

THE PHYSICAL PROPERTIES AND COOKIE-MAKING PERFORMANCE OF OLEOGEL

MADE WITH REFINED AND CRUDE OILS

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ABSTRACT

In this study, oleogels were prepared with crude plant oils using varying oleogelators. The role of oleogel was compared with refined oil oleogels as well as commercial shortening in the cookie making process. The plant oils used in this research include solvent-extracted crude soybean oil, refined soybean oil, expeller-pressed corn germ oil, and refined corn oil. β -sitosterol and/or monoacylglycerides, and rice bran wax were used either individually or in combination as the gelator to form oleogels using different oils. The physical properties of oleogels and corresponding cookies were investigated and compared. Overall, the incorporation of gelators into crude and refined oils could produce oleogels with solid-like properties. Refined oils showed higher gelling properties than crude oils. However, the cookie performance of crude and refined oil oleogels were comparable, which indicated that both crude and refined oleogels have the possibility to be used as shortening alternative in cookies.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. Lipid structure and function

Lipids can be defined as compounds that are soluble in non-polar organic solvents but insoluble in water (Bai ã & Lara, 2007). Lipid is a collective name for fat and oil and plays important roles in industrial applications and food applications for human and animal. From the edible perspective, people usually consume fats and oils through cooking oils, salad oils, butter, lard, margarine, and other processed fat-based products such as bakery goods, meat, cheese, and chocolates. In general, fat and oil are mainly the mixtures of triacylglyceride (TAG) molecules, which usually contain a glycerol backbone and three fatty acids (Davidovich-Pinhas, Barbut, & Marangoni, 2016). The key difference between fat and oil is their physical nature, i.e., fat is solid while oil remains liquid at room temperature. Such a difference in the physical nature of fat and oil is determined by the fatty acid composition of TAG (Bai ã & Lara, 2007; Marangoni et al., 2012).

Based on the presence or absence of double bonds in the molecular structure, fatty acids can be classified as saturated, monounsaturated, and polyunsaturated fatty acids, which represent zero, one, and more than one double bond, respectively (Bai ã & Lara, 2007; Dorni, Sharma, Saikia, & Longvah, 2018). Moreover, fatty acids also can be classified as short chain, medium chain, and long chain fatty acids depending on the chain length (Dorni et al., 2018). The unsaturated fatty acids including monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids can be further classified into omega series such as omega-3, omega-6, and omega-9 based on the position of first carbon double bond (Kostik, Memeti, & Bauer, 2013). Omega-3 and omega-6 fatty acids are essential fatty acids while omega-9 fatty acid is non-essential fatty acid

since the previous fatty acids cannot be effectively synthesized by the human body (Kostik et al., 2013).

The varying structure of fatty acids including the chain length, the degree of saturation, and the composition of fatty acids, not only affect the state of fat and oil, influence other physical and chemical properties of fat and oil such as thermal behavior, melting point, water solubility, solid fat content, viscoelasticity, cloud point, and oxidative stability (Bai ão & Lara, 2007; Kadhum & Shamma, 2017). In general, fat contains the fatty acid with longer carbonic chain have higher melting points and lower water solubility compared to fat with shorter chain fatty acids (Bai ão & Lara, 2007; Kostik et al., 2013; Marangoni et al., 2012). In addition to the chain length, as the number of double bonds increases, the lipid tends to be closer to the liquid state, causing a lower melting point (Marangoni et al., 2012). Moreover, fatty acids with *cis* double bonds have a lower melