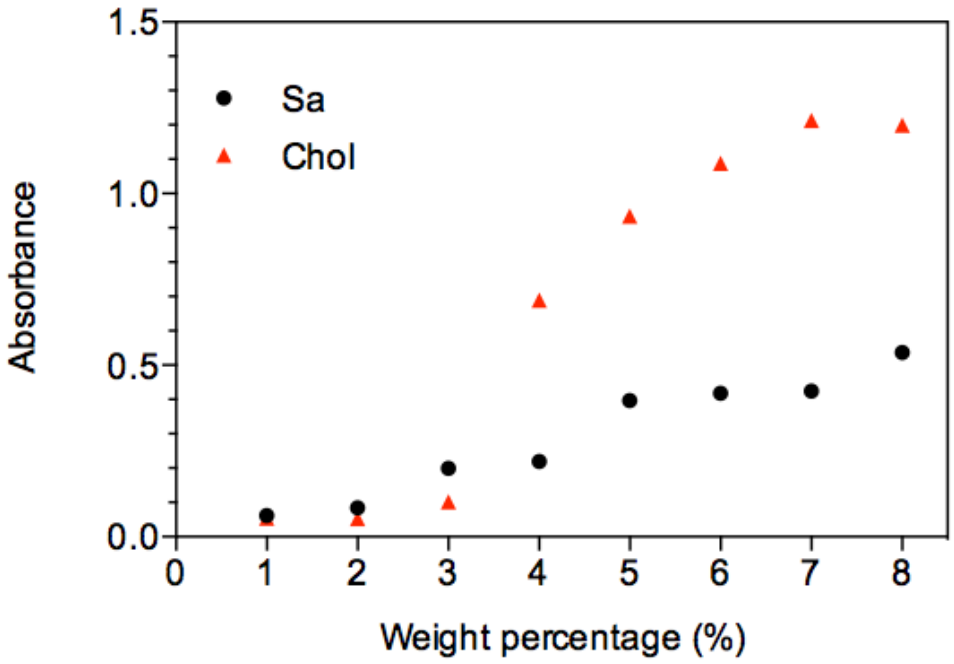

Fig. 6-2 Light absorbance at 500 nm of mono-component oleogels as function of concentration for representing the degree of opacity of oleogels.

6.2.2. Ternary phase diagram of Sa, β-Ss, and canola oil

To investigate the CGC of a mixed system (Sa + β-Ss) and corresponding mixing ratio for oleogelation, a series of total solid concentrations ranging from 1% to 8% (w/w) was tested in canola oil with varying mixing mass ratio, since this ratio seems to be a critical criterion for performance of an oleogel in a two-component structuring system.[39,96,129] All samples were

processed the same using a heating-cooling process. Depending on appearance, samples were described and classified generally as clear liquid (blue block), turbid solution (orange block), partial gel (white), and oleogel (green) (see Fig. 6-3). The first finding shown in Fig. 6-3 is that when the total solid content is up to 6 wt%, self-standing oleogels occur for the mixed system. Below 7% of total solid content used, only some cloudy turbid solutions and partial gels appear. For a two-component system, only 7 wt% of the total solid content has to be used to structure a vegetable oil is impressive. A higher total solid content (8, 16, or 32 wt%) of a popular two-component system, i.e., γ-oryzanol + β-sitosterol, was needed for producing oleogels. In addition, at a fixed 8% of total solid content, changes in mass fraction of Sa and β-Ss decide whether an oleogel is formed or not. For example, 3:5:92 (Sa:β-Ss:canola oil) was a turbid solution with clumps in it while 4:4:92 (Sa:β-Ss:canola oil) was an oleogel. It is surprising that the formation of an oleogel can be influenced by this subtle change in mass fraction of two components. Though stearic acid itself can structure canola oil into a gel at very low concentration (2 wt%), β-sitosterol is our main target to be used for structuring oil due to more health benefits. Also, addition of β-sitosterol interferes the self-assembly of stearic acid. It refers that the sitosterol co-crystallize with stearic acid upon cooling process. Therefore, a series of following analyses were done on the samples at 8% of total solid content, which referred to the CGC of the Sa-β-Ss system in canola oil.

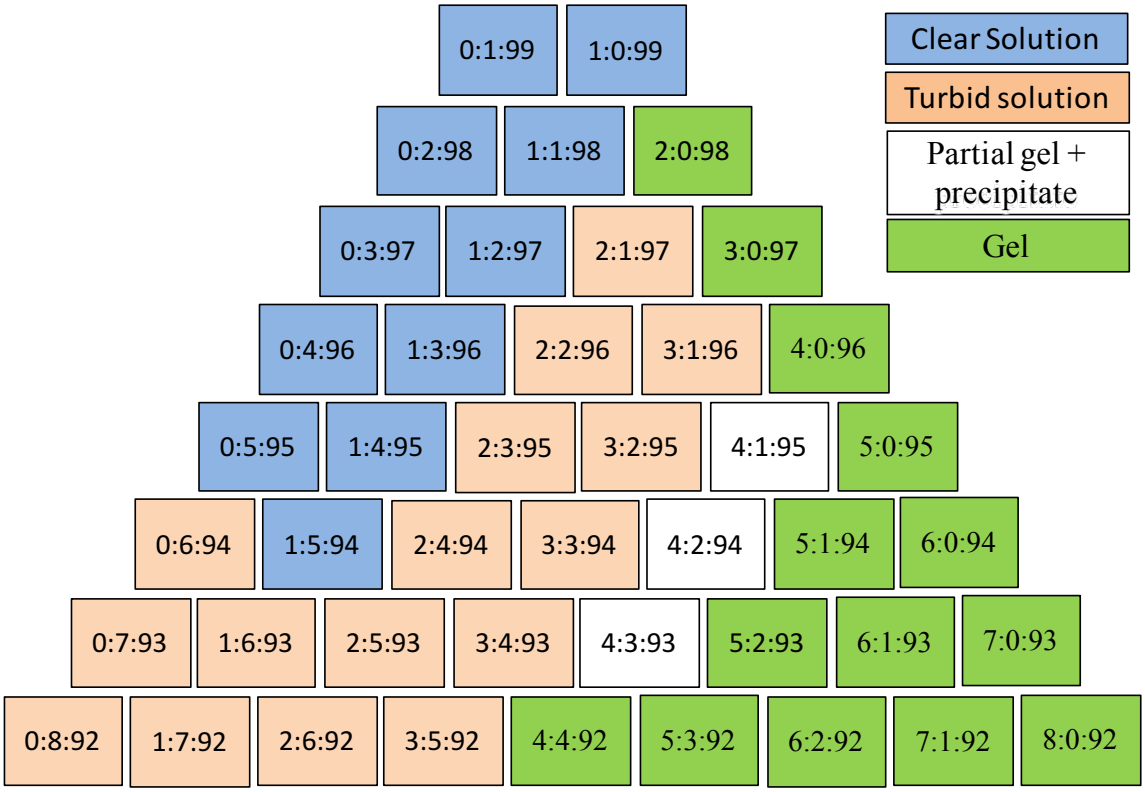

Fig. 6-3 Ternary phase diagram of Sa:β-Ss:Canola oil. Each number represents the weight percentage of Sa, β-Ss, and Canola oil respectively (e.g., 3:5:93 represents that the sample is composed of 3% Sa, 5% β-Ss, and 92% canola oil). Depending on the appearance observed, four groups are generally classified for clear liquid (blue), turbid solution (orange), partial gel (white), and oleogel (green).

The ternary phase diagram illustrates the fact that Sa-β-Ss is a promising binary system for structuring canola oil, resulting in self-standing oleogels with a similar characteristic of solid fat at certain combination ratios. Thus, how the mass fraction of two components plays the role in oleogel formation was of interest. Since Sa is a LMOG and β-Ss is not, Sa-β-Ss is naturally recognized as a gelator-additive system, while Sa-Chol is a gelator-gelator system which was used at the same concentration and mixing mass fraction as Sa-β-Ss, in order to illustrate the differences between gelator-additive and gelator-gelator systems.

At a fixed concentration of 8 wt%, every tested ratio in the Sa-Chol system was able to form a self-standing opaque oleogel (Fig. 6-4). One interesting result is that the degree of opacity of each

oleogel was subtly different. Instead of only visualizing this opacity, light absorbance was measured to show the differences in degree of opacity. In Fig. 6-5, a V-shape was observed, with the lowest point at the sample of S5B3 (5% of stearic acid and 3% of β-sitosterol). When the sample had either excess of Sa or Chol, the gels were more opaque due to the size of their crystals being larger or that the density of the crystals was higher. In other words, when the mass fraction of Sa and Chol was similar, the oleogel was more transparent. Based on the types of self-assemblies, gelator-gelator systems can be divided into two groups: 1) Co-crystals – two gelators self-assemble into repeated units for co-crystallization or random co-crystallization, and 2) Self-sorting – Two gelators self-assemble but somehow the individually formed crystals can self-assemble with each other to form a three dimensional network. This case commonly occurs in the situation where both crystals have an H-bonding donor or H-bonding acceptor and can connect with each other via hydrogen bonding. Cyclic structures can stack with each other as well via pi-pi stacking interactions. Based on this result, it is hard to judge whether Sa-Chol is a co-crystal or is self-sorting. This will be further discussed in the PLM section.

Even though Sa-Chol seems to be more promising than Sa-β-Ss because it can form an oleogel in any ratio at 8% concentration, the use of Chol is concerning, due to its association with cardiovascular disease. Thus, a compound with structural similarity to Chol, β-Ss, offers a promising alternative. In addition, β-Ss is a bioactive compound providing several functions, such as lowering cholesterol and providing anti-cancer properties.

Compared to Sa-Chol, not all ratios in the Sa-β-Ss system were able to form oleogels. It was found that S4B4 seems to be a critical gelation ratio. When the mass fraction of Sa was less than β-Ss (such as S1B7, S2B6, S3B5), only a turbid solution with clumps in it were seen (Fig. 6-4).

However, when the mass fraction of Sa was more than β-Ss including S5B3, S6B2, S7B1, oleogels were obtained. S4B4 was found to be an oleogel and also the ratio in between two regions. In addition, the results of the degree of non-transparency showed that most of the oleogels except S5B3 were more opaque than Sa-oleogel. This indicated that either the size of crystals was larger in Sa-β-Ss oleogel compared to Sa-oleogel or that more aggregates formed in Sa-β-Ss oleogels. S5B3 was the least opaque oleogel.

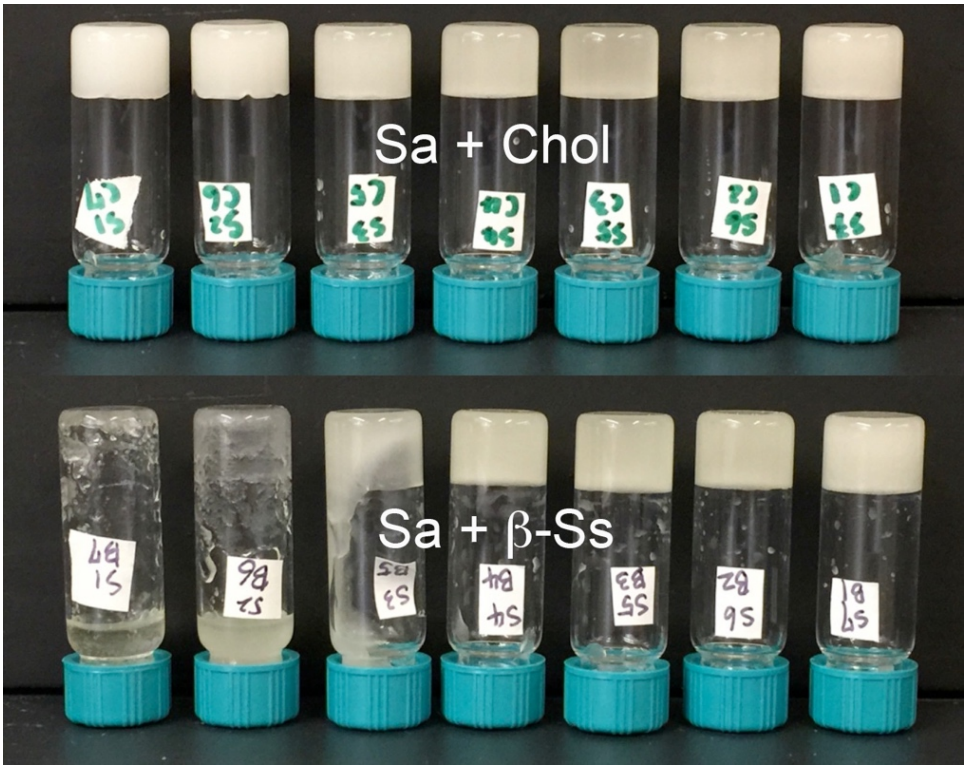

Fig. 6-4 The appearance of inverted samples of two Sa-Sterol (Chol or β-Ss) mixed systems with varying mass fraction in canola oil. From left to right, the mass fraction is 1:7, 2:6, 3:5, 4:4, 5:3, 6:2, and 7:1 (Sa:Sterol, w/w).

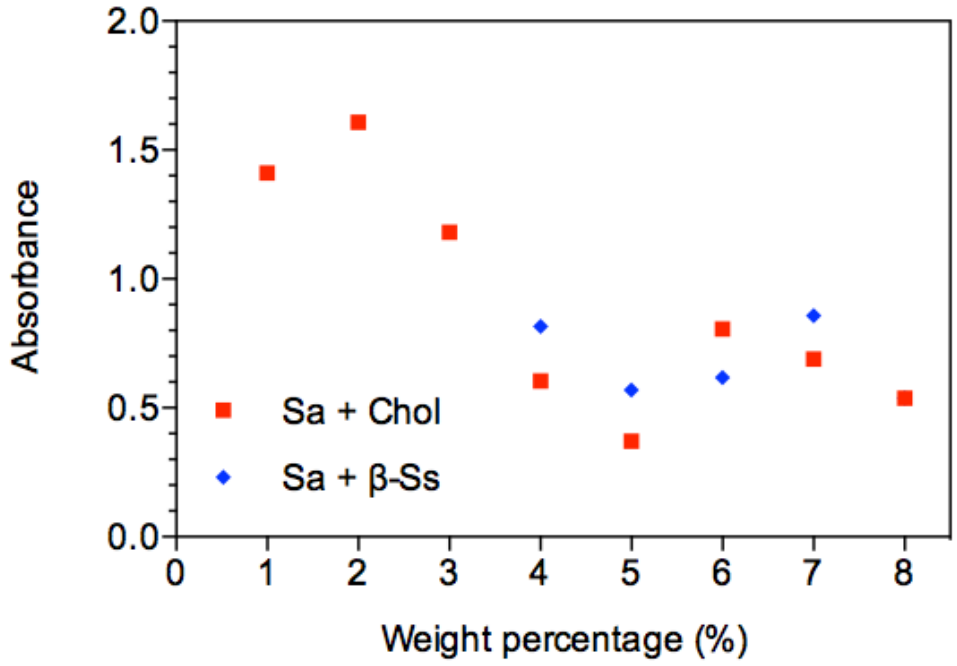

Fig. 6-5: Light absorbance at 500 nm of two-component gels for showing the changes in the degree of opacity of oleogels with a series of mixing ratio at a fixed 8% (w/w) of total concentration. X-axis shows the weight percentage of stearic acid. Red square is stearic acid + cholesterol, and blue diamond is stearic acid + β-sitosterol.

6.2.3. Crystal morphology is dependent on mass fraction of Sa and β-Ss in canola oil

Fig. 6-7 shows the microstructures of Sa-β-Ss samples with different mass ratio of Sa-β-Ss mixtures at 8% of total solid content in canola oil after 1 d of storage at 25°C (RT) from a hot solution. The oil phase, which is optically isotropic, appears completely dark and the presence of needle-like crystals is noticed by a bright white field. The similar microstructures were captured in S1B7 and S2B6, which are two non-oleogel samples. Ribbons and rosette crystals scattered the light, resulting in solutions with haziness. This result was in agreement with other studies[8,19] which found that low aspect ratio crystals are not likely to structure oils and subsequently form an oleogel. When the mass fraction is 4% of Sa and 4% of β-Ss in canola oil, smaller platelet crystals and some needle-like crystals were captured. These type of crystals were able to in some manner aggregate strongly enough to entrap canola oil; however, the strength of network was the weakest

compared to the remaining three oleogels according to their corresponding rheological behaviors. This finding is similar to Candelilla wax and beeswax in edible oils.[32,85,98,114] In addition, crystal morphology is highly related to gel formation[39,51,91,129,130]. For example, a high aspect ratio of crystals such as needle-, rod-, fibril-, and some platelet-like crystals are able to construct a network to immobilize liquid solvent[18,22,30,129]. Rice bran wax has been proposed in this context because it forms long (20–50 μm), needle-like crystals, which are easily able to entrap large volumes of liquid oil between the crystalline strands, resulting in oleogels.[104]

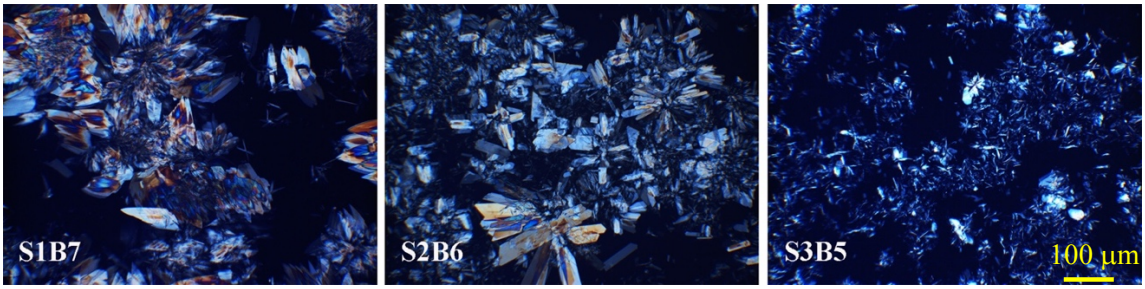

Fig. 6-6: Polarized light micrographs of three Sa-β-Ss non-oleogels. Rosettes were observed in non-oleogels (S1B7, S2B6, and S3B5). S1B7 is 1% (w/w) and 7% (e/e) of β-sitosterol. Scale bar is 100 μm.

During the cooling process, the Sa-β-Ss molecules with mass ratio of 5:3, 6:2, and 7:1 (Sa:β-Ss) self-assembled into relatively thin needle- and fiber-like crystals that intertwined and clustered, immobilizing the liquid canola oil (Fig. 6-7). These relatively thin crystals were ~2 μm in width compared to the ribbon crystals which were ~80μm in width and ~250 μm in length. The thin needle-like crystal though was not as long as tubules formed by γ-oryzanol + β-sitosterol, it still can form a three-dimensional network to entrap and solidify canola oil.

Altering the mass fraction of Sa and β-Ss affects the crystal morphology, particularly with respect to aspect ratio. This result is similar to the finding that the changes in crystal morphology of waxes in mineral oil was the result of altering the cooling rate[100]. Moreover, the increment of yield stress

(as will be shown by data to follow section 6.2.5) was found with decreasing particle size in our Sa+β-Ss system, which is consistent with the behavior for flocculated colloidal gels. Therefore, changes in mixing mass ratio of two components indeed tailor the physical properties of oleogels, including crystal morphology, crystal size, and the strength of the network.

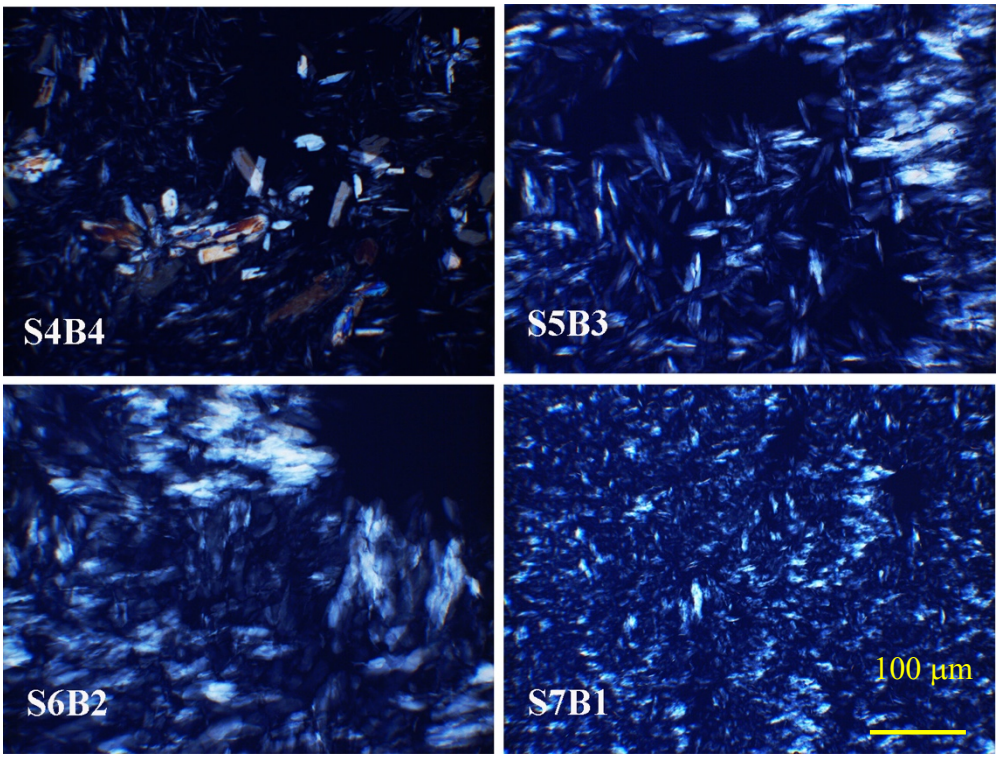

Fig. 6-7 Polarized light micrographs of Sa-β-Ss oleogels. Thin needle- and fiber-like crystals in oleogel samples (S4B4, S5B3, S6B2, and S7B1).

Sa-Chol was suggested to be a co-crystal system because its microstructures were not observed to have a combination of Chol crystals and Sa crystals. Thin fiber-like crystals were captured mainly with some thin and short needle-like crystals. Although every ratio of Sa-Chol was able to form an oleogel, there were subtle differences in microstructures between the ratios. S5C3 was the least opaque oleogel according to analysis of light absorbance. It showed significantly different microstructures compared to the others. It was apparent in the micrographs that bundles of thin fibers were gathering together. The resolution of the polarized light microscope is not sufficient

to investigate these kind of thin crystals. However, it did not have the strongest gel strength, which is in agreement with the statement that the yield stress increases with decreasing particle size. S7B1 showed the highest yield stress among Sa-Chol oleogels and it had a high amount of small crystal particles which aggregated together (see Fig. 6-8).

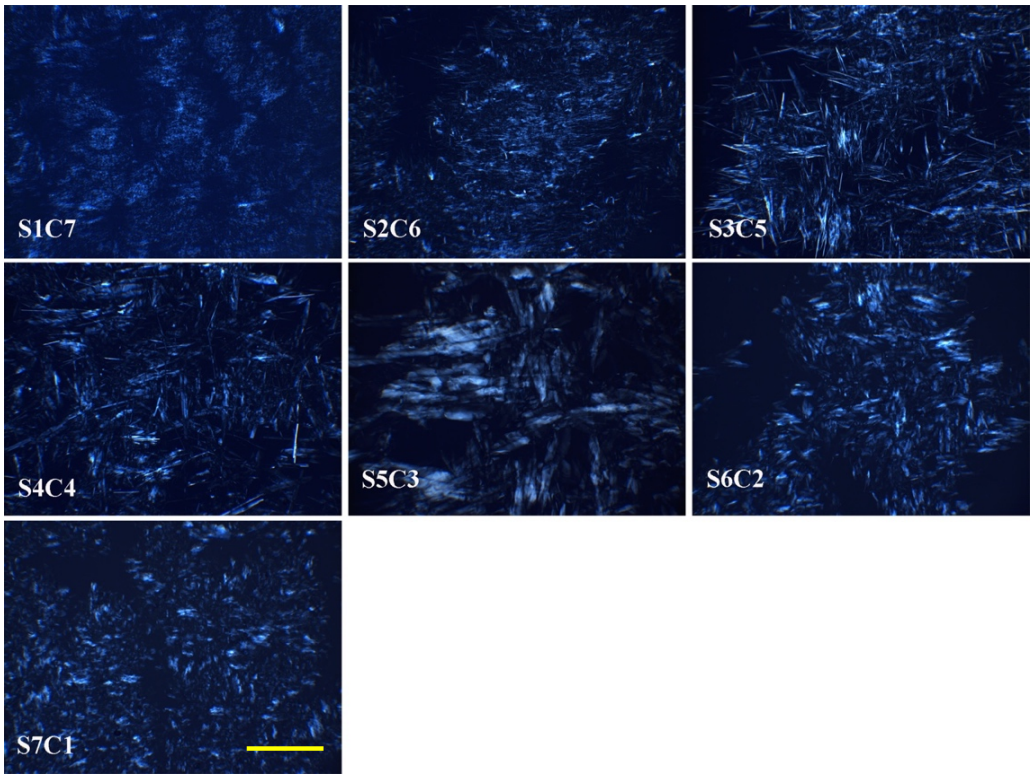

Fig. 6-8 Polarized light microscopic graphs of Sa-Chol oleogels. Thin needle- and fiber-like crystals and aggregates of crystals were observed in all gel samples. (Scale bar is100 μm)

6.2.4. Melting profiles of Sa-β-Ss oleogels

The thermal behavior of mono- and binary-component systems was investigated using differential scanning calorimetry analyses, with a heating program from 20℃ to 100℃ and a heating rate of 5°/min (Fig. 6-9). Phase transition from a slurry or an oleogel to a solution was observed using DSC when an endothermic peak was observed during the heating process. A melting profile represents specific thermal properties for each type of crystal because different energy is needed

for disassembling molecular packing in general, except occasionally for those with the same thermal properties.

The melting peak of stearic acid powder shifted from 71.2°C (peak temperature) to 53.5°C when it was in the presence of canola oil (Fig. 6-9a). In addition, the sharp peak became broader and smaller. This implies that the molecules of Sa are packed loosely when it is in canola oil, thus not much heat is needed for melting the ordered crystalline regions. A similar result was shown in β-sitosterol systems. As shown in Fig. 6-9b, pure β-Ss powder has a sharp melting peak at a high temperature of 131.6°C (peak temperature), which was shifted to 64.7°C and became a very small and broad peak. This result is consistent with its characteristic of non-oleogelation and irregular shape of crystals captured using PLM. Even though the crystals formed by β-Ss in the presence of oil had a higher melting temperature than those of Sa, these ribbon and rosette crystals were not able to self-assemble into a network to entrap canola oil.

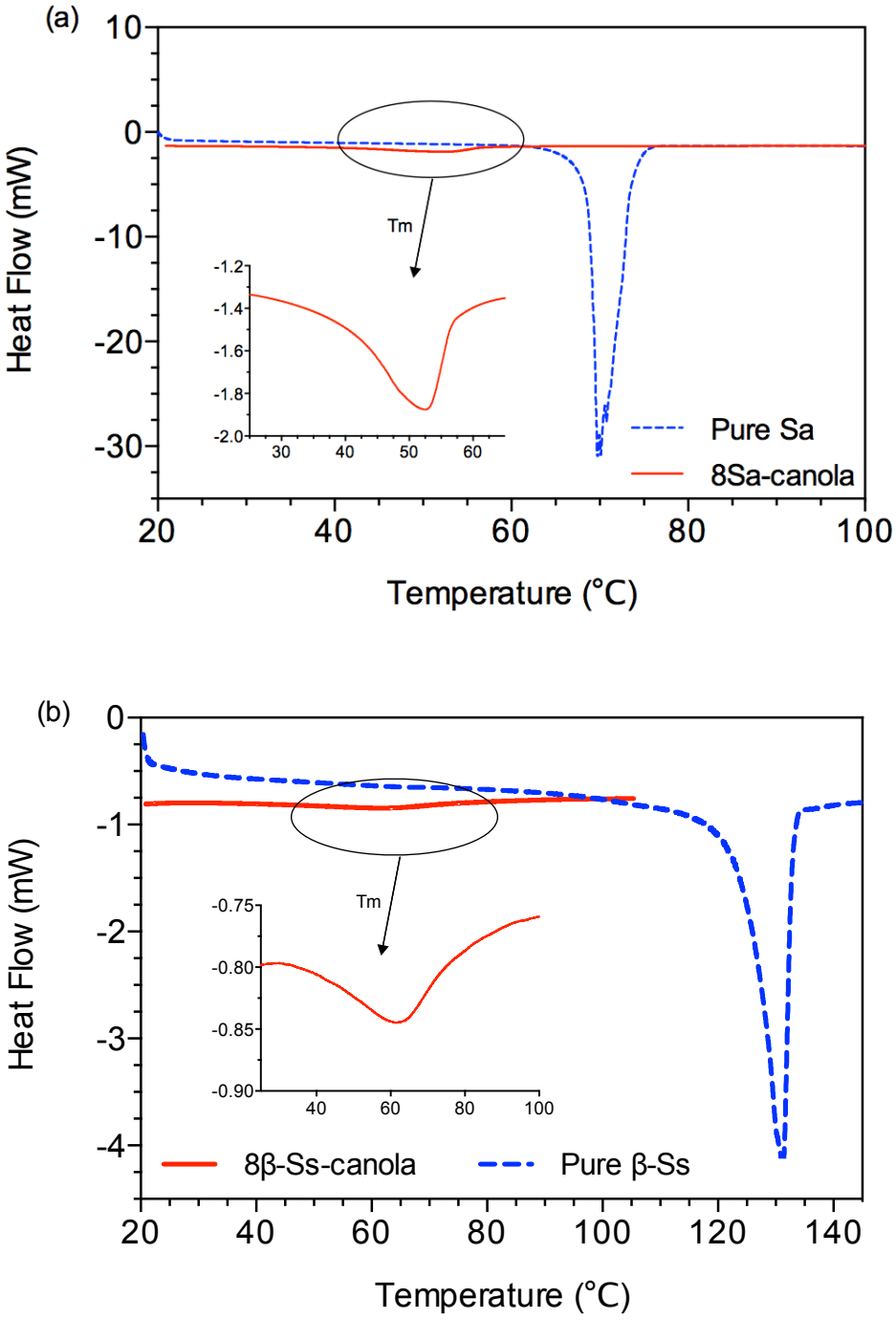

Fig. 6-9: Melting profile of pure Sa solids and Sa in canola oil (a), and pure β-Ss solid and β-Ss in canola oil (b).Both melting temperatures of Sa in oil and β-Ss in oil are lower than its pure crystal.

From the aforementioned results, changes in mass fraction of Sa and β-Ss led to different morphology of crystals formed and whether the oleogels formed. How two components self-assemble into crystals is of interest, and it has been found that co-crystallization or crystallization occurs independently. Fig. 6-10 shows the melting profiles of Sa-β-Ss in canola oil with varying mass fraction, which is able to illustrate how the co-crystallization happens. First of all, all the melting temperatures of mixed systems, irrespective of whether they are oleogels or just turbid solutions, had subtle or significantly lower melting temperatures than 8% of Sa in oil or β-Ss in oil. It indicated that Sa-β-Ss in the presence of canola oil behaved like a eutectic system, since the melting temperature of the final eutectic component is below that of its pure components[100],

Secondly, this eutectic system was sensitive to the changes in mass fraction of two components. When β-Ss was the major component (5-7%), the resulting non-oleogels had very similar melting profiles and the lowest melting point among all of the combination samples at a fixed concentration of 8% (w/w). These three ratios had similar microstructures as well in the shape of ribbons and rosettes. S5B3, the least hazy oleogel, had a relatively closer melting temperature (53℃) toward Sa-canola oleogel (53.5℃). Once Sa was used up to 7% with 1% of β-Ss, the melting point lowered subtly to 51.2℃. The oleogel self-assembly process of multi-components in oil-based systems is different from that of, for example, protein fibril formation in aqueous systems, which is driven by a change in entropy. Oleogelation is an enthalpy-driven process, and thus subtle changes in mass fraction of structurants occur, which leads to the differences in Gibbs free energy of a hot solution due to the solubility of structurants being influenced.

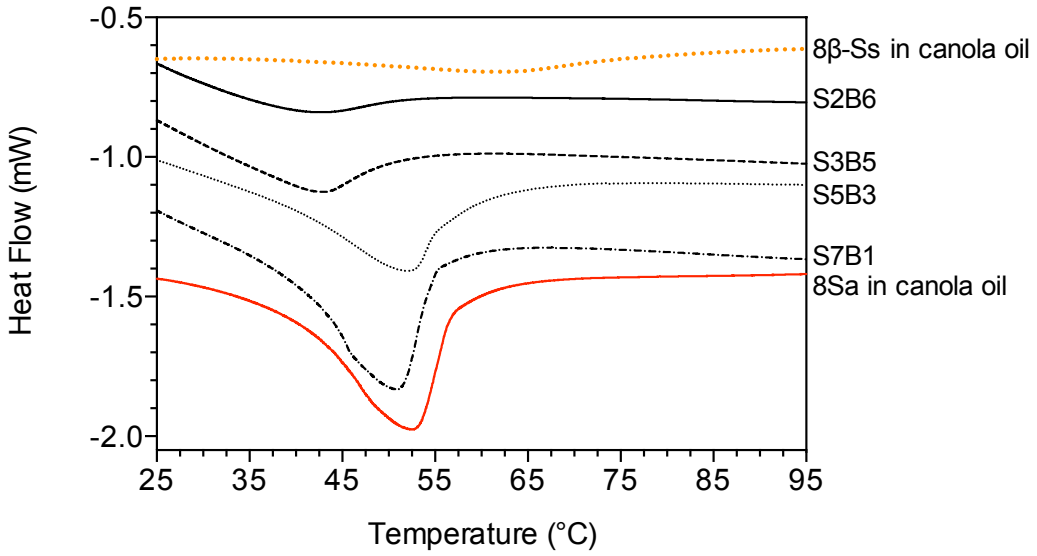

Fig. 6-10: Melting profiles of 8% Sa-oleogel, β-Ss-non-oleogel, and Sa-β-Ss-oleogel samples. Non-oleogel samples have lower melting temperature than oleogel samples.

6.2.5. Rheological behavior of oleogels

Rheological behavior is one of the most important physical properties of soft materials, which have both elastic and viscous properties. Therefore, four oleogels made of Sa-β-Ss with different ratios were prepared for measurement of rheology using a rheometer (Fig. 6-11). It can be clearly observed that rather than oleogels, commcercial butter and self-prepared lard were tested as the comparison groups to oleogels, in order to understand the feasibility of using animal fat or solid fat replacements due to their viscoelastic properties. All plots shown in Fig. 6-11 illustrate that storage modulus (G′) was higher than loss modulus (G″). If G′ is higher than G″ in a sample, this indicates that the sample is a solid-like material and is more characteristic of elastic. Four ratios of Sa-β-Ss system (S4B4, S5B3, S6B2, and S7B1) were analyzed for their oleogel properites. To compare the hardness of oleogels, the value of G′ can be an estimated indicator. The higher the value of G′, the harder the oleogel is. S4B4 and S5B3 were found to have a G′ of approximately 10^4, but for S6B2 and S7B1 this value was 10^6. These results are consistent with the results of

haziness of appearance and the crystal morphology. S4B4 not only needle-like crystals but also platelet-like crystals. S6B2 and S7B1 had more aggregates of needle-like crystals than S5B3. This may show that more crystals formed or more aggregrates of crystals may increase the strength of intermolecular forces in order to resist higher external stress for breaking the structures. At higher Sa concentrations, both polarized light microscopy and XRD analysis indicate the presence of needle-like crystals, which are expected in the junction zones between Sa-β-Ss self-assemblies (i.e., the microcrystalline domains).[131]

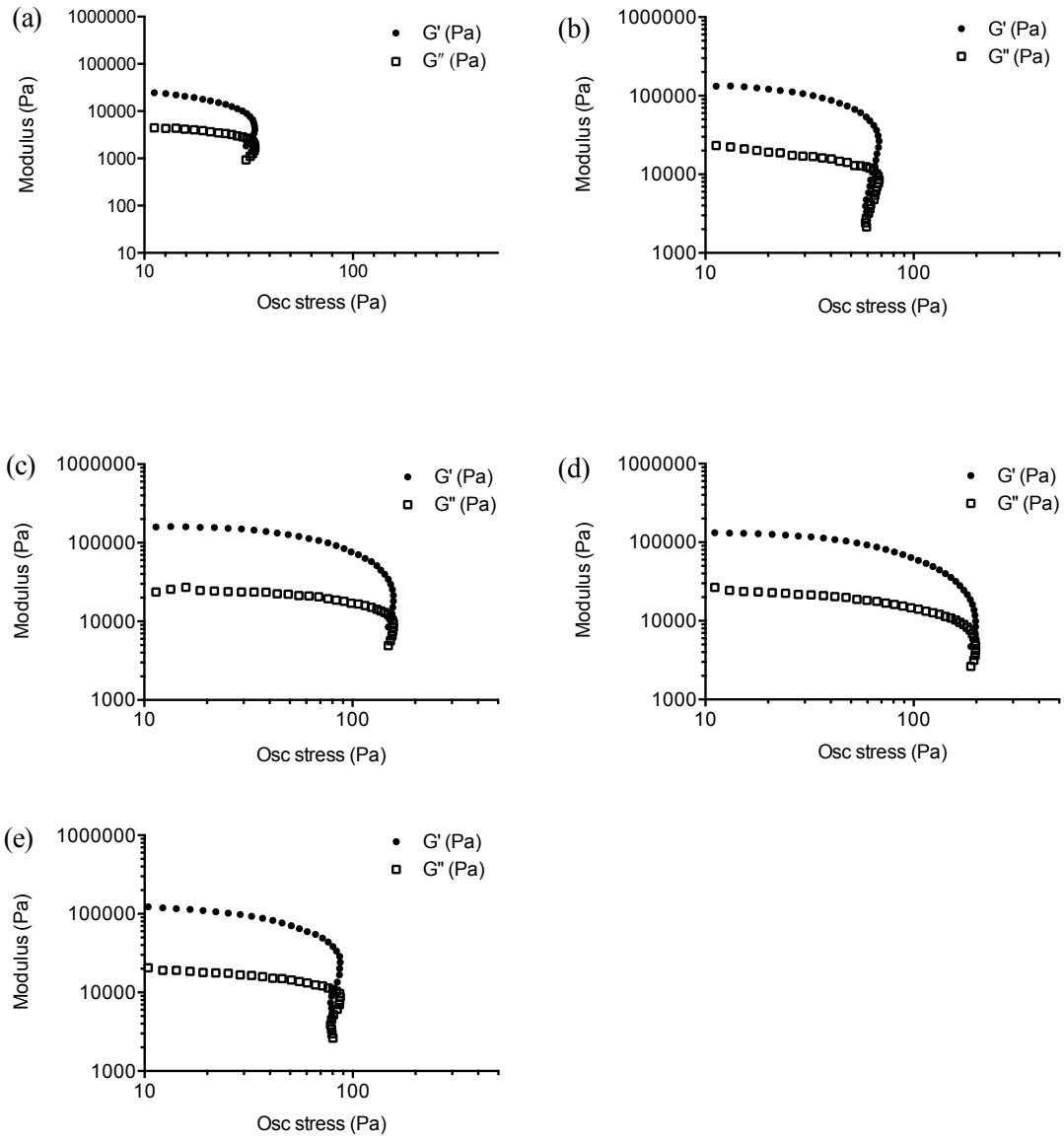

Fig. 6-11 Rheology of Sa-β-Ss-oleogels with mixing ratio of 4:4 (w/w) (a), 5:3 (b), 6:2 (c), 7:1 (d), commercial butter (e), and self-prepared lard (f).

Yield stress refers to certain given stresses of a solid-like material which starts to be destroyed without maintaining its structures, and the material starts to flow without the support of these structures. Yield stress was acquired by calculating with tangent analysis. Plot the applied stress versus modulus, a curve with a plateau region (linear region) was shown and followed by a dropped curve at certainly applied stress. In oscillatory tests, if a single tangent is applied to the

linear region of the curve then the yield stress is often taken as the stress at which the curve begins to deviate from this tangent. Yield stress can provide us the sense of strength of a gel. With a higher value of yield stress, it means that the harder you have to put on enable to breakdown the oleogel by destroying the framework consisting an oleogel. It was clearly observed when the mass fraction of Sa is higher, the yield stress is also higher (1000 Pa for S7B1; 10 Pa for S4B4). This indicates that increased numbers of needle- and fiber-like crystals are able to hold canola oil under higher external stress. In contrast, platelet crystals and lower numbers of crystals being formed led to a weaker strength of network to immobilize oil. In the literature, the strength of a gel has been found to depend on the nature of the bonds that form the network.[33,132,133] A strong gel is attributed to a network with permanent bonds, which are the connections of fibers through crystallographic mismatch branching; while a weak gel results from the network of termporary or transient bonds, which are formed through physical entanglements of elongated fibers.[22,30] Since no hydrogen bonding peak was shown in the IR spectrum of our Sa-β-Ss system (see Fig. 6-14), the network was stabilized by temporary or transient bonds instead of permanent bonds.

In addition, S6B2 and S7B1 had similar rheological behavior to butter and lard (animal fat) due to the combined effects of concentration and mixing mass ratio of Sa-β-Ss, and supramolecular network microstructure, including the shape, size, and the distribution pattern of the self-assembled crystals. We proposed that the rheological behavior of oleogels is able to be manipulated by varying the mass fraction of Sa and β-Ss. This can be an approach to produce the

desired texture of soft materials to apply these gels in a wide array of fields.

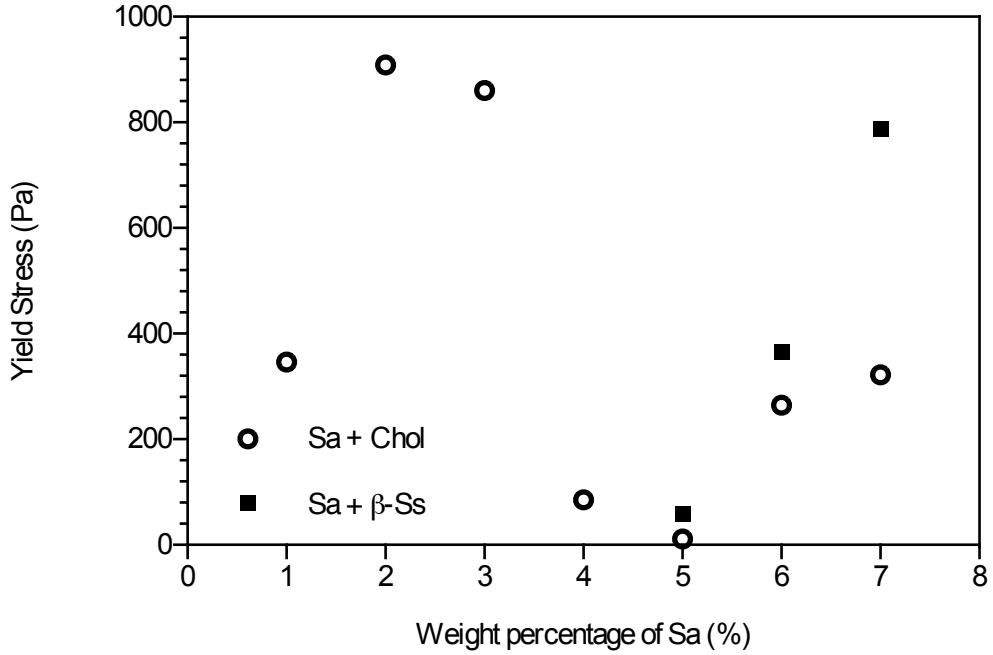

Fig. 6-12: Yield stress of two-component gels as a function of weight percentage of Sa in gels. Black square is Sa + Chol, White circle is Sa +β-Ss.

6.2.6. A predictable oleogel formation

As aforementioned, changes in mass fraction affect oleogelation, but these changes are not critical for determining whether or not gelation occurs. By analyzing the ternary phase diagram, only the ratio of Sa to canola oil mattered. The value of Sa/oil was found to be a critical number for determining whether or not it was an oleogel. A plot of wt% of Sa vs. wt% of β-Ss was used to investigate the correlation between the ratio of Sa, β-Ss, and canola oil and gel formation (Fig. 6-13). Red dots in Fig. 6-13 represent non-oleogel at this ratio and green dots represent the oleogel sample. Green dots tend to be observed in the lower right corner of the plot. The parameters meeting the requirements were related to the ratio of Sa to Canola oil. Once the Sa/oil ratio was

up to 0.0435, irrespective of whether β-Ss was more or less than this ratio, an oleogel would be expected to be produced.

To test this assumption, S4B5 and S4B6 were prepared to determine whether the gels formed or not. In fact, both compounds produced opaque oleogels (Fig. 6-13). These results suggest that the critical factor for forming an oleogel is not the total solid content, but rather the ratio of Sa to Canola oil in Sa-β-Ss belonging to a two-component system. It can be illustrated rationally that Sa has the main role for forming the self-assembled network, and when the ratio of Sa to Canola oil is up to 0.0435, network formation is not influenced by an additive – β-Ss in this case. So far, a predictable system has yet to be reported; thus, this is a recommended system which warrants further investigation.

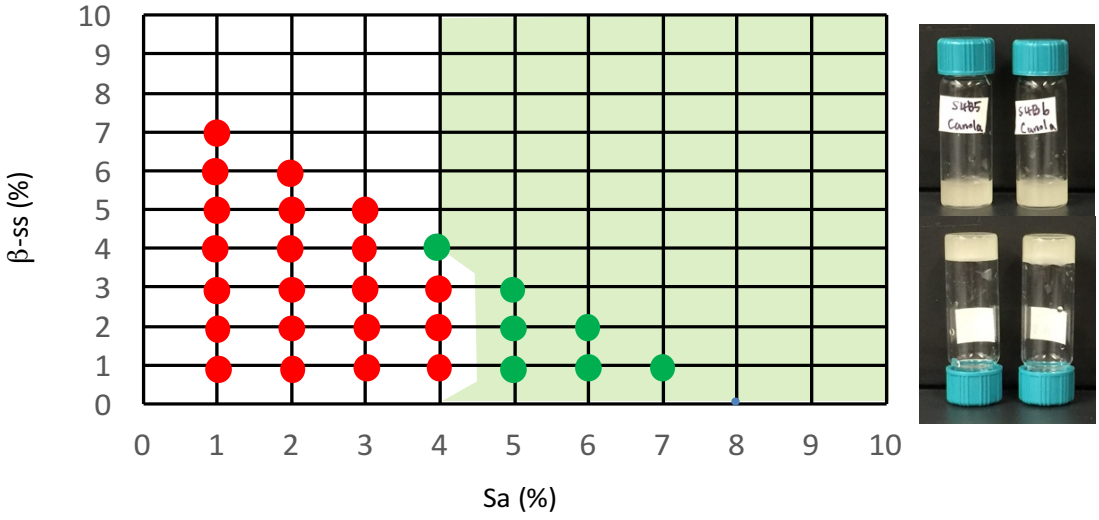

Fig. 6-13 When the ratio of Sa to Canola oil is up to 0.0435 (Green-filled area), the hot solution becomes a gel upon cooling in an ambient environment for 24 hours. Two predicted samples (S4B5 and S4B6) were tested to prove the assumption.

6.2.7. The relationship between mass fraction of two components, oleogel formation and crystal formation

The aforementioned results showed that the ratio of Sa to canola oil plays a key role for oleogel formation, and that the mass fraction of two components affects the size of crystals and the yield stress of oleogels. Previous studies illustrated that the minimum area per molecule at the 1:3 and 3:1 molecular ratio of fatty acid to fatty alcohol leads to a decrease in the interfacial tension and which results in a smaller critical radius and significantly increased nucleation rate.[100] Thus, in our case, altering the mass fraction of Sa and β-Ss can be an approach to modify the nucleation and crystallization and subsequently affect the oleogel formation and its physico-chemical properties.

In many cases of molecular gels, FT-IR has been used to investigate the changes in formation of H-bonding, and environmental changes in atoms at bonds, such as stretching and rotation of bonds. Infrared spectroscopy has been applied to solid lipids to provide information about polymorphism, crystal structure, conformation and chain-length. Previously, FT-IR has been applied for studying the nature of molecular interactions, such as H-bonds and pi-pi stacking, using various gelators or solvents.[134,135] For example, when H-bonds are required for network formation, the spectra show absorbance in the 3500-3600 cm^{-1} region (-OH stretching)[129]. In Fig. 6-14, no absorbance peak at the range of 3500-3600 cm^{-1} was observed indicating the absence of H-bonding interaction. Thus, the molecular packing and crystal network in our tested gelled samples were stabilized by other non-covalent interactions. Thus, the strength of gelation network of our systems may be weaker than other molecular gels, which are stabilized by H-bonding.

In gelled samples, no peak formed or shifted in the range of 1000-3600 cm^{-1}. Besides, no characteristic peaks account for the functional groups of canola oil disappearing (Fig. 6-14). In other words, the FT-IR spectrum of neat canola oil was the same spectrum of gelled samples,

indicating that there was no breakdown of chemical bonds or any newly formed chemical bonds after supersaturation. Therefore, a molecular gel is a suitable alternative way to develop fat replacers with conserving the chemical composition of canola oil.

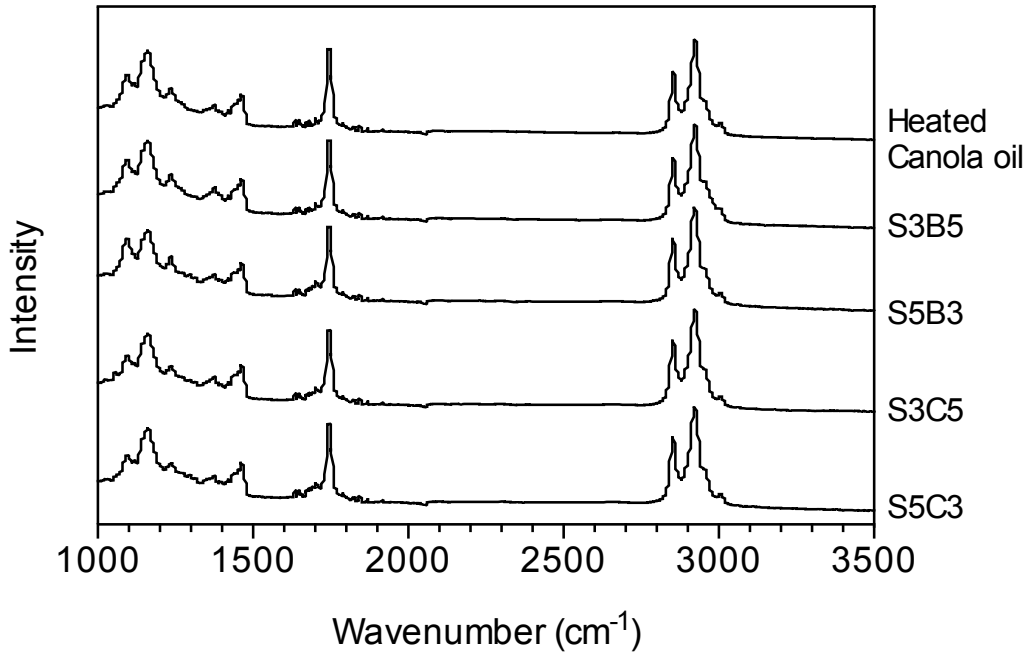

Fig. 6-14 FT-IR spectrum of Canola oil after heating, and two mass ratios of Sa + sterol systems representing non-oleogel and oleogel samples. S3B5 is a non-oleogel sample and the remaining ratios shown are oleogels.

X-ray diffraction in wide angle regions was used to explore the structural differences between non-oleogel and oleogel samples made of Sa-β-Ss mixtures with varying mixing ratios and canola oil. The diffraction peaks identified (Fig. 6-15) correspond to *d*-spacing for all samples. The distance between crystalline planes is called *d*-spacing. In molecular gels, the value of *d*-spacing can refer the distance between relative ordered structures. Overall, irrespective of whether they are oleogels or a non-oleogel, the amorphous background peaks from canola oil (19˚ and 41˚ of

2θ) and the multi-peaks corresponding to polycrystalline material are shown. The peaks corresponding to canola oil confirmed the existence of liquid canola oil in these three samples. Also, the result of polycrystals supported what we had observed under polarized light microscopy (see Fig. 6-7).

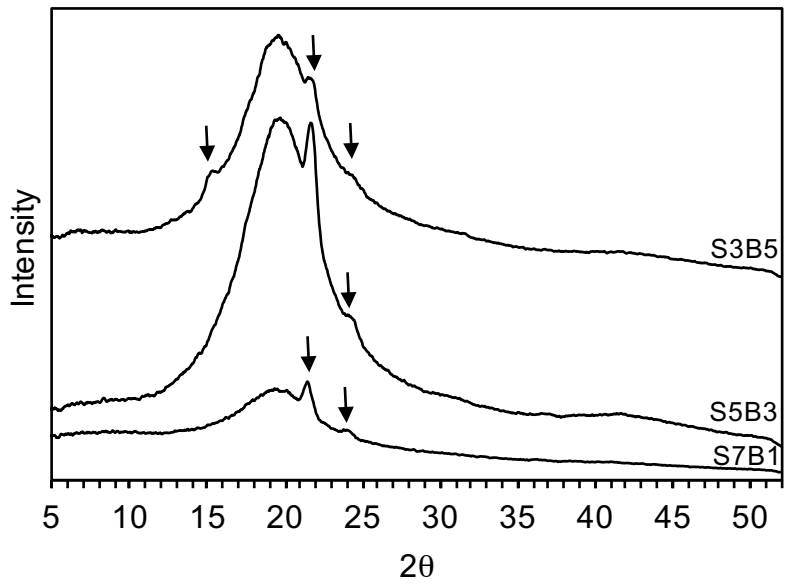

Fig. 6-15: X-ray diffraction patterns for the non-oleogels (S3B5) and oleogels (S5B3, S7B1) prepared from varying mass ratio of Sa-β-Ss in canola oil. Non-oleogel has one more peak at 14˚ than oleogels having peaks at 21˚, and 24˚. The broad peak refers to canola oil.

In comparing a turbid solution (S3B5) to oleogels (S5B3, S7B1), S3B5 had a small peak presenting at around 14˚, but this was not the case for oleogels, which showed peaks at 21˚ and 24˚ of 2θ. According to Bragg's law, a *d*-spacing of S3B5 with a peak at around 14 ˚ corresponds to ~5.10-5.80 Å, which was larger than the ~3.66-4.15 Å obtained from an oleogel. A larger value of *d*-spacing indicates that the crystal is larger. This was consistent with the crystal morphology captured in microscopic images. Increasing mass ratio of stearic acid to β-sitosterol leads to crystals being formed and changing from large platelet- to thinner fiber-like crystals with different

d-spacing obtained using X-ray diffraction. In addition, the diffraction peaks at 21˚ and 24˚ of 2θ are not only similar to those oleogels made of waxes and oil[104] but also to those of β´ forms of triacylglycerols. Among the three polymorphisms of fat crystals, the β´form with orthorhombic perpendicular subcellular-packing exhibits better functionality properties, such as smooth and pleasant mouth feel in margarine and shortening due to its crystal morphology, melting temperature close to mouth temperature, and dispersability. The results of X-ray diffraction patterns indicate that the changes in the mixing ratio of Sa and β-Ss significantly influence the arrangement of molecules during crystallization.

6.2.8. Crystallization is affected by mass fraction of Sa and β-Ss

For measuring the inducing time of crystallization, the scattered light intensity of S3B5, S4B4, S5B3, and S7B1 solutions in canola oil were recorded through the cooling process from hot solutions using spectrometry. Fig. 6-16 shows that the curves of all samples were of all samples was sigmoidal and the light absorbance stayed more or less constant during cooling, depending on the mixing ratio of Sa and β-Ss, then a sudden and sharp increase in the intensity was observed. After a few minutes, maximum absorption was observed, indicating that no more significant crystal growth was occurring. The change in intensity was attributed to a significant increase in light absorbance. Based on this, the induction time for crystallization was taken as the time when the structurants started to crystallize, at which time the light absorbance significantly increased.

Fig. 6-16 shows that the induction time of crystallization is dependent on the structurant ratio; the crystals occurred at the shortest time, 5 min, (i.e., the earliest time period in the cooling process) when the mixing ratio was 7 wt% to 1 wt% (Sa: β-Ss). On the other hand, the slowest

crystallization (8.5 min) was associated with a mixing ratio of 3 to 5 (wt%). In addition, among these four samples, only S3B5 started lately at 8 min and reached equilibrium at 12 min, which was distinguished from the others. Oleogels (S4B4, S5B3, and S7B1) only needed 5 or 6 minutes to initiate crystallization and reached equilibrium at 8 min. The later the crystallization happened, the bigger the resultant crystals were, resulting in a non-oleogel sample. In contrast, with a reduced time of 5 min for initiating crystallization, a higher aspect ratio of crystals formed, which led to the formation of an oleogel. These results are similar to the findings of Dassanayake et al.[104]

Each induction time of crystallization corresponds to certain temperatures during cooing. The shorter the induction time, the higher the temperature when mixed Sa-β-Ss started to transform from free molecules to self-assembled crystals. This result indicates that the changes in Sa–β-Ss ratio affects the condition of supersaturation and subsequently the size and structure of formed crystals, which possibly explains the effects of the ratio on transparency and firmness of the oleogel, as shown by spectrometry (see Fig. 6-5) and rheology, as shown in Fig. 6-11, and also as previously reported by Bot et al.[89,96]

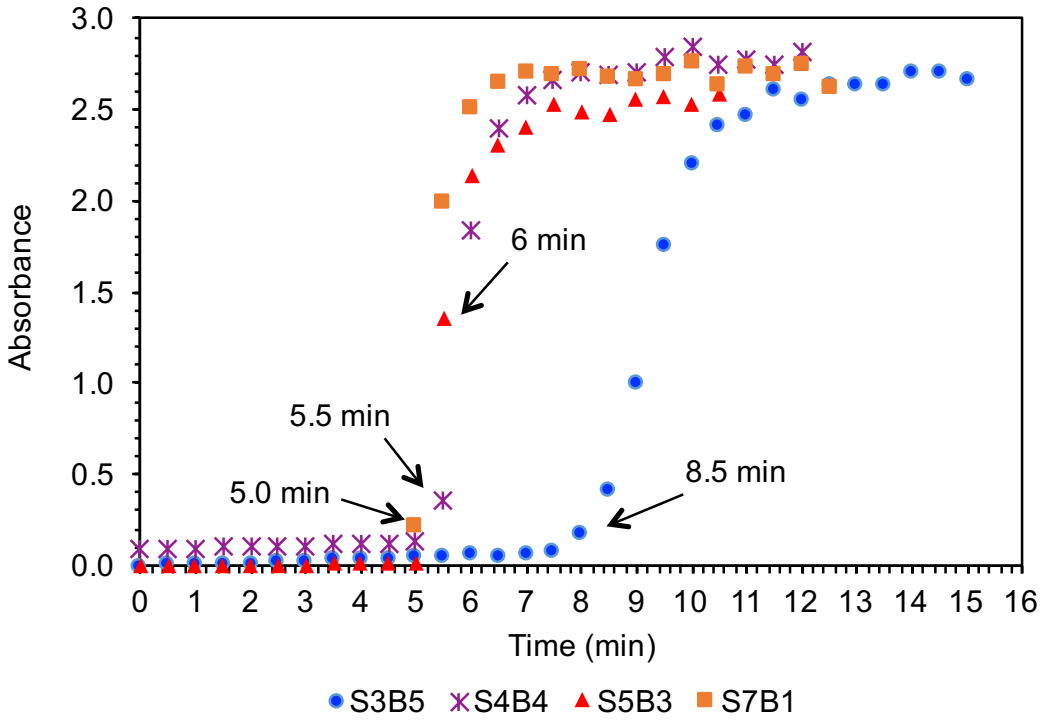

Fig. 6-16: Intensity of light absorbance vs time during cooling of S3B5, S4B4, S5B3, and S7B1(S: stearic acid; B: β-sitosterol) structurant solutions in canola oil as measured by spectrometry. Gel sample has shorter induction time than non-gel sample.

6.2.8.1. Micrographs of crystallization as a function of time

Even though gelation does not occur immediately after a solution is cooled below the crystallization temperature, the kinetics of the initial stage of crystallization were of interest in order to visualize the differences observed from the results of induction time of crystallization. Thus, two non-oleogels (S2B6 and S3B5) and two oleogels (S5B3 and S7B1) were prepared to study the kinetics of growth of the crystals/aggregated in the first three hours during the cooling process using polarized light microscopy. Overall, the results showed a high correlation between Sa-β-Ss ratio, growth rate of crystallization, and the nature of Sa-β-Ss self-assemblies (Fig. 6-17– Fig. 6-20).

S2B6, a non-oleogel sample, was a turbid suspension with clumps and cloudy aggregates. The large ribbon aggregates, rosettes, and a few small rod- and needle-like crystals were captured after storage at room temperature for 24 h; however, only small platelet aggregates were captured 3 hours after removal from the hot plate (Fig. 6-17). The transfer time was neglected, so 0 min meant the time the sample began to be monitored. A large amount of dots was observed at 5 min, and an aggregation of dots were seen at 10 min. Short rod- or platelet-like crystals were observed at 15 min. After 40 min, the growth of crystals and aggregates tended to slow down, resulting in clusters of short rod-like crystals. The crystals were not dispersed evenly over the viewing area, differing from the situation for oleogel samples.

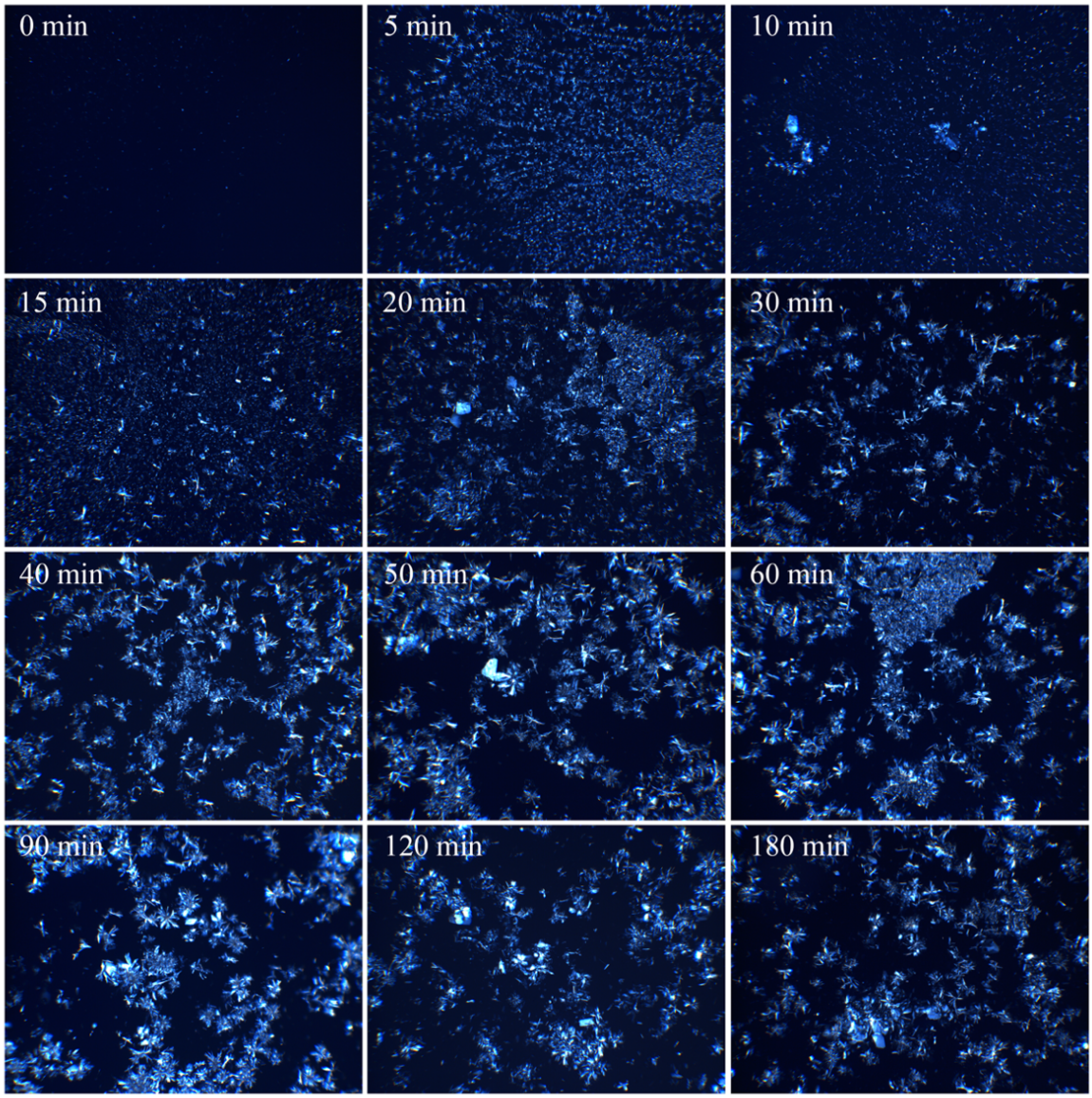

Fig. 6-17: Polarized light microscopic graphs of S2B6 with time lapse. Width of each microscopic graph is 475 μm. From upper left to bottom right are 0, 2, 10, 15, 20, 30, 40, 50, 60, 90, 120, and 180 min.

Another non-gelled sample, S3B5, was observed using a polarized light microscope from 0 to 180 min (see Fig. 6-18). At 0 min, a few dots were already present. At 5 min, more dots occurred, and were recognized as nuclei for promoting crystal growth. From 8 to 40 min, few events occurred. This was consistent with the observation in light absorbance spectra showing that S3B5 had a sharp increase in light absorbance at 8 min and reached maximum light absorbance at

approximately 12 min. Therefore, no differences were seen from 10 to 40 min. Nevertheless, micrographs showed apparent aggregation occurring at 50 min. There were no ribbons and rosettes observed during three hours, but crystals were seen when S3B5 was stored overnight. These findings provided support that the longer the time needed for crystallization, the bigger the crystals were that formed. Moreover, bigger crystals are not favorable in terms of self-assembly into a network for entrapping liquid oils.

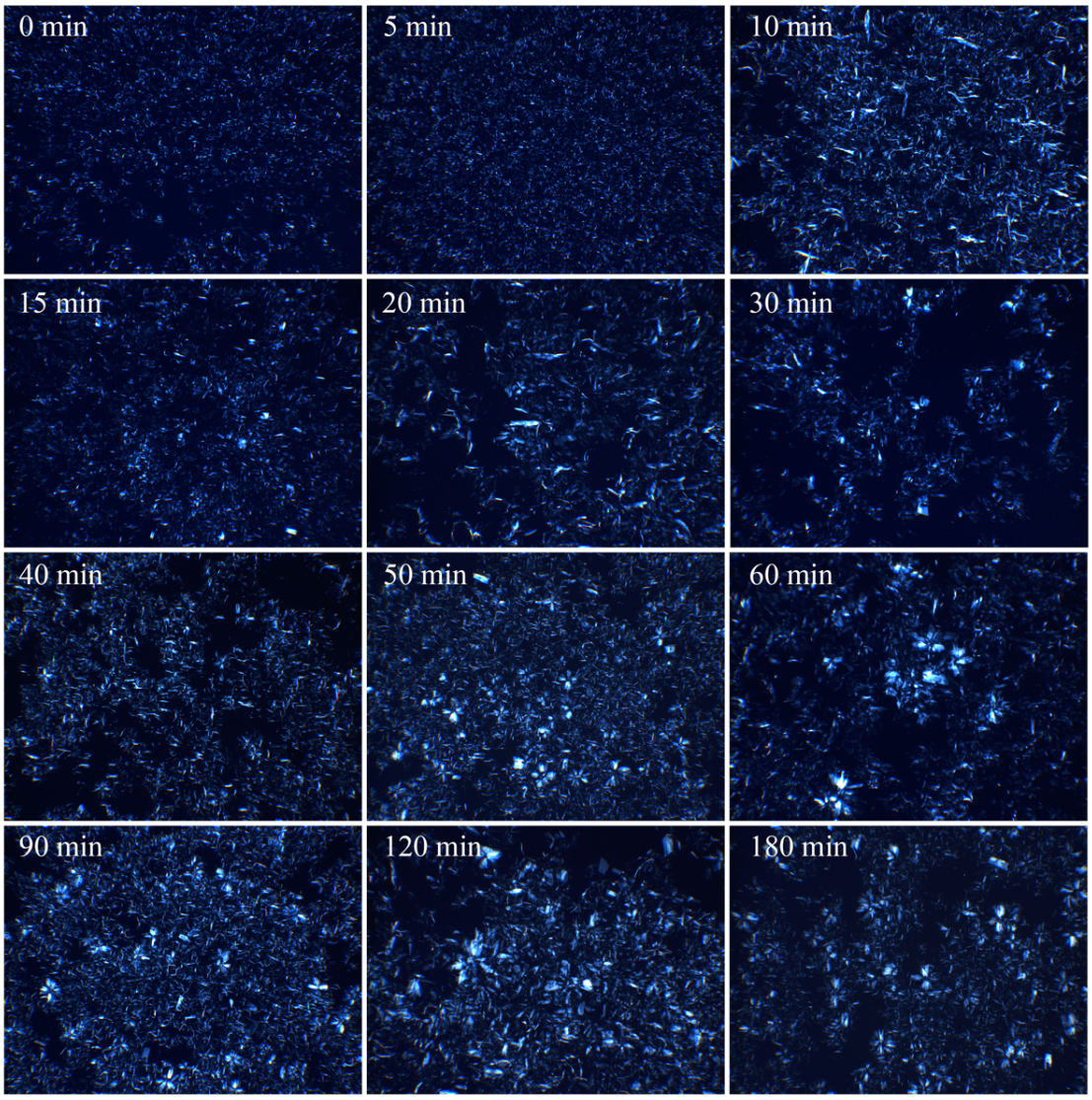

Fig. 6-18 Polarized light microscopic graphs of S3B5 with time lapse. Width of each microscopic graph is 475 μm. From upper left to bottom right are 0, 2, 10, 15, 20, 30, 40, 50, 60, 90, 120, and 180 min.

S5B3 and S7B1 were both oleogel samples and there were no apparent differences observed under PLM during three hours (Fig. 6-19–Fig. 6-20). At 0 min, high density and evenly dispersed dots were seen in both micrographs of S5B3 and S7B1. Longer needle- and fiber-like crystals were seen from the micrograph at 10 min. Induction time for crystallization for both S5B3 and S7B1 were very similar (5.5 and 5 min, respectively). This matched our observations for PLM. Furthermore, the crystals showed the same shape and size at both one and 24 hours.

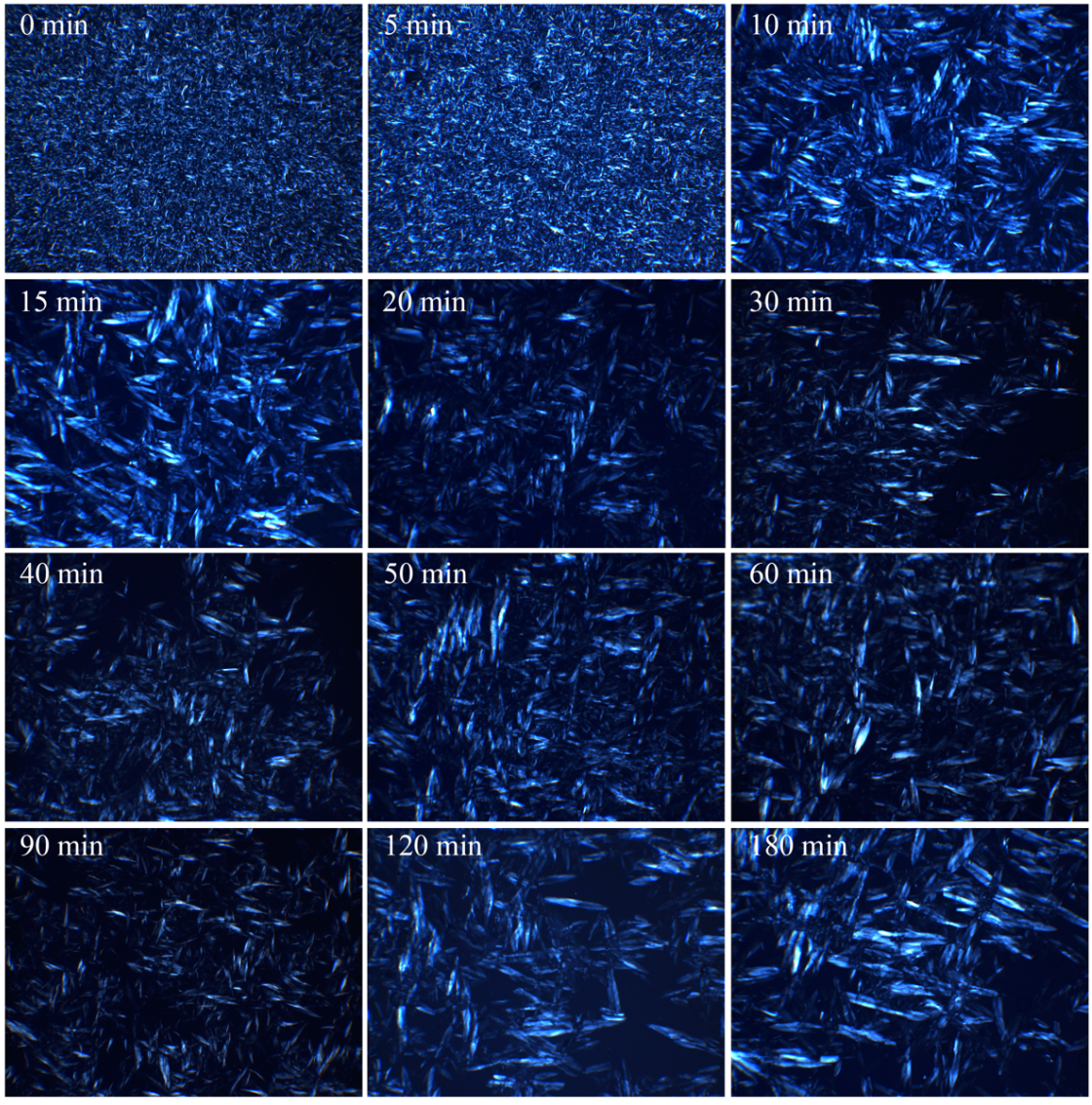

Fig. 6-19: Polarized light microscopic graphs of S5B3 with time lapse. Width of each microscopic graph is 475 μm. From upper left to bottom right are 0, 2, 10, 15, 20, 30, 40, 50, 60, 90, 120, and 180 min.

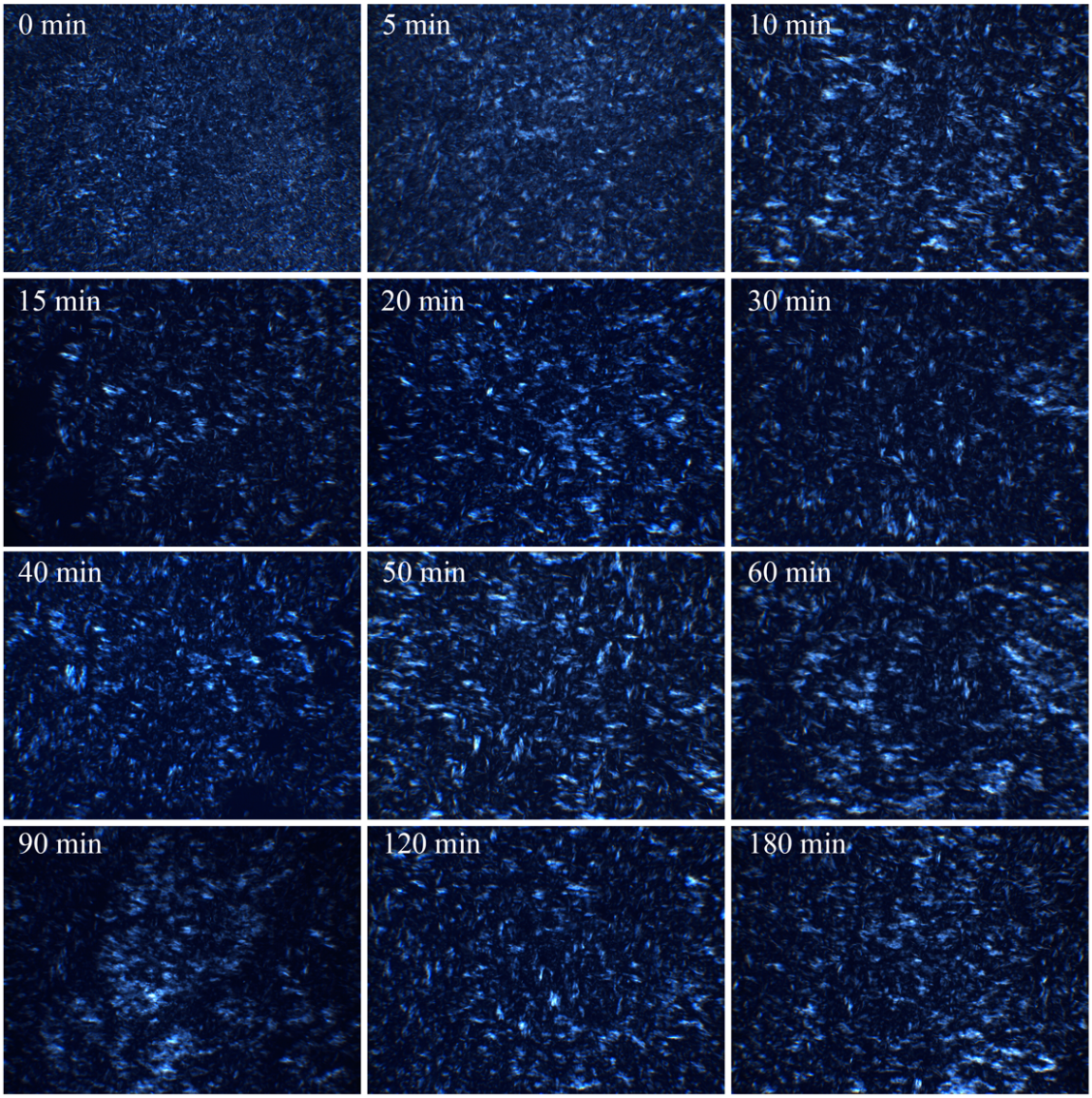

Fig. 6-20: Polarized light microscopic graphs of S7B1 with time lapse. Width of each microscopic graph is 475 μm.

6.3.Conclusion

The results obtained in this study show that it is possible to structure canola oil by enabling molecular gelation with the use of a mixture of β-sitosterol and stearic acid at specific combination ratios (i.e., S4B4, S5B3, S6B2, and S7B1) with a total solid content of 8% (w/w). In addition, the criteria to form an oleogel in this Sa-β-Ss-canola system involves a ratio of SA:canola oil of

>0.0435. This finding suggests that stearic acid is a successful co-gelator to β-sitosterol by changing the condition of supersaturation in terms of altering large platelets, ribbons, and rosette crystals (of a width of ~80μm) into thinner needle- and fiber-like crystals (of a width of ~2 μm). Altering the mass fraction of Sa and β-Ss in canola oil leads to changes in the condition of supersaturation, which causes different crystallization induction times. As a result of these changes, the subsequent crystal sizes are different. The shorter the crystallization induction time, the smaller the size of the crystals that are formed. This mechanism has been proposed to explain why increased supersaturation results in the formation of a larger number of critical nuclei and thus smaller mean crystal size. Crystal size is highly related to gel strength. The findings show that when crystal size decreases, gel strength increases.

Another advantage of forming oleogels as a replacement of fat solids is that canola oil is not chemically modified through the entire heating-cooking process for making oleogels, which means that fatty acid profiles of TAGs in canola oil remain unchanged. Therefore, this study suggests that oleogel is a promising material as a substitute for solid fats by physically entrapping canola oil via the use of Sa-β-Ss, a binary system in canola oil. Finally, this study also points out that multi-component systems provide increased opportunity for the formation of oleogels with different physical properties for a wide range of food and pharmaceutical applications.

7. Effects of Oil Phase on Oleogel Formation

Abstract

Oleogel, a relatively new semi-solid substance, has been suggested to be used as a solid fat alternative. A critical requirement for forming oleogel is believed to be the balance between gelator-gelator and gelator-solvent forces. Many studies have been done to find suitably low molecular weight molecules such as gelators, and studying the self-assembles formed. In contrast, comparatively few studies discussed the role of edible oils in oleogel formation. Twenty-five commercial edible oils (canola oil, two flaxseed oils, hemp oil, two avocado oils, roasted almond oil, roasted hazelnut oil, safflower oil, cottonseed oil, soybean oil, blend oil, grapeseed oil, extra virgin olive oil, olive oil, corn oil, sunflower oil, pumpkinseed oil, peanut oil, macadamia nut oil, two rice bran oils, sesame oil, two coconut oils) and two non-edible castor oils were used with a promising two-component gelator system, stearic acid + β-sitosterol, to study the influences of oils on oleogel formation. Only 11 edible oils were able to solidify resulting in self-standing oleogels. Four of the prepared samples formed partial oleogel samples. Tiny crystals and ribbon-like crystals were found in the samples of non-oleogels where a three-dimensional network cannot be formed in order to immobilize oil. In contrast, the needle-like crystals were shown in gelled samples, which aligns with most studies. Based on the results of the hierarchal clustering analysis, the fatty acid compositions were found to be related to oleogel formation. As oil contained a low percentage of saturated fatty acids (ca. 7-14%), it had a greater potential to be structured in terms of forming an oleogel. In addition, an oil that had a high amount of linolenic acid seemed to have the same results. This provided further information for understanding the mechanism of oleogelation, especially the gelator-solvent interaction.

7.1.Introduction

Oleogels are a group in the family of organogel consisting of structurants and organic solvents. Depending on the nature of structurants and organic solvents, several categories are classified. Oleogels are made of liquid oils and usually low molecular weight molecules as structurants. In the previous chapter, a two-component system was studied and found to be a successful structurant to solidify a large amount of canola oil in a specific mass ratio. Although the self-assembled crystals formed highly depend on the nature of structurants, such as the existence of hydrogen bonding, solvents have been proposed as having a role in helping or interfering the crystallization of structurants. It is believed that the interaction of the gelator-solvent determines the final status of samples becoming a solution, a gel, or a precipitate, as well as the interaction of gelator-gelator. Since the field of edible oleogels is relatively new and has not been studied thoroughly, only some studies have been reported for finding an edible low molecular weight gelator. The role of the gelator in crystallization and gel formation has not even been mentioned in the systematic research for studying the role of edible oils in oleogelation.

Nevertheless, only few studies using edible oils as solvents has been studied. The critical gel concentration of the same low molecular weight gelator was reported differently depending on the given oils. In addition to the CGC, the hardness of oleogels was reported differently due to the use of a different oil as well. Wright and Marangoni[136] reported that only 1.5% of ricinelaidic acid was needed to structure canola oil while 4% was needed to structure sesame oil. A possible reason was that there are more sterols and tocopherol in sesame oil. More hydroxyl groups might participate in the hydrogen bonding and then interfere with the formation of the self-assembled network leading to a failure to network forming at low concentration. Moreover, steric hindrance also provides obstacles for aggregation since sterols and tocopherol have ring structures.[131,136]

Gandolfo et al. have reported that among three oils used in the fatty acid + fatty alcohol system, the oleogel made of soybean oil was the harder gel then those made of sunflower oil and rapeseed oil due to intrinsic differences in these oils. Soybean oil has 11% of saturated fatty acids (comprising palmitic acid), which is higher compared with sunflower oil and rapeseed oil. In addition, this gel was formed and stored at 5°C in the study. At as low as 5°C, a part of soybean oil is crystallized naturally, which contributes to the structure providing some hardness as it would when refrigerated by a consumer.

The effects of other organic solvents rather than an oil on gelation have been reported as well. Zhu et al. reported that the changes in the structural transition of a single diphenylalanine from resulting organogels to flower-like microcrystals deposited at the bottom of the container appeared when changing the mixing ratio of ethanol and toluene. The formation of self-assembled network of diphenylalanine in toluene is proposed to be driven by the pi–pi stacking between aromatic moieties and hydrogen bonding of peptide main-chains. The additional solvent, ethanol, which has a higher polarity than toluene, is involved in the formation of hydrogen bonds during molecular self-assembly of the diphenylalaine in the mixed solvents, thus leading to the transformation of organogels into microcrystals. Another study has provided some clarification regarding the effect of solvents on crystal morphology. Wang and Hao[59] found that a surfactant molecule, sodium laurate, self-assembled into a fibrillar network that entraps only certain solvents (i.e. alcohols). Among those alcohol-containing gels, different crystalline morphologies were found as a result of scanning electron microscopy analysis. This showed the influence of solvents on the microstructures of molecular gels. Wang and Hao proposed that alcohol chain lengths are longer, and the gels can be more easily formed due to the solvophobic force of sodium laurate decreasing with the increasing length of alcohol molecules. Sodium laurate fibers would dissolve

more easily at room temperature; therefore more fibers would entangle with each other, leading to stronger gels.

Edible plant oils are mixtures of lipids composed mainly of triacyclglycerols (TAGs) with a content of up to 95%-98% and other minor components, such as fatty acids, fatty alcohols, and unsaponifiable matter including tocopherols, sterols and waxes, and etc.[137,138] TAGs are composed of a glycerol backbone with three fatty acid moieties esterified to the alcohol groups. Each vegetable oil has its own specific fatty acid composition, which is formed by a mixture of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) depending on the plant sources and the process during extraction and refining of oils. The differences in fatty acids composition not only affect the physicochemical properties of vegetable oils, but also show the impact on human health due to fatty acids' different influences on human health, including the risk of serious diseases. Since a human body cannot synthesize long-chain (C_{16} to C_{20}) fatty acids and has no ability to desaturate long-chain fatty acids at either C_3 or C_6 from the methyl end, n-3 and n-6 PUFAs have been termed as essential fatty acids. It has been proven that the metabolites of these essential fatty acids are important to human health. For instance, PUFAs with 20 carbons (i.e., eicosapentaenoic acid) can be metabolized to a variety of eicosanoids, such as prostaglandin E2 and leukotriene B4, which act in immune and inflammatory response regulation.[139]

The major vegetable oils for global supplying and consumption are soybean, palm, palm kernel, rapeseed, olive, sunflower seed, coconut, peanut, and cottonseed are listed in the report by the USDA-NASS in 2017. Soybean oil accounted for approximately 80% of total edible oil consumption in the US (USDA-NASS) in 2017 because of its availability and its many desirable characteristics, including compositional and functional properties. The typical

composition of refined soybean oil includes more than 99% of TAGs, 0.003-0.045% of phospholipids, 0.13% of phytosterols, 0.11-0.18% of tocopherols, and less than 0.05% of free fatty acids. The major fatty acid in TAG structure in refined soybean oil is linoleic acid ($C_{18:2}$), a n-6 PUFA, followed by oleic acid ($C_{18:1}$), a n-9 MUFA. In contrast to soybean oil, main fatty acids in the TAG structure in canola oil, from a modified variety of rapeseed, are oleic acid followed by linoleic acid. In addition, canola oil is well known for its very low saturated fatty acid content (< 7%) and a small amount of erucic acid (< 2%) and glucosinolates (30 μmol/g) compared with traditional rapeseed oil. The presence of high levels of erucic acid ($C_{22:1}$) and glucosinolates in early rapeseed oils, before the mid-20th century, was blamed for producing fatty deposits in the heart, skeletal muscles, and adrenals of rodents as well as impairing growth. Thus, through the efforts of the breeding program, the first low-glucosinolate and low-erucic cultivar of *B. rapa* (Candle) was released by Keith Downey belonging the National Research Council of Canada in Saskatoon in 1977. The next year, the Western Canadian Oilseed Crushers registered the name of canola and then transferred to the Canola Council of Canada in 1980.[140]

Sunflower (*Helianthus annuus* L.) is another major botanical source for producing edible oils in the United States as well as globally. Like soybean oil and canola oil, different sunflower oils of various compositions from different sunflower hybrids are available in the market developed from the techniques of mutagenesis and breeding strategies. For instance, high-oleic sunflower oil (up to 75-90%) was developed by K.I. Soldatov in Russia and produced commercially in the United States in 1984. Regular sunflower oil only has about 14-39% of oleic acid. The following mid-oleic (~43-71%) sunflower oil has been promoted by the US National Sunflower Association in 1995. For unsaponifiable matter in three types of sunflower oil, a high level of α-tocopherol content (400-1000 mg/kg) enhances the nutritional value of sunflower oil as a vitamin E source,

compared with other commercial vegetable oils; only an intermediate value of sterols (1700-5200 mg/kg)) is in sunflower oils, compared with other vegetable oils. The highest sterol content is found in rapeseed oil, 4800-11300 mg/kg, followed by maize oil (8000–22100 mg/kg) and sesame oil (4500–19000 mg/kg), provided by the Codex Alimentarius.

Cottonseed oil, a linoleic acid-rich oil (n-6 PUFA), is derived from the seeds of the cotton plant (*Gossypium* sp). 98-99% TAGs, 0.2-0.4% diacylglerols, 0–0.4% sterols, 0.05% free fatty acids, and 0.05% tocopherols are in cottonseed oil in general. Cottonseed oil has high levels of palmitic acid (~25%). Due to this characteristic and unequal distribution of saturated fatty acids along the glycerol backbone, cottonseed oil crystallizes into a β2 form with very small, fine crystals associated with a desirably smoother consistency and a more fluid plasticity in solid-fat products.[140] Rice bran oil has the same characteristic. Rice bran oil is extracted from rice bran, a by-product of rice milling and has been used for centuries in many South East Asian countries. Rice (*Oryzae sativa* L.) is one of the staple foods of about half of the world's population.[141] Due to high production of rice, availability becomes a significant advantage of rice bran. In addition to the high level of palmitic acid ($C_{16:0}$) at 12-28%, rice bran oil has the highest sterol content of all commercial vegetable oils. Among unsaponifiable components in rice bran oil, oryzanol (a group of ferulic acid esters of triterpene alcohols and plant sterols) and β-sitosterol are the most abundant and have physiological/biological effects. In addition to the bioactive function, the mixtures of γ-oryzanol and β-sitosterol have been reported as successful structuring agents for solidifying several vegetable oils resulting in oleogels.[34,39,87]

Since few studies have investigated the role of vegetable oils in oleogelation, the primary objective of this study is to investigate the effects of vegetable oils on oleogelation. Twenty-five edible oils

and two non-edible castor oils were structured by a two-component system, stearic acid + β-sitosterol, which proved to be a promising structuring agent with canola oil as reported in the previous chapter. Due to the major difference between oils are their fatty acid profiles, fatty acid profiles are the main focus to find the relationship between oils and oleogelation. It is also believed that once the relationship is found, not only can an oleogel be formed, but an oleogel with desirable physicochemical properties can also be manipulated. It contributes an important approach for fat substitution in the future.

7.2.Results and Discussion

7.2.1. Effects of oil used on oleogelation

To investigate the influence of oils on oleogelation, 25 edible oils and 2 non-edible oils (castor oils) were prepared with the same structuring system, i.e., 4% (w/w) stearic acid and 4% (w/w) β-sitosterol. In chapter 4, S4B4 was reported as critical gelation ratio in canola oil, thus it was chosen for studying the influence of oils. All prepared samples were inverted for 1 h and were divided into three groups according to different results after dragging using gravity. The first group comprised self-standing oleogels as shown in Fig. 7-1.

Twenty-five oils were used. Only eleven were able to form gels under the tested conditions. Eleven oils were used, including flaxseed oil, hemp oil, canola oil, blend oil, almond oil, safflower oil, flax oil, hazelnut oil, vegetable oil (soybean oil), cottonseed oil, and one avocado one in this group. This meant that these oils can be structured using S4B4 resulting in oleogels. The samples in the second group were defined as partial oleogels because they did not completely drag with gravity. It indicated that the force for aggregation of self-assembles was not strong enough to keep

all particles and oil whole. Sunflower oil, grapeseed oil, corn oil, and extra virgin olive oil belonged to this group. The remaining twelve oils (two brands of rice bran oil, avocado oil, pumpkinseed oil, peanut oil, sesame oil, olive oil, macadamia nut oil, two coconut oils, and two castor oils) could not be structured by S4B4, which resulted in non-oleogel with clumps in it. The happening of non-oleogels may be caused by the unbalancing between the force of gelator-gelator and the force of gelator-solvent.

Similar results were reported that only 1.5% of REA was needed to structure canola oil while 4% was needed to structure sesame oil.[136] The reasons proposed were that there were more sterols and tocopherols in sesame oil. More hydroxyl groups can participate in the hydrogen bonding and then interfere with the formation of the self-assembled network leading to the failure of network formation. In addition, steric hindrance also provided obstacles for aggregation since sterols and tocopherol have ring structures.[131,136]

Fig. 7-1 Effects of oils on oleogelation. All the samples were prepared with given oil and S4B4, a two-component structuring system. According to the appearance of inverted vial, samples are classified to three groups: oleogels, partial oleogels, and non-oleogels.

7.2.2. Microstructures of oleogels, partial oleogels, and non-oleogels

The microstructure of the network was observed using polarized light microscopy since crystals formed by low molecular weight gelators during supersaturation are anisotropic specimens. Polarized light microscopy has a high degree of sensitivity and can be utilized for qualitative studies targeted at an anisotropic specimen. It has been reported that crystal morphology has high relevance to gel formation. Thus samples prepared from different oils were studied for their crystal morphology and crystal aggregation. Fig.7-3 shows microscopic graphs of nine oleogels made of flax oil, flaxseed oil, canola oil, blend oil, almond oil, safflower oil, hazelnut oil, vegetable oil (soybean oil), and avocado oil in B brand. In alignment with previous studies, although no long fibrils were seen, needle and platelet-like crystals were mainly captured in these oleogels. However, there were tape and ribbon-like crystals in some oleogels as well. In flax oil, aggregates of ribbon-like or big platelet-like crystals were observed. Clusters of platelet-like crystals were seen in the commercial blend (canola oil + extra virgin olive oil), safflower oil, and vegetable oil (soybean oil). In the hazelnut oil sample used, few big platelet-like crystals were seen. These results were similar to the results described in chapter 4. S4B4 was the critical gelation ratio; needle-like crystals and a few bid platelet-like crystals were observed in canola oil. Although there were ribbons or big platelet-like crystals, they did not interfere with the aggregation of crystals in order to form oleogels. It has been reported that the successful formation of molecular gels because of the formation of specific self-assembled crystals as building blocks which subsequently form a three-dimensional network via non-covalent interactions. Fibrils, tubules, needle-like, rod-like, and platelet-like crystals have been found in molecular gels playing a role as a building block.[31,52,85,90,95,98] Among these micro-crystals, fibrillar crystals have received the most interest,

due to their higher aspect ratio leading to a stronger network. This network formed by fibrils is called specifically a self-assembled fibrillar network.

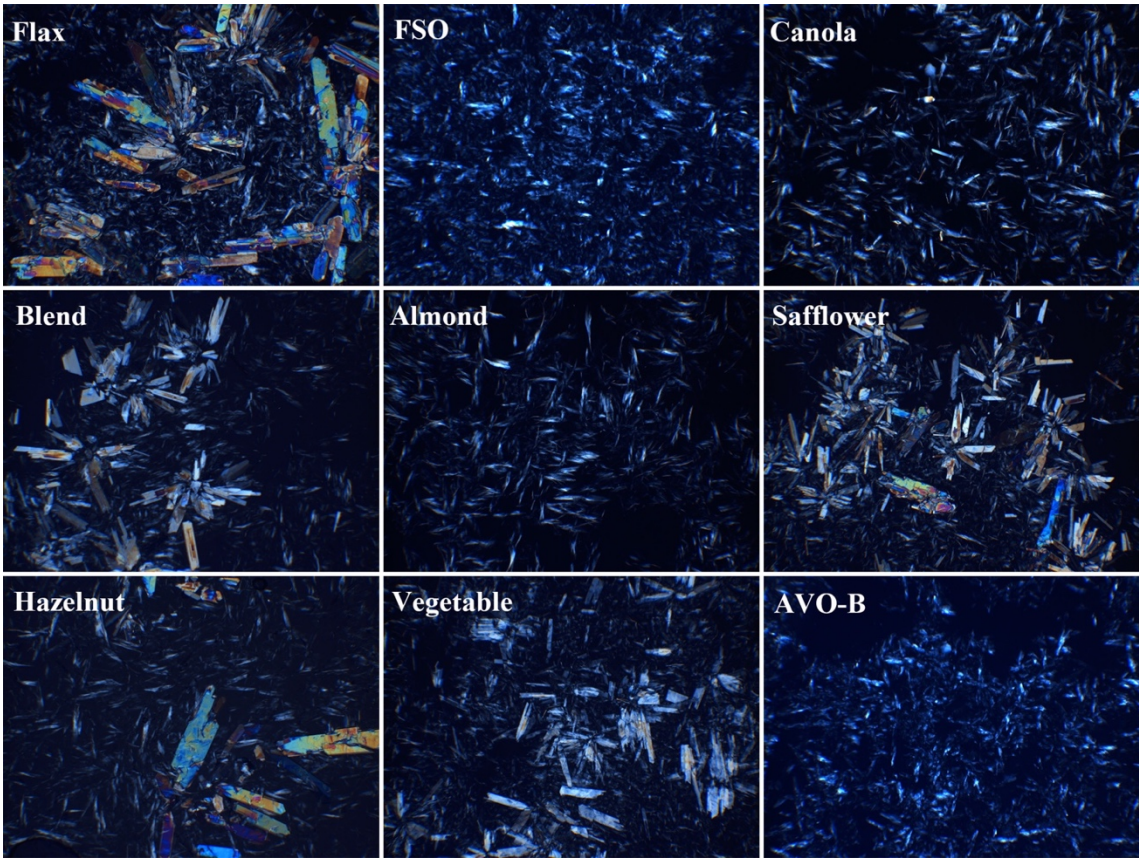

Fig. 7-2 Microstructures of oleogel samples using polarized light microscopy. Black field is the background. Flax refers to high lignan flaxseed oil; FSO is flaxseed oil, canola is canola oil; Blend is commercial blend oil containing canola oil and extra virgin olive oil; almond is roasted almond oil; safflower is safflower oil; hazelnut is roasted hazelnut oil; vegetable refers to soybean oil; AVO-B is avocado oil in B brand. Width of wash graph is 475 μm.

In Fig.7-3, microstructures of partial oleogel samples showed the same crystal morphology as for most oleogels (Fig.7-3). This indicated that S4B4 in grape seed oil, corn oil, and extra virgin olive oil was able to self-assemble into potential building blocks of a three-dimensional network, while the network was not strong enough to entrap the oils for resisting the force of gravity. They may refer that more β-sitosterol and/or stearic acid was miscible in oil causing not enough formed

crystals to tangle together[50,142,143] or molecules in oils interacted with structurants in order to interfere with the crystallization and network formation.

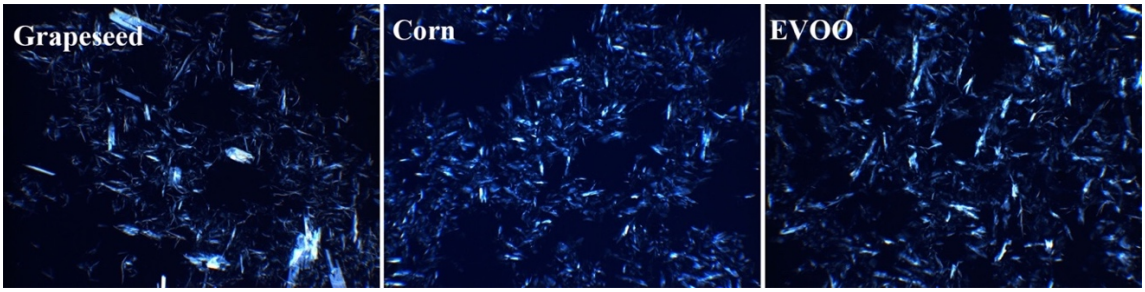

Fig.7-3 Microstructures of partial oleogel samples. (FSO: flaxseed oil; AVO-B: B brand of avocado oil; EVOO: extra virgin olive oil) Width of wash graph is 475 μm.

Morphology of dots were captured substantially in non-oleogels, as shown in Fig. 7-4. It indicated that S4B4 cannot grow well in either one dimension or two dimensions resulting in small dots crystals. It showed that small dot-like crystals were not able to entangle and aggregate with each other resulting in formation of non-oleogels. This can be explained by structurants having higher solubility in these oils and subsequently no more crystal growth upon supersaturation. However, short needle-like crystals were shown in macadamia (MNO) and sesame oil. It referred to the clumps observed in non-oleogels, turbid viscous solutions. S4B4 continued to co-crystallized to form needle-like crystals and to enable network formation in order to entrap oil, but the number was not even enough to gel a half of oil. This was attributed to the higher solubility of S4B4 in these oils. For non-edible castor oils (castor oils), although aggregates of needle-like crystals were shown, no oleogel formed in this case either. It seems that the viscous of castor oil does not help network formation. The property of viscous might even interfere with the mobility of structurants during crystallization; thus it failed to form a continuous self-assembled network.

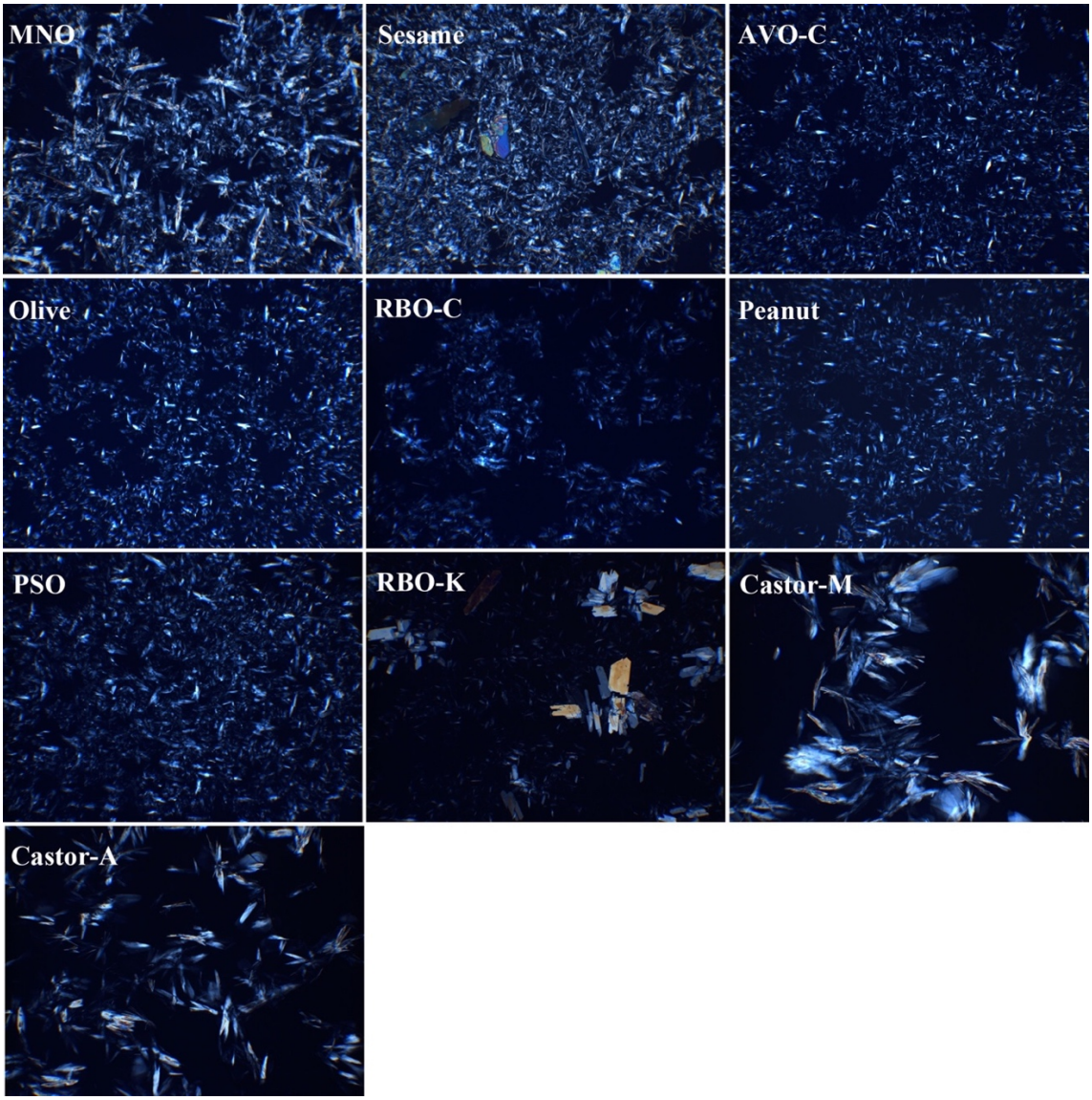

Fig. 7-4 Microstructures of non-oleogel samples using polarized light microscopy. (MNO: macadamia nut oil; AVO-C: C brand of avocado oil; RBO-C: C brand of rice bran oil; PSO: pumpkinseed oil; RBO-K: K brand of rice bran oil; Castor-M: M brand of castor oil; Castor-A: A brand of castor oil). Width of wash graph is 475 μm.

7.2.3. Differences in thermal behaviors between oleogels, partial oleogels, and non-oleogels

Melting profiles of prepared samples were obtained using differential scanning calorimetry. A broad peak was observed in every oleogel with the range starting from approximately 30°C to 60°C, as shown in Fig. 7-5. No difference was observed between oleogels with a variety of oils, except flax oil, which had broad peak shift slightly to the right. This was in alignment with the results of the microstructure that showed that flax had big-platelet aggregates compared with other oils. A broad peak indicated that there were more than one kind of ordered crystals in a sample. In other words, many crystals contribute to a wide melting range. This results also aligned with the results of the microscopy graphs where needle-like, fiber-like, and platelet-like crystals were captured in one sample. Partial oleogels showed the same result as the oleogel samples. They had a broad peak with the similar melting range, as shown in Fig. 7-6.

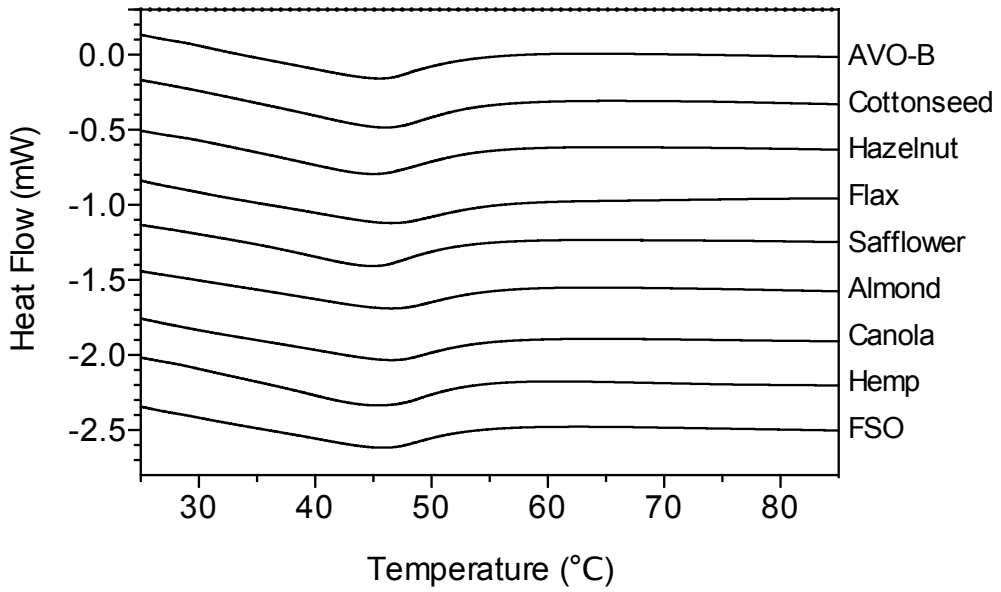

Fig. 7-5 Melting profiles of oleogels using DSC with a heating profile from 20°C to 155°C at a rate of 5°C/min. From top to bottom is avocado oil in B brand, cottonseed oil, hazelnut oil, flax oil, safflower oil, roasted almond oil, canola oil, hemp oil, and flaxseed oil, respectively.

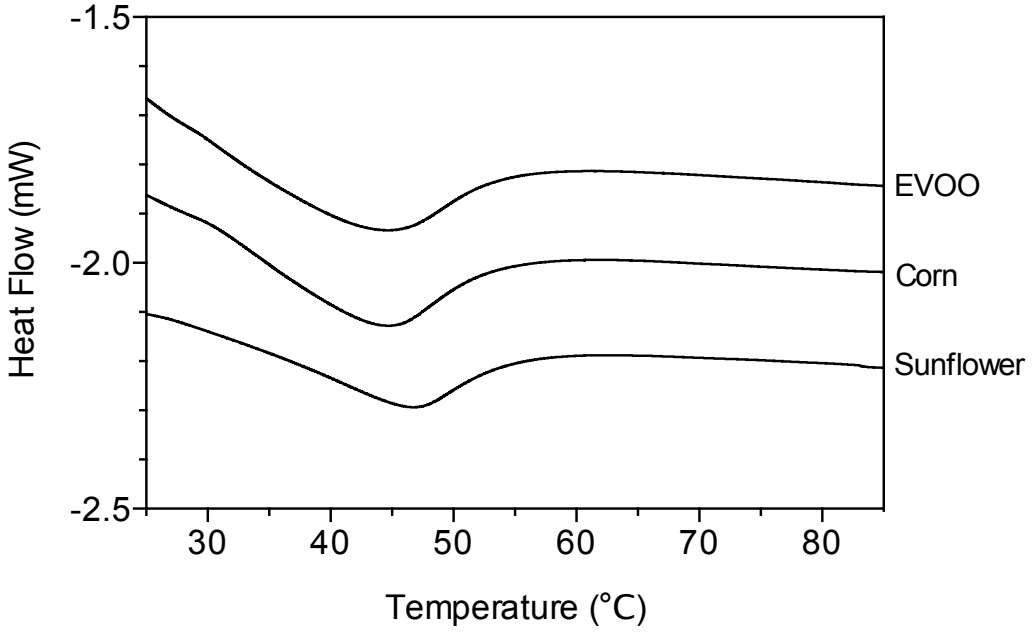

Fig. 7-6 Melting profiles of partial oleogel samples using DSC with a heating profile from 20℃ to 155℃ at a rate of 5℃/min. From top to bottom is extra virgin olive oil, corn oil, and sunflower oil, respectively.

However, non-oleogels had the smaller broad peak at lower temperature compared to the oleogels and partial oleogels (Fig. 7-7). This indicated that non-oleogels and oleogels had significantly different crystals due to different molecular packing. Dot-like and some big platelet crystals were observed in non-oleogels. It indicated that the melting range of dot-like and big platelet-like crystal is lower than needle-like crystals. In addition, pure 8% of β-sitosterol crystallized into dot-like crystals in canola oil as did the crystals here in non-oleogels with olive, peanut, rice bran oil in C brand, and avocado oil in C brand. It might reveal that very little stearic acid participated in the crystallization. Thus, both the crystal morphology and melting profile were close to pure β-sitosterol crystallized in canola oil. The results of microstructures and melting profiles showed that oil type influenced crystallization and subsequently influenced formation of oleogels.

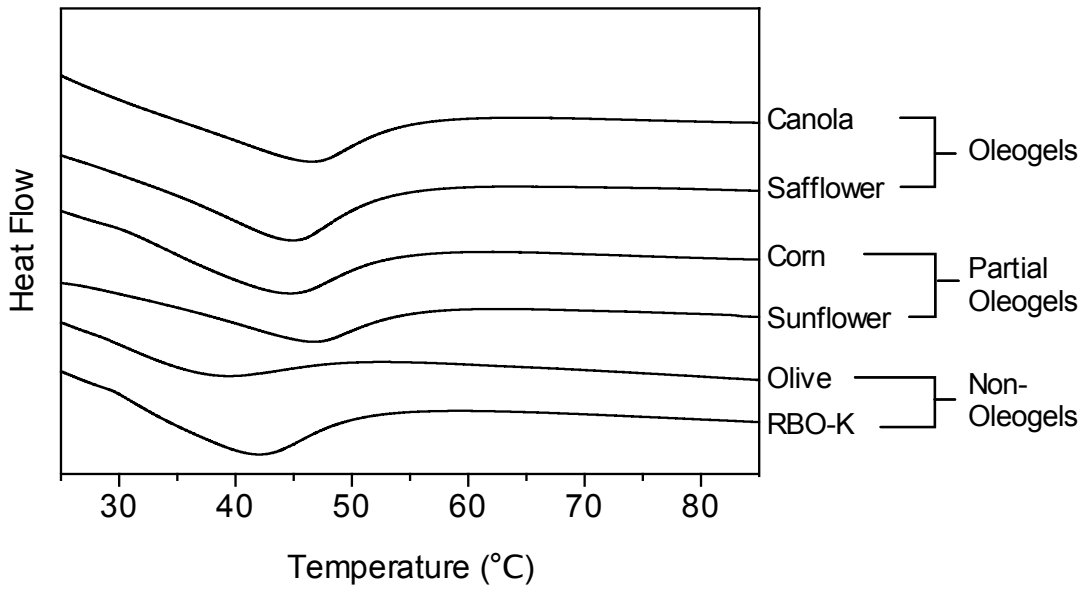

Fig. 7-7 Effect of oils on melting profiles of samples. The top two lines are canola oil and safflower oil, respectively, representing the group of oleogels; the middle two lines are corn oil and sunflower oil representing the group of partial oleogels; the bottom two lines are olive oil and rice bran oil in K brand representing the group of non-oleogels.

7.2.4. Relationship between fatty acid compositions and oleogelation

Few studies reported that oils affect oleogelation. For instance, sesame oil and canola oil were used with ricinelaidic acid to prepare oleogels. Under the same preparation conditions, 1.5% of ricinelaidic acid started to structure canola oil resulting in an oleogel at 25℃, but not for sesame oil. It required as much as 3% to start to form a thick liquid oil and 4% to form oleogel in sesame oil. In addition, the solvent nature, polarity, and presence of co-surfactants can influence the aggregation of LMOGs.[21] To our knowledge, no study showed how a gelator or multi-gelators perform in a variety of oils. In this study, we discovered there were three oils of twenty-seven oils according to the appearance of inverted vials. To correlate the fatty acid compositions of oils (Fig. 7-8) and oleogelation, a summary table was shown in Table 1.

Even oils can be pressed and refined from many botanical sources, oils have similar compositions in general, including triacyclglycerols, fatty acids, phospholipids, phytosterols, etc. In nature, common fatty acids in oils are saturated fatty acids (from carbon 12 to carbon 24), monounsaturated fatty acids (C16:1, C18:1, C20:1, and C22:1), and polyunsaturated fatty acids (C18:2, C18:3, C20:2). Fatty acid profiles determine the physical properties of oils and fats. Generally, a high percentage of saturated fatty acids (>35%) leads to fats, vice versa. Therefore,

fatty acid profiles of oils were analyzed to determine the correlation with oleogel formation. All of the twenty-seven liquid oils were purchased from a local market or an online shop. From the nutrition fact labels on the bottles of oil, fatty acids were categorized into three groups (i.e., saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid). In Fig. 7-8, saturated fatty acid ranged from 7% to 25% in a variety of oils. Monounsaturated fatty acid ranged between 14.3% and 86.1%, and 3.6% to 78.6% was found for polyunsaturated fatty acid. Flaxseed oil, hemp oil, canola oil, blend oil (canola and extra virgin olive oil), almond oil, and safflower oil showed the lowest SFA. Coconut oil, rice bran oil, and pumpkinseed oil showed relatively high levels of SFA.

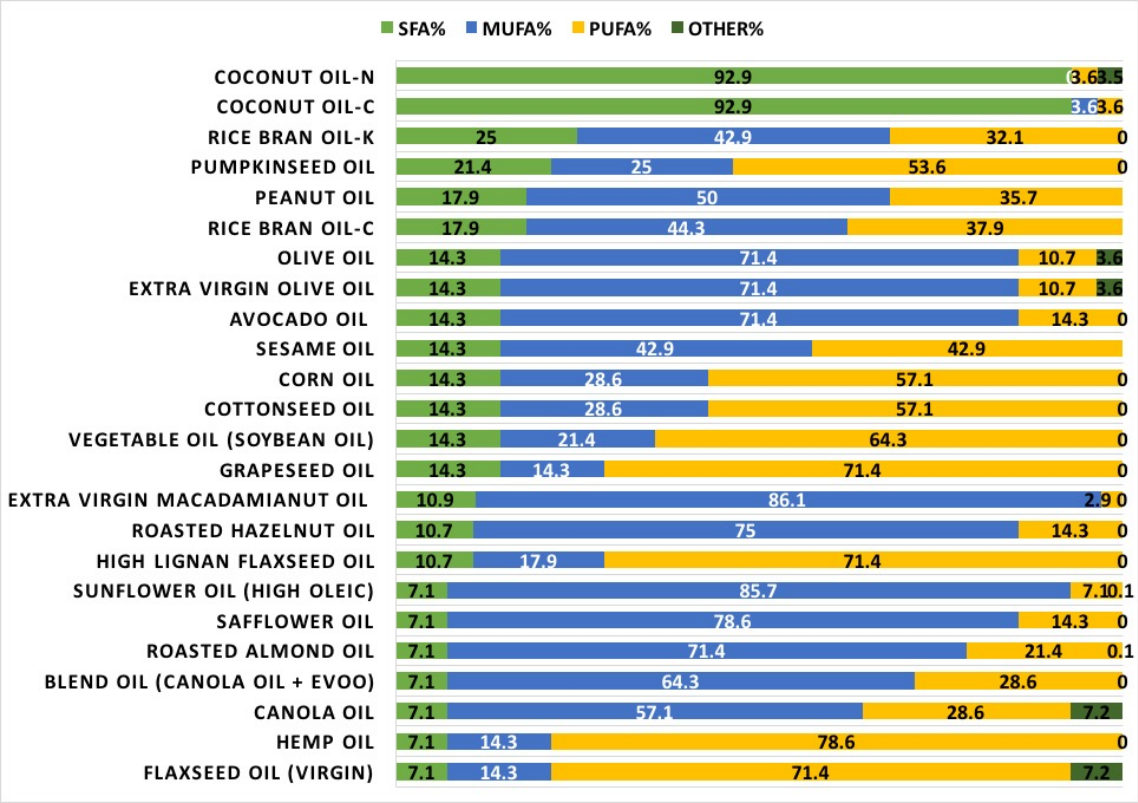

Fig. 7-8 Labeled fatty acid compositions of a variety of oils obtained from a local market or an online store (Amazon.com). Green bar represents the amout of saturated fatty acids in %, blue bar represents monounsaturated fatty acids, and yellow bar represents poluunsaturated fatty acids.

Not all the oils can be structured by the same gelator systems (S4B4). The samples were divided into three groups based on appearance: oleogels, partial oleogels, and non-oleogels. As shown in Table 1, the content of saturated fatty acids was relatively low, from 7-14% in the oleogel group, and the percentage of saturated fatty acids was relatively high in non-oleogel samples (14, 21, or even 52%). However, there was no clear tendency in the percentage of MUFA and PUFA. We inferred rationally that the percentage of saturated fatty acids played a major role in oleogel formation. The analysis of hierarchy clustering was further executed to see the correlation between fatty acid compositions and oleogel formation in mathematical ways.

Table 1 Summary of fatty composition of oils and their corresponding results after undergoing

supersaturation, i.e., oleogels, partial oleogels, and non-oleogels. The number of the first column corresponds to the hierarchal clustering analysis.

NO	Oil (Product name)	Food Grade	Labeled SFA%	Labeled MUFA%	Labeled PUFA%
Oleogels formed					
1	Flaxseed oil (Virgin)	v	7.1	14.3	71.4
2	Hemp oil	v	7.1	14.3	78.6
3	Canola oil	v	7.1	57.1	28.6
4	Blend oil (Canola oil + EVOO)	v	7.1	64.3	28.6
5	Roasted Almond oil	v	7.1	71.4	21.4
6	Safflower oil	v	7.1	78.6	14.3
7	High Lignan Flaxseed oil	v	10.7	17.9	71.4
8	Roasted Hazelnut oil	v	10.7	75.0	14.3
9	Vegetable oil (soybean oil)	v	14.3	21.4	64.3
10	Cottonseed oil	v	14.3	28.6	57.1
11	Avocado oil-B	v	14.3	71.4	14.3
Almost Oleogels formed					
12	Sunflower oil (High oleic)	v	7.1	85.7	7.1
13	Grapeseed oil	v	14.3	14.3	71.4
14	Corn oil	v	14.3	28.6	57.1
15	Extra Virgin Olive oil	v	14.3	71.4	10.7
Turbid Solutions formed					
16	Extra virgin macadamianut oil	v	10.9	86.1	2.9
17	Sesame oil	v	14.3	42.9	42.9
18	Avocado oil-C	v	14.3	71.4	14.3
19	Olive oil	v	14.3	71.4	10.7
20	Rice bran oil-C	v	17.9	44.3	37.9
21	Peanut oil	v	17.9	50.0	35.7
22	Pumpkinseed oil	v	21.4	25.0	53.6
23	Rice bran oil-K	v	25.0	42.9	32.1
24	Coconut oil-C	v	92.9	3.6	3.6
25	Coconut oil-N	v	92.9	0.0	3.6
	Castor oil-M	Not	N/A	N/A	N/A
	Castor oil-A	Not	N/A	N/A	N/A

7.2.5. Hierarchal clustering analysis

7.2.5.1.Based on labeled fatty acid composition

Hierarchical cluster analysis of 25 objects (edible oils) is defined by a stepwise algorithm that merges the most similar oils at each step. The information of oils for running hierarchical clustering analysis is their labeled fatty acid compositions and their gelation ability, as shown in Table 1. Similarity in this study is in accordance with fatty acid compositions and gelation ability. The result of hierarchical cluster analysis was displayed as a tree diagram called a dendrogram. It began with each oil in a separate cluster. At each step, the clusters that were most similar were joined into a single new cluster. As the analysis shows, #24 and #25 had shorter vertical lines, thus it meant that #24 (Coconut oil-C) and #25 (Coconut oil-N) were the most similar oils among all the oils. Moving up from the bottom of dendrogram to the top, these two coconut oils separate themselves entirely from all others. However, these two coconut oils could not reach a semi-solid state (gel) with the presence of the S4B4 gelator system. They showed a huge difference in fatty acid composition compared with other oils. Coconut oils had more than 90% of SFA, and really low percentages of MUFA and PUFA (~3.6%). #18 (avocado oil-C) and #19 (olive oil) joined the second most similar group. Olive oil and avocado oil-C had almost the same distribution of fatty acids, i.e., 14% of SFA, 71% of MUFA, and around 10% of PUFA. In addition, they belonged to non-oleogels with the presence of S4B4.

To move up the vertical line, four clusters appeared based on similarity. Canola oil (#3) and blend oil (#4) were grouped with gel formed and similar fatty acids distribution (7.1% of SFA, 57-64% of MUFA, and 28.6% of PUFA). Avocado oil-B (#11), hazelnut oil (#8), and safflower oil (#6) were in a cluster, and they were able to become a gel with the presence of S4B4. Thus far, we

could infer that FA distribution for 10-14% of SFA, 71-78% of MUFA, 10-14% of PUFA did not fulfill the criteria of gel formation. Rice bran oil-C (#20) and peanut oil (#21) were in a cluster with the same gelation ability (non-gelled) and similar fatty acid percentages (17.9% of SFA, 44.3-50% of MUFA, and 35-38% of PUFA). Flaxseed oil (#1), hemp oil (#2), and high lignan flaxseed oil (#7) were in a cluster with the same gelation results (gel) and similar fatty acid percentages, i.e., 7-10% of SFA, 14-17% of MUFA, and 71-78% of PUFA. Toward the top, sesame oil (#17) joined the group of peanut oil (#21), and rice bran oil-C (#20) followed by rice bran oil-K (#23). Sesame oil had 14% SFA, 42% MUFA, and 42% PUFA, and rice bran oil-K had 25% of SFA, 42% of MUFA, and 31% of PUFA.

After the hierarchal clustering analysis, one of these fatty acid combinations was highly related to the potential of gel formation. It was 7-10% of SFA, 14-17% of MUFA, and 71-78% of PUFA. Three oils (high lignan flaxseed oil, hemp oil, and flaxseed oil) whose fatty acid combinations were in these ranges became gels with the presence of S4B4. Thus, we could infer that once oil has its combination of fatty acids as low in SFA, low in MUFA, and high in PUFA, it has the highest possibility to be structured by S4B4 resulting in an oleogel. Conversely, when FA combinations are 17% (SFA), 44-50% (MUFA), 35-38% (PUFA) and 14% (SFA), 42% (MUFA), 42% (PUFA) and 14% (SFA), 14% (MUFA), 71% (PUFA), the oil could not become a gel by using S4B4. Furthermore, these three oils that nearly became gels have particular ranges of fatty acid combinations as well. 14% of SFA, 28% of MUFA, and 57% of PUFA were found in corn oil; 7% SFA, 85% MUFA and 7% of PUFA were found in sunflower oil. Thus, a high percentage of PUFA (71-78%) and low percentage of SFA (7-10%) were positively related to gel formation. In the future, we might be able to predict oleogel formation using S4B4 as a gelator system.

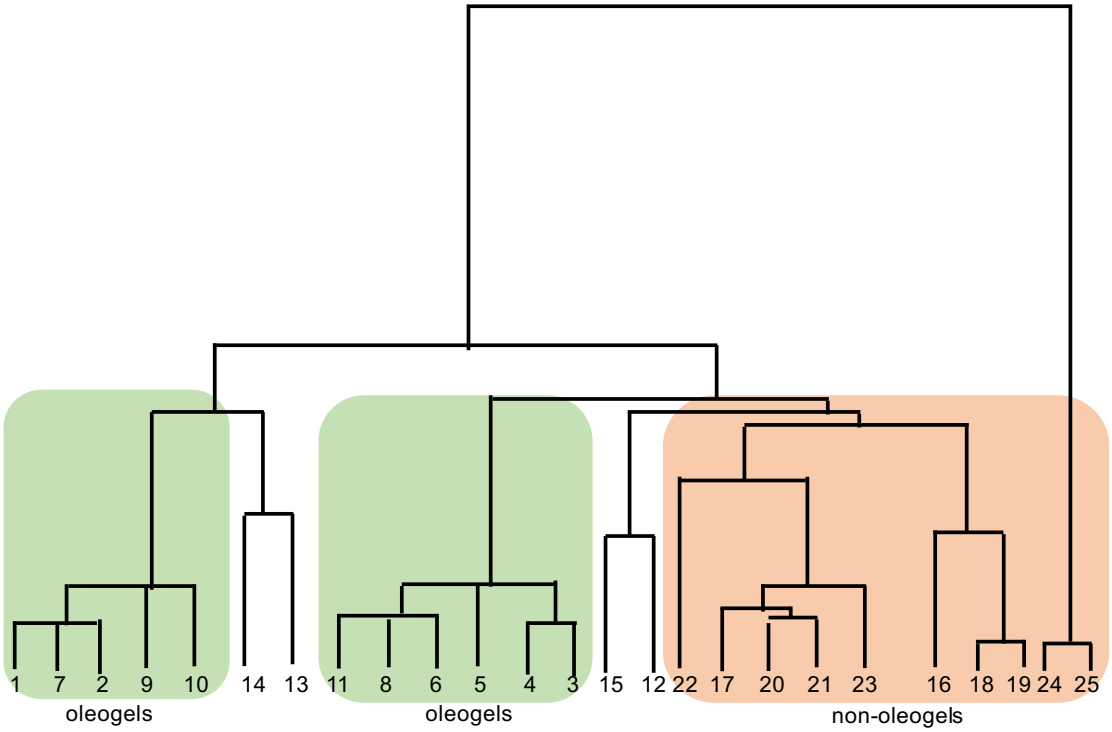

Fig. 7-9 Hierarchal clustering analysis of 25 edible oils based on their labeled fatty acid compositions. Twenty-five edible oils are flaxseed oil (#1), hemp oil (#2), canola oil (#3), blend oil (canola oil + extra virgin olive oil, #4), roasted almond oil (#5), safflower oil (#6), high lignan flaxseed oil (#7), roasted hazel nut oil (#8), vegetable oil (soybean oil, #9), cottonseed oil (#10), avocado oil-B (#11), sunflower oil (#12), grapeseed oil (#13), corn oil (#14), extra virgin olive oil (#15), macadamianut oil (#16), sesame oil (#17), avocado oil-C (#18), olive oil (#19), rice bran oil-C (#20), peanut oil (#21), pumpkinseed oil (#22), rice bran oil-K (#23), coconut oil-C (#24), and coconut oil-N (#25), respectively. (Detail information of oils is shown in Table 1, p.106)

7.2.5.2.Based on reference fatty acid composition

From the result of label FAs dendrogram, three certain regions had been distinguished in accordance with gelation formation. Hence individual fatty acids percentage was interested in seeing if any species of fatty acids may contribute to gel formation. As Figure 2 shown, #3 (High lignan flaxseed oil) and #10 (Flaxseed oil) were not having the shortest vertical line, but these two ended up grouping in a higher level and separated themselves entirely from all the others. Flaxseed oil contains the highest percentage of linolenic acid (C18:3), 56.1 and 47.5%, respectively among oils listed in Table 2. The high amount of linolenic acid may be a decisive factor for gel formation

in the presence of S4B4. When the shortest vertical line (the most similar) was observed, two clusters appeared. One group contained #5 (Roasted hazelnut oil) and #6 (Safflower oil), and the other group has #18 (Rice bran oil-Reference 1) and #19 (Rice bran oil-Reference 2). Number 5 and 6 were gel and were Hazelnut oil and Safflower oil respectively. Both of them had a high amount (> 70%) of oleic acid (C18:1); however, other oils having high oleic acids, such as sunflower oil, were not formed a gel. Thus, oleic acid could not be seen a critical composition solely for gel formation. But when the oil had at least 70% of oleic acid and over 10% of linoleic acid (C18:2), it was structured with highly chance. Number 18 and 19 were rice bran oil from different references to represent two different sources. Rice bran oils had the highest amount of palmitic acid (C16:0) among oils listed in Table 2 and were not able to be a gel with the presence of gelators. We may infer that palmitic acid is negatively related to gel formation. But avocado oil and cottonseed oil which had 16-20% of palmitic acid were able to be gelled, palmitic acid is not directly responsible for gelation ability. Furthermore, an oil having 20% of palmitic acid combined with 30-40% of linolenic acid or 40% of oleic acid, it was a potent combination for enabling structured resulting in a gel. Overall, only the high amount of linolenic acid (3-50%) is positively related to gel formation.

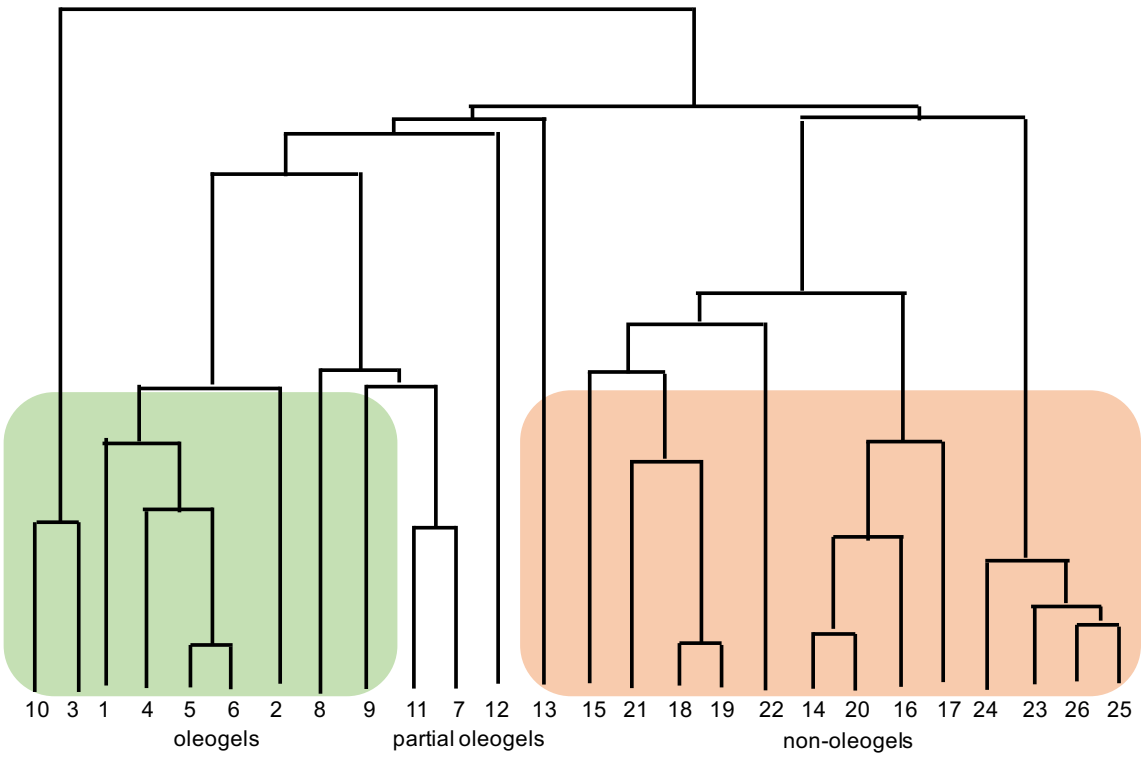

Fig. 7-10 Hierarchal clustering analysis of 25 oils based on their labeled fatty acid compositions. Twenty-five edible oils are canola oil (#1), avocado (#2), high lignan flaxseed oil (#3), roasted almond oil (#4), roasted hazelnut oil (#5), safflower oil (#6), high lignan hemp oil (#7), cottonseed oil (#8), vegetable oil (soybean oil, #9), flaxseed oil (#10), grapeseed oil (#11), corn oil (#12), sunflower oil (#13), olive oil (#14), pumpkinseed oil (#15), peanut oil (#16), extra virgin macadamia oil (#17), rice bran oil (#18), rice bran oil (#19), avocado oil (#20), sesame oil (#21), coconut oil (#22), coconut oil (#23), castor oil (#24), and castor oil (#25), respectively. (Detail information of oils is shown Table 2).

Table 2 Summary of fatty composition of oils and their corresponding results after undergoing supersaturation, i.e., oleogels, partial oleogels, and non-oleogels. The number of the first column corresponds to the hierarchal clustering analysis.

NO	Oil (Product name)	Food Grade	C16:0	C18:0	C20:0	(n-9) C18:1	(n-6) C18:2	(n-3) C18:3	(n-9) C20:1
Oleogels									
10	Flaxseed oil (Virgin)[2]	v	5.5	3.5	0.65	22.1	20.5	47.5	N/A
7	Hemp oil[1]	v	6.4	2.6	nd	11.5	59.4	3.36	N/A
1	Canola oil[2]	v	5.2	4.4	nd	59.5	18.8	11.9	N/A
	Blend oil (Canola oil + EVOO)	v	N/A						
4	Roasted Almond oil[1]	v	6.8	2.3	0.09	67.2	22.8	nd	0.16
6	Safflower oil[1]	v	5.6	2.3		78.35	13	0.1	N/A
3	High Lignan Flaxseed oil[5]	v	6	3.15	nd	17.8	16.3	56.1	N/A
5	Roasted Hazelnut oil[3]	v	5.6	2.5	0.2	75.1	12.9	0.4	0.2
9	Vegetable oil (soybean oil)[2]	v	9	4	nd	28.5	49.5	8	N/A
8	Cottonseed oil[2]	v	20	2	nd	35.4	42	nd	N/A
2	Avocado oil[3]	v	16.9	0.8	0.1	57.5	10.8	nd	0.1
Partial oleogels									
13	Sunflower oil (High oleic)[5]	v	3.8	4.55	1.05	82.85	8.55	N/A	N/A
11	Grapeseed oil[3]	v	6.9	3.9	0.3	19.4	63.9	0.4	0.3
12	Corn oil[2]	v	10	3.5	N/A	26.8	48	nd	N/A
	Extra Virgin Olive oil	v	N/A						
Non-oleogels									
17	Extra virgin macadamianut oil[5]	v	8.4	3.2	2.3	65.1	2.3	0.1	2.25
21	Sesame oil[1]	v	9.7	6.5	0.63	41.5	40.9	0.21	0.32
20	Avocado oil[5]	v	16.9	0.8	0.1	57.5	10.8	nd	0.1
14	Olive oil[1]	v	16.5	2.3	0.43	66.4	16.4	1.6	0.3
18	Rice bran oil[1]	v	20	2.1	nd	42.7	33.1	0.45	1.11
16	Peanut oil[1]	v	7.5	2.1	1.01	71.1	18.2	nd	nd
15	Pumpkinseed oil[1]	v	13.1	5.7	0.47	24.9	54.2	0.12	1.08
19	Rice bran oil[5]	v	19.8	1.9	0.9	42.3	31.9	1.2	0.5
22	Coconut oil[1]	v	nd	2.7	nd	6.2	1.6	nd	nd
23	Coconut oil[2]	v	9.2	2	0.5	8.8	0.5	nd	N/A
24	Castor oil[4]	Not	1.3	1.2	N/A	5.5	7.3	0.5	N/A
25	Castor oil[4]	Not	0.7	0.9	N/A	2.8	4.4	0.2	N/A

[1]Orsavova et al., 2015[144]
[2]Kostik et al., 2013[145]
[3]Vingering et al., 2010[146]
[4]Salimon et al., 2010[147]
[5]Gunstone, 2011[140]

7.3.Conclusion

Twenty-five edible and two non-edible castor oils were prepared with S4B4 to investigate the relationship between fatty acid compositions of oils and formation of oleogels. Three defined appearance, oleogels, partial oleogels, and non-oleogels was based on the result of the inverted vial method. Not every oil could be structured by the structuring agents, which means oil phase participates in the formation of oleogels. Through an analysis of polarized light microscopic graphs, crystal morphologies showed differences in different appearance in general. Needle-like crystals were primarily shown in oleogel samples. Even a small portion of big platelet-like and ribbon-like crystals was seen in few oleogel samples. It did not affect oleogels formation; however, it indicated that these oleogels have as many functional building blocks as possible for constructing a strong enough network to avoid the falling of oil phase from gravity. Either dot- or big platelet-like crystals were captured in non-oleogels, which aligns with the low aspect ratio of crystals. They have a small chance of aggregating or interacting with each other because they do not have a network to immobilize an oil phase. With using hierarchal clustering analysis, fatty acid composition of oils was highly related to the oleogel formation. It was found that a small portion of saturated fatty acids (7-14%) was showing positive oleogel formation in general. Furthermore, a whole profile was distinguished i.e., 7-10% of SFA, 14-17% of MUFA, and 71-78% of PUFA for providing a greater chance of forming oleogels with the presence of S4B4 among all the profiles of oils. This study reported more evidence that fatty acid compositions play an important role in leading oleogel formation.

8. Mango carotenoid-enriched oleogel as a potential fat alternative

Abstract

With the increasingly successful use of low molecular weight molecules for structuring liquid oils, oleogels have become a particular focal point. Their potential as a substitute for solid fats in many food products, particularly those involved in baking, is an area that requires further research. Some studies have examined incorporating oleogels into foods; however, a significant issue concerns the risk of oil oxidation during heating for oleogel production and during storage. In this study, extracting mango source carotenoids by blending with canola oil was proposed as a way to increase the antioxidative activity in liquid oil and also the target oleogel. β-carotene and zeaxanthin were observed in the canola oil after blending with fresh mango pulp based on the analysis of the visible spectrum, which is in agreement with previous studies showing that β-carotene is the main carotenoid (48-84% in dry base) in mango. The optimum extraction mass ratio of mango to oil, 1.2 (w/w), was determined and used for making oleogels. This carotenoid extract-enriched canola oil was prepared to subsequently be structured by a two-component system resulting in a nutraceutical-enriched oleogel. A two-component system was the combination of 5% (w/w) stearic acid and 3% (w/w) β-sitosterol, which was proposed as a promising gelling system for structuring eleven commercial edible oils. The change in canola oil did not affect the ability of the two-component gelator system to produce oleogels. Further evidence of this was illustrated by the observation of needle-like crystal microstructures, which were the same as those observed in an oleogel made of control unmodified canola oil. No significant difference was found in melting profile, but the rheological behavior seemed to depend on the concentration of mango crude extract. Surprisingly, oleogels had slightly higher yield stress in higher concentration of crude extract. Overall, the described method is a feasible way to form

nutraceutical-enriched oleogels by having high antioxidant activity, due to the presence of carotenoids, in the oil phase and it offers an appropriate approach for retarding oxidation to improve oleogel shelf-life. However, the temperature susceptibility of carotenoids must be considered when carotenoid-enriched canola oil and oleogels are prepared. It was found that heating at above 90°C for a period of time, such as 10 min, seemed to lead to isomerization and epoxidation for carotenoids and subsequent conversion from β-carotene and zeaxanthin to *cis*-violaxanthin, according to the analysis of visible spectra.

8.1. Introduction

Mango (*Mangifera indica* L.) is native to South and Southeast Asia and is the second most important tropical fruit (behind banana) in terms of production, marketing, and consumption. Its yellow-orange characteristic color is attributed to the presence of a remarkably high carotenoid content. Mango is recognized as a good source of carotenoids, although the amount and composition of carotenoids is more or less affected by cultivars. For example, it has been found that β-carotene content is much higher in cultivars of Haden and Uba (~2000 ug/100g) than in Tommy Atkins and Palmer (~500 ug/100g).[148] In addition to the differences in cultivars, the degree of ripeness and post-harvesting conditions also contribute to these differences.[149–151]

Carotenoids are lipid-soluble pigments responsible for color, particularly yellow-orange, of a wide variety of foods and have been thoroughly investigated due to their free radical scavenging properties. Approximately 700 carotenoids have been found in nature. All of them, to a greater or lesser degree, show antioxidant capacity which is associated with a number of health benefits, such as protection against cancer, heart and vascular disease, and degenerative diseases (e.g., Alzheimer's disease).[148,152,153] Besides, approximately 50 carotenoids have pro-vitamin A activity

which means they transform into vitamin A for meeting the needs of maintaining human functionalities. Of those 50, the three most important precursors of vitamin A in humans are α-carotene, β-cryptoxanthin, and β-carotene. Deficiency of vitamin A causes serious eye diseases, rough scaly skin, and retarded tooth and bone development. In addition, carotenoids cannot be synthesized in the human body, and all carotenoids come from the diet. It has been found that deeply pigmented fruits, juices, and vegetables constitute the major dietary sources. For example, mango, pumpkin, and carrots are the food sources of β-carotene.[154]

Oleogels made of mainly oil phase and a small amount of low molecular weight structurants have been proposed for the high potential of being alternatives to solid fat for fat-rich food products. Low molecular weight molecules in oleogels play a role like the triacylglycerols in solid fats that crystallize to specific structures giving a space-filling network capable of supporting its weight against the gravity resulting in a gel.[155] Under an applied stress, oleogels start to yield or flow owing to breakdown of the network. The breaking of these crystal interactions in oleogels by flow increases oleogels' spreadability, which shows their potential as substitutes for spreadable butter. This space filling network consisting of crystals gives oleogels firmness. Therefore, crystal formation, crystal size, crystal morphology, and interaction between crystals all affect the rheological properties of oleogels and further affect oleogels' application in foods.

Crystallization and melting profiles of fat blends affect their application in food products, particularly bakery products.[156] Firmness, the most direct characteristic of fat, is affected by crystal size, shape, and alignment, the degree of formation of mixed crystals and even the ability of crystals to flocculate into a network. As a result of considering oleogels as fat substitutes, crystallization, melting profiles, and viscoelastic properties of oleogels should be discussed. It has

been reported that smaller and finer β′ crystals in solid fat were more preferred due to their stabilization of more air and liquid components compared to relatively larger β crystals. Self-assembled three-dimensional networks of oleogels comprising self-assembled crystals have been studied, with their formation having been found to be influenced by the forces between gelator-gelator and gelator-solvent. The composition of the liquid phase (solvent) may affect the polarity of the solvent, causing changes in interaction between gelator-gelator and gelator-solvent. Formation of self-assembled crystals could fail due to changing solvent conditions.

In this study, there are two main objectives: (1) investigating the effects of carotenoid-enriched canola oil on oleogel formation; (2) discussing the effect of the heating process on carotenoid structures, because carotenoids are susceptible to heat and a heating process cannot be removed from producing oleogels. Authors suggest that including carotenoids in the oil phase provides antioxidative activity of oleogels in order to retard the lipid oxidation either during the preparation process or storage.

8.2.Results and Discussion

8.2.1. Preparation of carotenoid-enriched canola oil

Mango is recognized for its antioxidant activity due to high amounts of antioxidants such as polyphenols, carotenoids, and ascorbic acid. Since the target oleogel has over 90% of oil as its major ingredient, the oil phase is a good source to be modified for increasing the functionality, such as having oil soluble antioxidants in the oil phase. Thus, a series of fresh mango pulp concentrations were blended with canola oil using a small-scale ball miller, in order to determine miscibility efficiency between carotenoids and canola oil. A series of mass ratio of mango pulp to canola oil (0, 0.2, 0.4, …, 1.6, w/w) were prepared. One observation of note is that when mango

pulp was used up to 1.2 (w/w), mango pulp pieces had difficulty contacting the oil evenly and were difficult to blend. This led to decreased extraction efficiency. The mango/oil ratio of 1.6 (w/w) was the maximum for the ball miller to work functionally, with higher concentrations leading to the sample being too condensed for blending. After blending and centrifugation, clear golden yellow colored canola oils were obtained. As shown in Fig. 8-1, the color of the very left sample (canola oil only) was close to colorless and the gradient of yellow color was obviously seen from left to right, with color saturation in 1.0 or 1.2 mass ratio samples. This indicated that oil soluble carotenoids were successfully extracted from mango due to their corresponding color. Moreover, they produced good miscibility with canola oil, and no impurity or phase separation was observed.

After mango pulp and canola oil were milled together, canola oil changed its color from colorless to light yellow and even to golden yellow, as shown in Fig. 8-1. We concluded that this milling extraction method was a successful method to extract target functional compounds (i.e., carotenoids) which contribute to the color of yellow. It has been reported that types of carotenoids in mango are influenced by cultivar difference, climatic effects, stage of maturity at harvest, and processing after harvest. However, *all-tans-β*-carotene, *all-trans-β*-cryotoxanthin, zeaxanthin, luteoxanthin isomers, violaxanthin (*all-trans* and *cis*), 9-*cis*-violaxanthin and neoxanthin (all-tans and *cis*) have been identified as major carotenoids in mango using HPLC analysis.[149,152,154] Visible spectral analysis is the key for identifying which HPLC peak refers to what carotenoid. The more or less shifting of λ_{max} has been observed in the visible spectrum because of the change in extraction solvents. Only a few nm shifting was observed for *all-trans-β*-carotene.

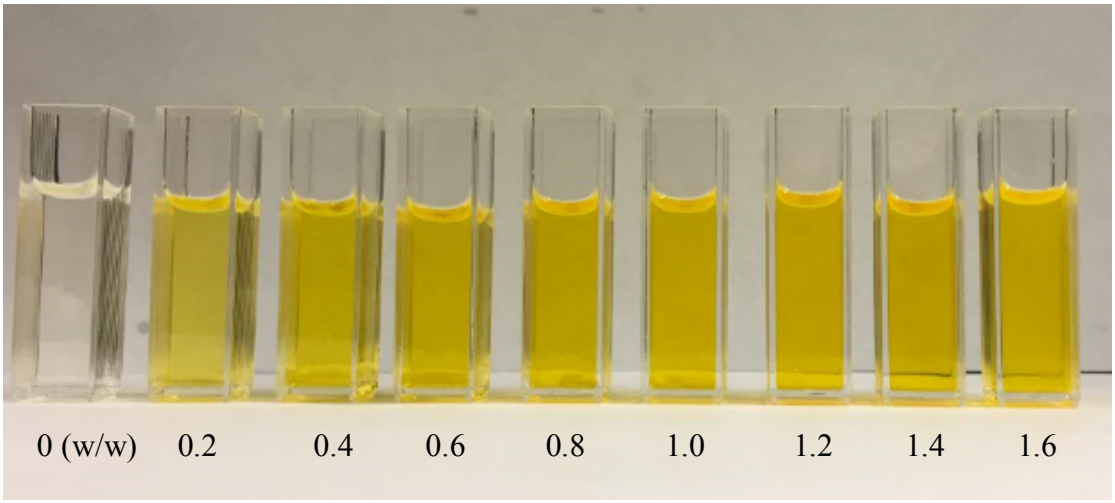

Fig. 8-1 Appearance of canola oil containing mango carotenoids with different preparing mass ratio of fresh mango pulp to canola oil ranging from 0, 0.2, 0.4, …, to 1.2 (w/w).

The presence of *β*-carotene and zeaxanthin in carotenoid-enrich canola oil was proposed due to the matching visible spectrum. In Fig. 8-2, the spectra of carotenoid-enrich oils had three main large peaks at around 428 nm, 450 nm, and 480 nm respectively with different intensity. These three wavelengths perfectly matched *all-trans-β*-carotene and zeaxanthin's corresponding wavelengths, as shown in Table 3. Numerous studies have reported that λ_{max} of *all-trans-β*-carotene are 424, 448, 476 nm in petroleum ether and 422, 450, and 477 nm in a gradient of acetone in hexane. Several sets of λ_{max} for zeaxanthin have been reported as well: 425, 478, 476 nm in petroleum ether and 423, 450, 478 nm in a gradient of acetone in hexane. In addition to that, among all carotenoids in mango pulp *β*-carotene is the most abundant one at 48-84%.[149] In addition, the intensity of the light absorbance was increased with the increment of mango/oil ratio from 0.2 to 0.6. The intensity of light absorbance was out of accurate range where the mango/canola oil ratio was up to 1.2, which means the sample is too concentrate. This confirmed that the decreased extraction efficiency occurred when less surface contact was in between fresh mango pulp and canola oil.

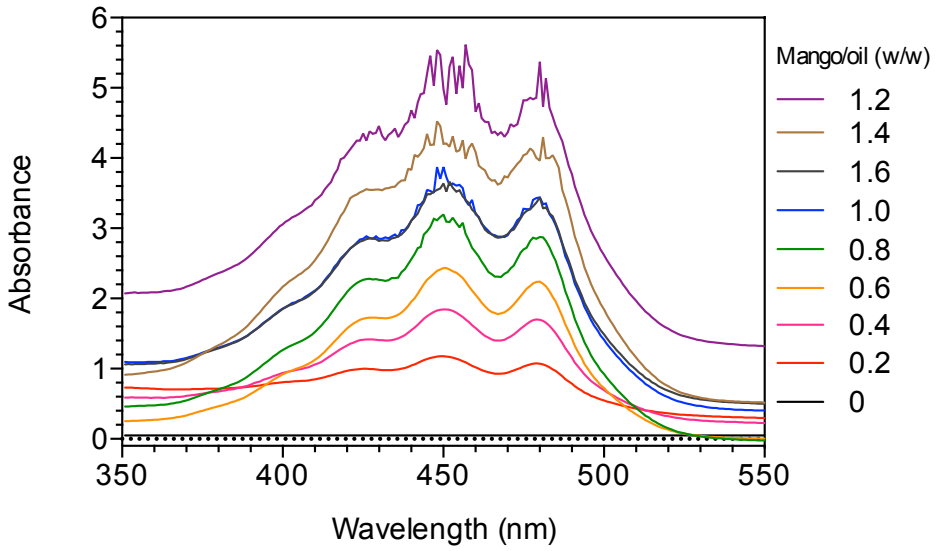

Fig. 8-2: Spectra of canola oil after blending with different blending mango/oil ratio (w/w) from 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 (w/w). The noise shows in the ratio above 0.6 because the concentrate of samples is beyond the detection range of the spectrometer used in the study.

Table 3 Identifying λmax for the carotenoid-enriched canola oil.

Concentration of mango pulp used (wt%)	**λ_{max} (nm)**
0 - Pure canola oil	Not detectable
0.2 Carotenoids-enrich canola oil	426, 450, 478
0.4 Carotenoids-enrich canola oil	427, 450, 478
0.6 Carotenoids-enrich canola oil	428, 450, 480
0.8 Carotenoids-enrich canola oil	427, 450, 480
1.0 Carotenoids-enrich canola oil	428, 450, 480
1.2 Carotenoids-enrich canola oil	429, 448, 480
1.4 Carotenoids-enrich canola oil	429, 448, 480
1.6 Carotenoids-enrich canola oil	428, 450, 480
λ_{max} from reference	
All-trans-β-carotene	424, 448, 476*
	422, 450, 477**
All-trans-zeaxanthin	425, 448, 476*
	423, 450, 478**
Cis-violaxanthin	410, 434, 461*
	412, 437, 465**

*Azevedo-Meleiro, and Rodriguez-Amaya, 2004. λ_{max} (nm) in petroleum ether, obtained with a spectrophotometer.
**Mercadante et al., 1997. Obtained with diode array detector in a gradient of acetone in hexane.

8.2.2. Preparation of oleogels with carotenoid-enriched oil

With the successful preparation of carotenoid-containing oils, the promising two-component structuring agent, S5B3, was added into it to prepare the expected resulting oleogel. 5% Sa and 3% β-Ss were weighed in a vial containing oils with different amounts of carotenoids in them. The mixtures were heated until complete dissolution of particles of both Sa and β-Ss and subsequently cooled to RT for 1 day. After one-day quiescent standing, all samples were investigated for their gelation ability through the inverted vial method. Fig. 8-3 shows the results of inverted vials. All samples are shown as oleogels. These carotenoid-containing oleogels were referred to as mangoleogels in the following contexts. An even-larger scale of sample was prepared (Fig. 8-4). This result indicated that olgeogelation was not influenced by the presence of carotenoids, which can theoretically slightly increase the oil's polarity. Additionally, formation of mangoleogels was independent of the amount of carotenoids, although carotenoid quantification was not performed in this study. We can only infer that the actual amount of carotenoids may be too low to affect the polarity of canola oil, resulting in no interference on forming mangoleogels. The quantitative study is suggested to be done for more information. The other proposed reason was that the S5B3 may be able to structure a wide range of polarity of oils according to the findings from previous chapters.

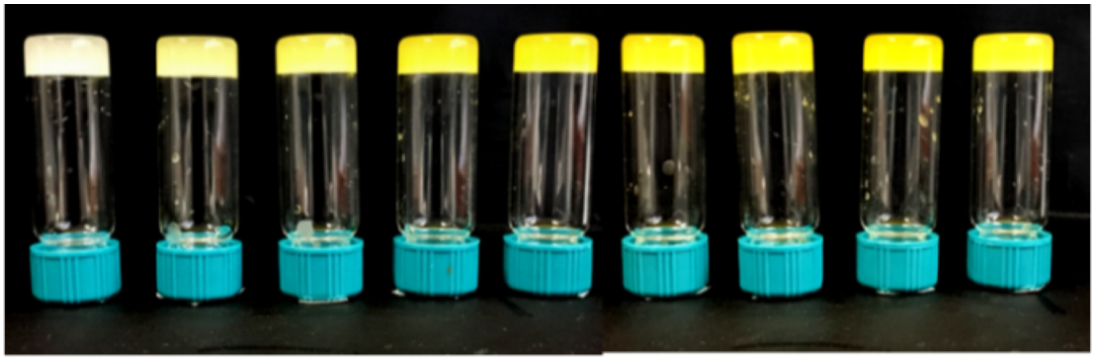

Fig. 8-3 The appearance of oleogels made of carotenoid-enriched canola oil and S5B3 with a series of mass ratio of fresh mango pulp to canola oil used during extraction. From left to right: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 (w/w).

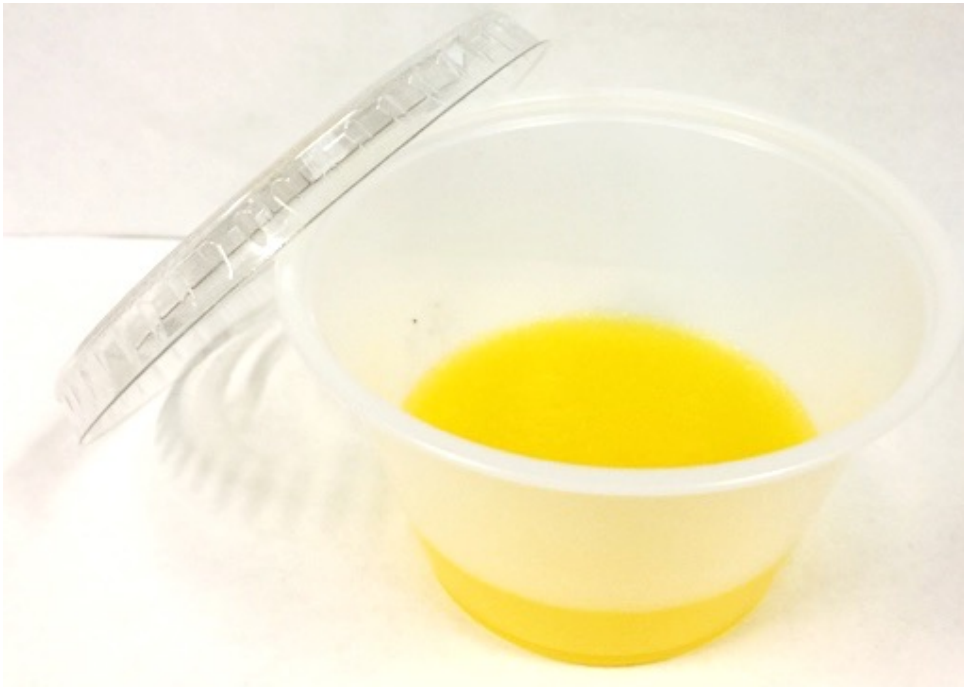

Fig. 8-4: A larger-scale oleogel sample containing mango carotenoids.

8.2.3. Effect of carotenoids on oleogel formation

Although every sample was able to become a mangoleogel, the microstructure of each was rather interesting. Fig. 8-6 illustrates that oleogel microstructures with different mango pulp weight in canola oil (0, 0.2, 0.4, ..., 1.4, 1.6 (w/w)) were identical in their needle-like shape. In comparison with oleogels made of pure canola oil, microstructures did not show any difference. Only subtly

higher amounts of crystals were observed in the mangoleogels. Specific needle-like crystals enabling the formation of networks to structure oils have been reported. In addition, the needle-like crystals were similar to the β′ polymorph which is believed to be the most functional polymorphic form of crystalline fat for fat-enriched products due to its small size and needle-like shape. Compared to large crystal aggregates, these small needle-like crystals provide a smoother texture and better mouth-feel[157]. This result indicates that the presence of carotenoids did not affect the self-assembly of Sa and β-Ss in terms of not destroying the balance between two interactions (i.e., gelator-gelator and gelator-solvent interactions). Moreover, the small and needle-like crystals could contribute to the desirable mouth feel required for foods, especially high fat content bakery products.

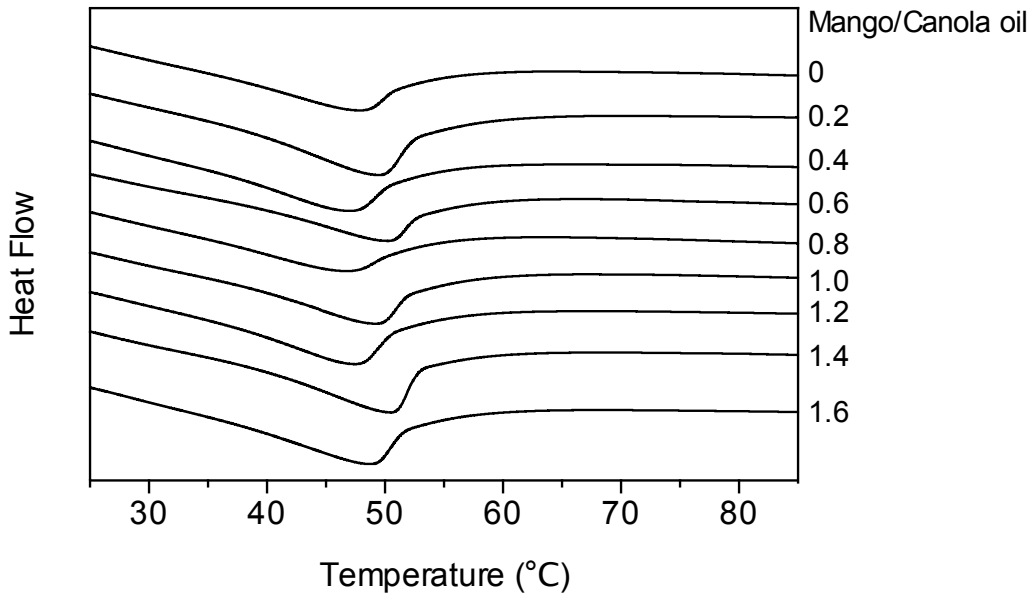

Fig. 8-5 Melting profiles of oleogels made of different carotenoid-enriched canola oil. From top to bottom: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 (mass of fresh mango pulp/ mass of canola oil) used to prepare carotenoid-enriched oils.

Thermal properties of mangoleogels were analyzed using differential scanning calorimetry to obtain the melting profiles. The range of peak melting temperature was 48–51°C. There was no

significant difference between all the mangoleogels and regular oleogel, which is consistent with microstructure observations. Regarding the 48–51°C melting range, it is similar to the range of commercial margarine and butter products. In most bakery products, the melting range of butter or margarine affects the preparation of dough and subsequently affects the texture and mouth feel of products. For example, if the melting point is low, the dough has decreased stiffness, causing preparation difficulties. Furthermore, interactions between water and flour are influenced, leading to formulation changes, which is not welcome for food manufacturers. In comparison with other reported oleogels, this melting range though is lower but is suitable for food applications.

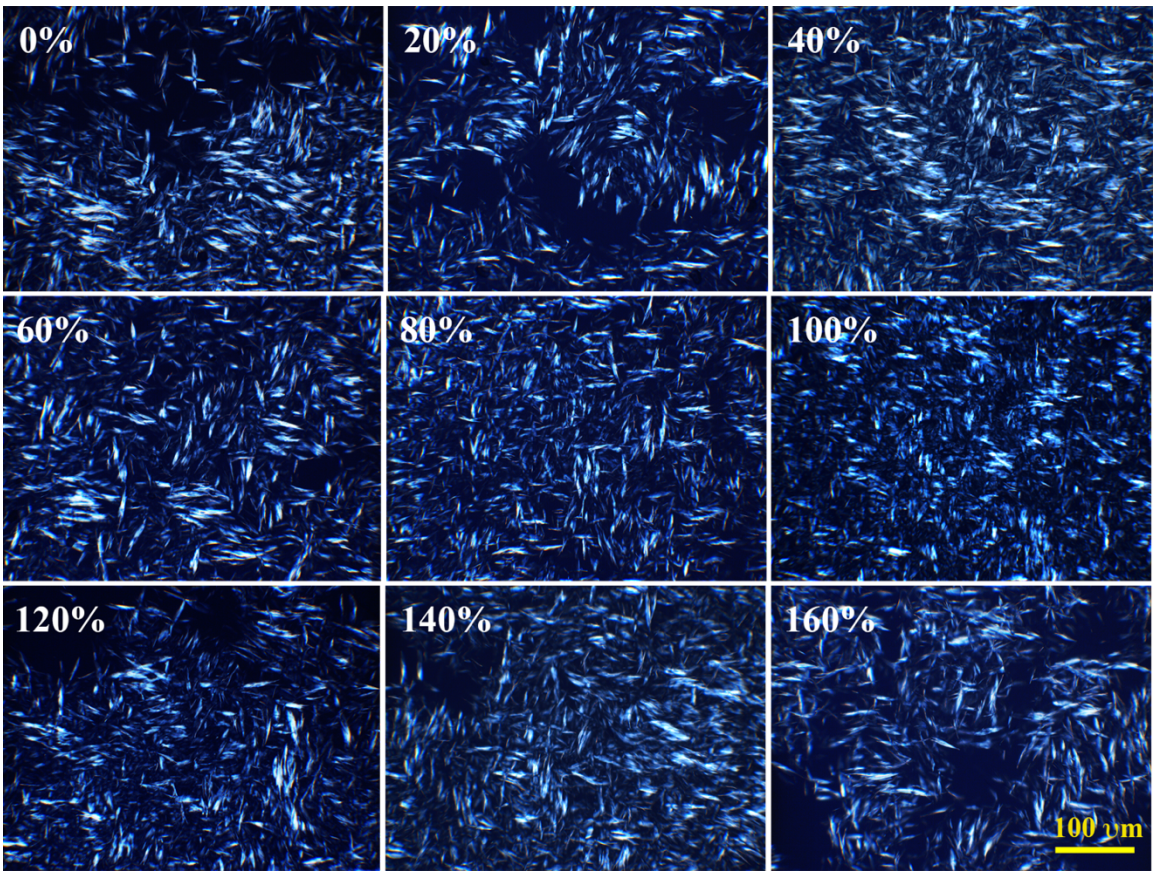

Fig. 8-6 Polarized light microscopic graphs of oleogels containing mango carotenoids with different percentages of mango used during extraction.

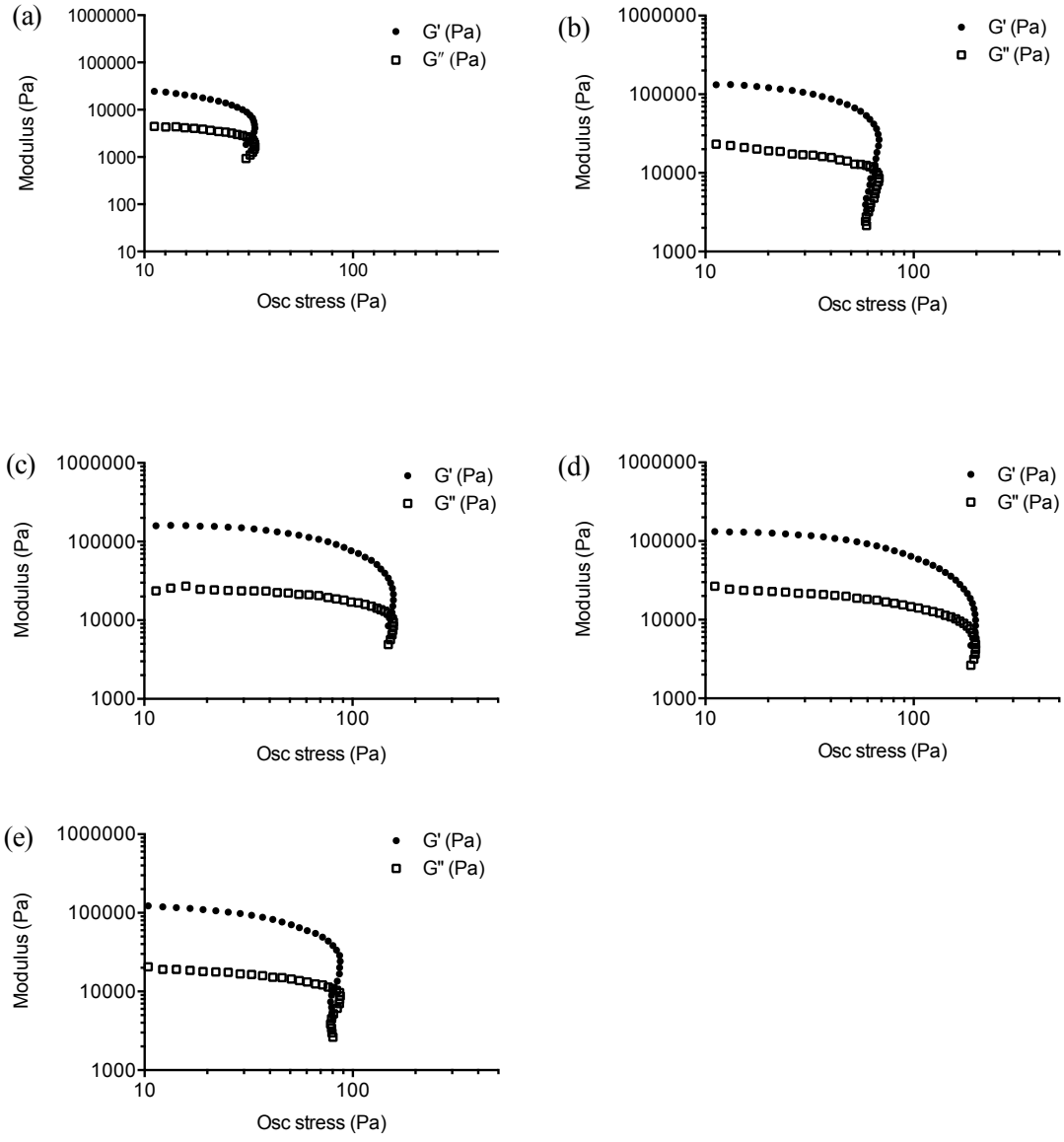

Fig. 8-7: Rheology oleogels containing different amount of mango carotenoids by preparing the carotenoid-rich oil with different amount of fresh mango pulp. Mango/canola oil mass ratio (w/w) is 0 (a), 0.4 (b), 0.8 (c), 1.2 (d), and 1.6 (e).

Selected mangoleogels and oleogel with pure canola oil were investigated for their rheological properties by using a rheometer with oscillation mode. Mangoleogels showed a viscoelastic solid structure with storage modulus (G′) higher than loss modulus (G″) throughout the measured oscillatory stress until touching the crossing point.

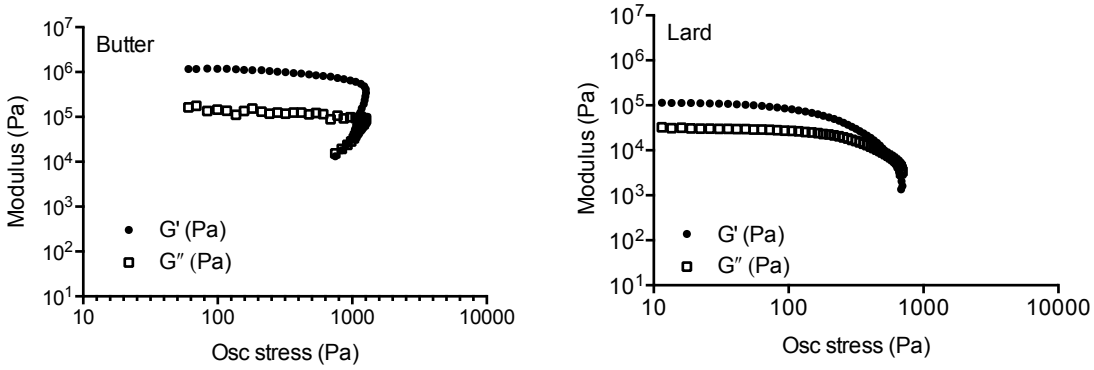

Fig. 8-8: Rheology of commercial butter and self-prepared lard. G′ is greater than G″indicating both butter and lard tested are solid-like materials.

No significant differences were found between all tested samples in terms of maximum G′ values. This result indicates that the hardness was similar in all samples, irrespective of whether the oleogel was prepared using pure canola oil or mango-extracted canola oil. However, the overall network strength was stronger for mangoleogels (yield stress of 80-100 Pa) and weaker for oleogel with pure canola oil (yield stress of 50 Pa), which was observed by the value of yield stress. Yield stress means the pressure applied to destroy the structure of materials resulting in becoming a liquid-like material. Generally, the higher value of yield stress, the stronger network is indicated. Also. Compared to the commercial butter and self-made butter (see Fig. 8-8), the hardness of oleogels containing mango carotenoids was closer to lard rather than butter in terms of G′ values. But the strength of network was smaller than both butter and lard. One thing was noticed that the

curve of modulus looked like boomerang because the stress decreased after the network of gel was destroyed casusing the backward curve.

8.2.4. Effect of blending and processing temperature on carotenoids

Upon studying the efficiency of carotenoid extraction and the influence of carotenoid-containing oil on olgeogelation, the blending and processing temperatures were found to impact the structure of β-carotene and zeaxanthin significantly. For increasing the extraction efficiency, higher temperatures rather than room temperature, such as 30°C, 60°C, and 90°C were suggested. A mango/oil ratio of 1.2 mixture was heated to 30°C, 60°C, and 90°C for 10 min and then blended using an IKA ultra turrax Tube Drive control equipped with 30 stainless balls. The blended sample solution was centrifuged to remove solid particles, such as mango fibers, and to obtain a clear golden yellow liquid. The same batch of fresh mango pulp was used to avoid carotenoid level differences due to the use of different mangoes. As shown in Fig. 8-9, only pure canola oil was colorless, the color of the rest of the samples with different blending temperatures is identical – golden yellow. A spectrometer was used to obtain carotenoid-enriched canola oil visible spectra in order to distinguish carotenoid types.

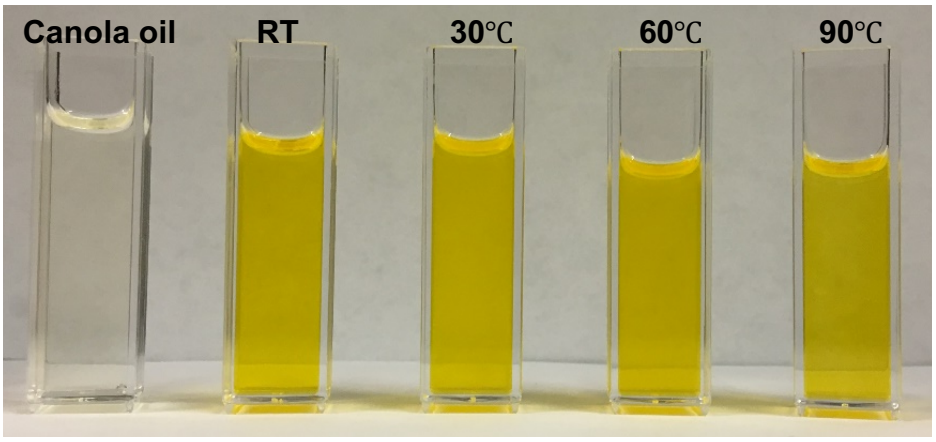

Fig. 8-9 Effect of temperature on carotenoid extraction efficiency. From left to right: pure canola oil, extraction at room temperature, 30°C, 60°C, and 90°C, respectively.

Fig. 8-10 shows the spectra of mango-extracted oils with mass ratio of 1.2 which were extracted at RT, 30°C, 60°C, and 90°C. Results show that intensity of light absorbance significantly decreased when the blending temperature was up to 60°C. The amount of extracted carotenoids did not increase with temperature; in contrast, carotenoids decreased, particularly at 90°C. Moreover, the spectrum has a significant hypsochromic shift of 15 nm which means that peaks of λ_{max} shift toward shorter wavelengths. This indicated that β-carotene and zeaxanthin were destroyed and converted to other carotenoids. Hypsochromic shifts in the absorption spectra of mango carotenoids during processing have been reported by Brekke et al., and Ranganad and Siddappa. They noted a change in carotenoid composition due to the effects of processing, such as light exposure, heat, and acid. Carotenoids exposed to light, heat, and acid may undergo isomerization and epoxidation.[158–160]

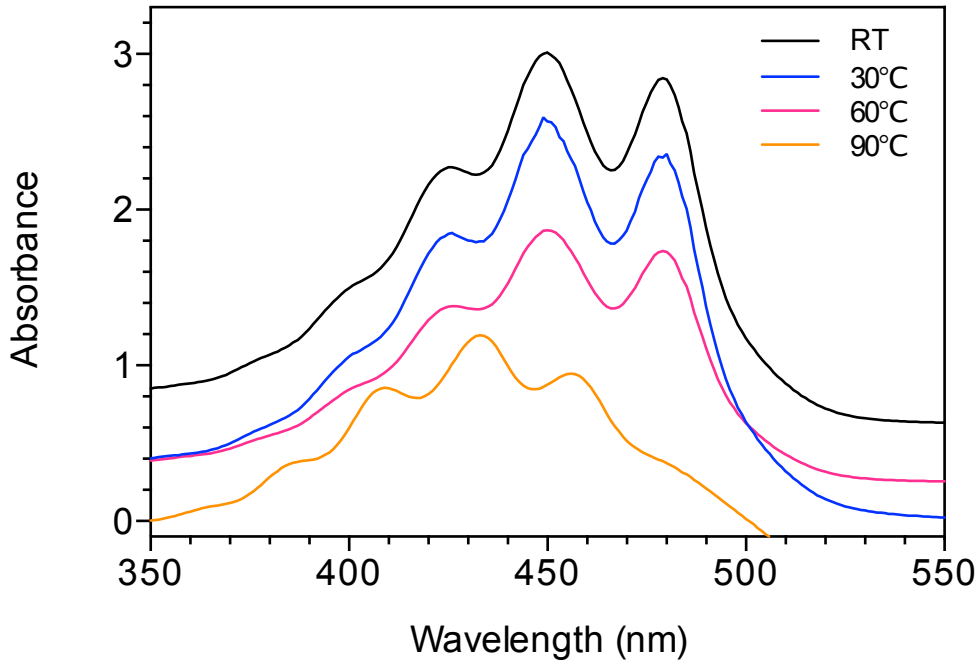

Fig. 8-10: Spectra of carotenoid-enriched canola oil with different blending temperature treatments. From top to bottome is RT (25°C), 30°C, 60°C, and 90°C, respectively.

At 90°C blending temperature, a different visible spectrum with a new λ_{max} was observed at 409, 435, and 463 nm. New λ_{max} were similar to those reported for λ_{max} of the *cis* form of violaxanthin, which are 410, 434, and 461 nm or 412, 437, and 465 nm. β-carotene and zeaxanthin may have been oxidized during the blending process at high temperatures (90°C) perhaps resulting in the formation of *cis*-violaxanthin, which is one of the xanthophyll pigments having oxygen in its structure.[161] *Cis*-violaxanthin is a common oxidized derivative from zeaxanthin and β-carotene in biosynthetic pathways of plants or in post-harvest processing with heat or acid, as shown in Fig. 8-11.[149,162,163] Chen et al.[149] published an article discussing the effect of drying treatments on stability of carotenoids in Taiwanese mango. The highest temperature they studied was 60°C using hot-air drying with or without soaking ascorbic acid as pretreatment. Zeaxanthin was not detected in any hot-air drying treatment, while for β-carotene, the compound was not destroyed as much as zeaxanthin. This result clearly indicates that hot-air drying has a destructive effect on zeaxanthin at 60°C. In agreement with this, the spectrum of carotenoid-enriched canola oil prepared at 60°C had lower intensity of light absorbance compared to those prepared at RT and 30°C. This indicates that blending at 60°C has some destructive effects on β-carotene and zeaxanthin. Food products with high carotenoid content, such as mango, orange, and cashew apple juice[164] tend to lose varying amounts of carotenoids and develop new isomers or oxidative derivatives of carotenoids during heating due to isomerization and epoxidation in aqueous-based systems. The presence of *cis*-violaxanthin, a epoxide as a result of isomerization and epoxidation, in carotenoid-enriched canola oil prepared at 90 °C indicates that isomerization and epoxidation occur in oil-based systems as well.

In addition to blending temperature, the temperature used to prepare oleogels is another important factor to be considered. In order to avoid destroying or converting the carotenoids' structures,

room temperature was used for preparing carotenoid-enriched canola oil for further oleogel preparation. Before making oleogels, a simple heating test was applied. The same batch of carotenoid-enriched canola oil prepared at RT was distributed to five treatments: no further heating, and heating up to 30, 60, 90, and 120°C. Once the oil reached the target temperature, the heating process was stopped. Results of visible spectra are shown in Fig. 8-12. A slight hypsochromic shift appeared for oils which were treated at 90 and 120°C. However, the shift was not as noticeable in comparison to a 90°C blending temperature. This result was due to the very short heating process time applied which did not allow the carotenoids to undergo isomerization and epoxidation. Although carotenoids were not changed significantly, a mild shift still occurred, thus indicating that high temperature (above 90°C) has a significantly destructive effect on carotenoids.

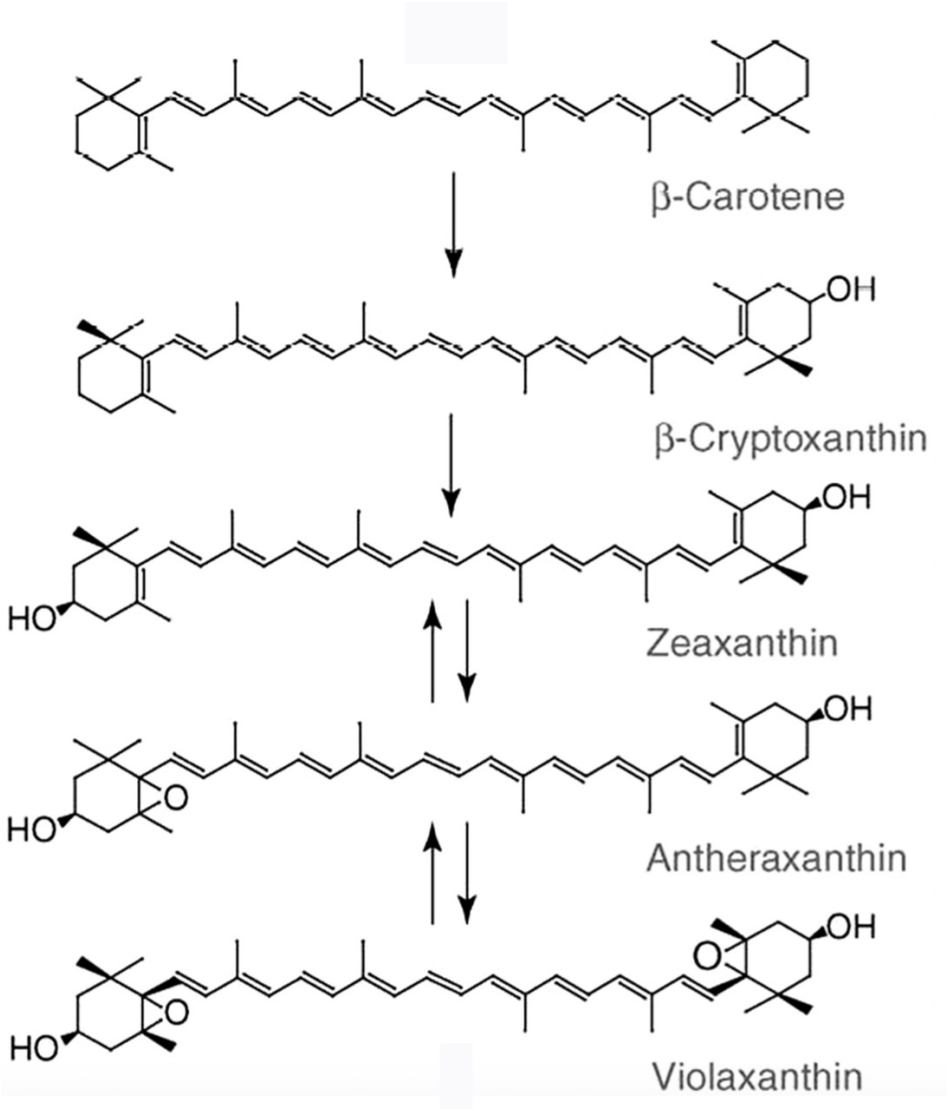

Fig. 8-11: Part of the carotenoid biosynthetic pathway in plants.[162]

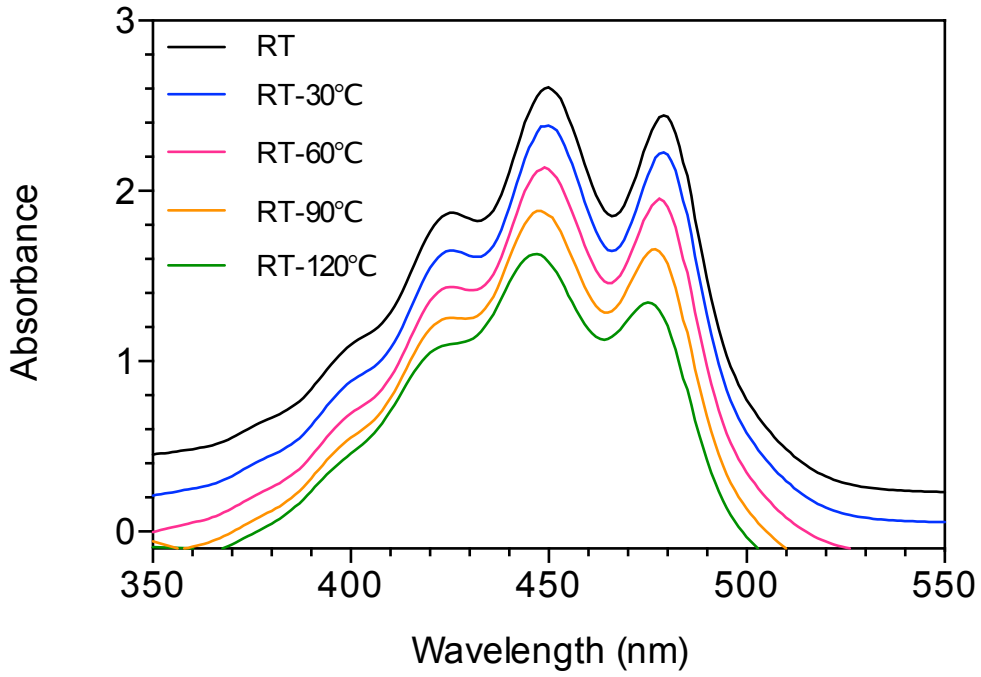

Fig. 8-12: Effect of further heating process on visible spectra of carotenoid-enriched canola oils.

Regarding the study of the effects of temperature on carotenoids in mangoleogels, carotenoid-enriched canola oil prepared at RT was used to prepare mangoleogel in order to maintain β-carotene and zeaxanthin. For preparing oleogels at lower temperatures and ensuring a lack of undissolved solid particles of β-sitosterol, 5% stearic acid was used alone at 65°C, since the melting temperature of β-sitosterol in oil was 120°C. For high temperature treatments (120°C) both 5% Sa and S5B3 (total 8%, w/w) were used to form oleogels. As shown in Fig. 8-13, the oleogel with 65 °C as preparing temperature for 40 min had the same visible spectrum as carotenoid-enriched canola oil itself having λ_{max} at 428, 450, 480 nm. This result indicates that 40 min heating at 65°C and the presence of 5% stearic acid has no destructive effects on *β*-carotene and zeaxanthin. The two oleogels prepared at 120°C for 40 min showed a hypsochromic shift in their visible spectra. New λ_{max} were 409, 435, and 463 nm, which were the same as for the

carotenoid-enriched oil blended at 90°C. Although the co-existence of stearic acid, β-sitosterol, and carotenoids does not affect the formation of oleogels, the high temperature for dissolving low molecular weight molecules in order to form an oleogel does affect the type and amount of carotenoids. *Cis*-violaxanthin is not absent in natural mango; however, *β*-carotene and zeaxanthin are the ones we would like to have in food products due to their high antioxidative activities. *β*-Carotene even has high pro-vitamin A activity. Including *β*-carotene and zeaxanthin rather than *cis*-violaxanthin is ideal from both chemical and nutritional viewpoints. Overall, the presence of *β*-carotene in oleogels is beneficial because it can retard lipid oxidation; however, the suitable low molecular weight gelators, which can melt at temperatures below 90 °C or even 60 °C, are suggested for use in this case.

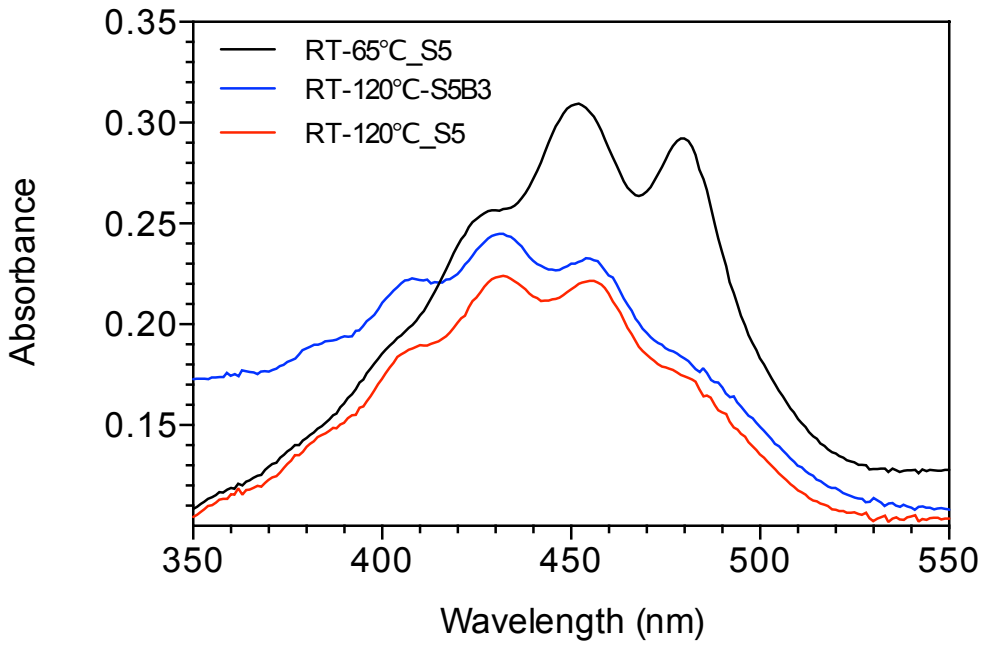

Fig. 8-13 Visible spectra of oleogels using different heating temperatures and gelator systems. At high temperature, 120 °C, a hypsochromic shift is shown referring the changes of chemical structures of carotenoids.

8.3.Conclusion

This research has found that is feasible to extract carotenoids from fresh mango pulp by blending with canola oil. A clear golden yellow oil was obtained and the color was attributed to *β*-carotene and zeaxanthin based on the matched λ_{max} shown in the visible spectrum with peaks at 428, 450, and 480 nm. Canola oil with the modification of dissolving carotenoids in it did not interfere with the crystallization of mono- and two-component structurants during the cooling process from a hot solution. Irrespective of the amount of carotenoids in canola oil, oleogels were formed with even slightly higher yield stress characteristics compared to the control oleogel made from pure canola oil. The rheological properties of oleogels with carotenoids were comparable to commercial butter and lard, thus indicating that oleogels are indeed good potential substitutes for solid fats. Although including carotenoids in the oil phase is a highly appropriate idea and is feasible, the processing temperature should be considered. In order to incorporate the carotenoids in oleogels, the heating temperature for making an oleogel is suggested to be below 90°C, or even below 65°C, in order to prevent destructive effects on carotenoids. Thus, 5% stearic acid is more suitable for preparing carotenoid-enriched oleogels because high temperatures are not required for dissolving stearic acid, rather than mixtures of stearic acid and β-sitosterol, which need as high as 120°C to dissolve all the solid particles in order to make an oleogel.

Overall, this study has illustrated that even canola oil is modified by having carotenoid compounds, and it still can be structured, resulting in an oleogel.

9. Future work

9.1.Quantitative of extracted carotenoids from fresh mango pulp to canola oil

The last section of the thesis incorporate the idea of addition of nutraceutical to enhance the health benefits for the oleogel that is proposed to be a fat substitute. Through a obvious coloring changing of canola oil after blending with fresh mango pulp from pale yellow to golden yellow, it is believed that carotenoids were extracted out from the fresh mango. Therefore, the measurement of visible spectra was done to investigate what types of carotenoids are. The results showed that *β*–carotene and zeaxanthin are the most possible carotenoids in carotenoid-enriched canola oil due to specific corresponding peaks. The quantitative study which was not done is equally important to qualitative study. Therefore, author suggests here that few more studies related to quantification of carotenoids in oil can be done, such as HPLC, HPLC-MS/MS.

9.2.Effect of heating temperature on carotenoids

It was found that there was a change in chemical structure of carotenoids after heating over 90°C for more than 10 min or in a short time at 120°C due to heat susceptible of carotenoids. It will be suggested firstly that using HPLC to specify what carotenoids formed after heating and what the amount is. Secondly, finding another preparing method to prepare oleogels involved β-Ss is suggested, since the melting temperature of β-Ss is higher than the carotenoids' sensitive temperature. To resolve this issue, two solutions are proposed. Firstly, small amount of pure oil is used to melt β-Ss under high temperature and followed by adding carotenoid-enriched oil and mixing well in a very short time (less than 1 min). To reduce the exposing time of carotenoids to high heating temperature. The second approach is to find another co-crystal compound for β-Ss

to reduce its melting temperature in oil. Though there are some studies on multi-component systems with β-Ss, it is still a trial and error study on finding a matching co-crystal.

9.3.Effect of oils on the efficiency of carotenoids extraction and on the antioxidative capability of carotenoids.

According to the results of this study, eleven of twenty-five edible oils can be structured by Sa+β-Ss, a two-component system. It means that not only canola oil, bust also the rest of ten edible oils have the feasibility to dissolve carotenoids and to form a carotenoid-enriched oleogel for more widen application in the food industry. Since carotenoids are well known for their antioxidative capacity, it is suggested to investigate how mango carotenoids in oil will extend the shelf-life of oils by retarding the lipid oxidation during processing and storage.

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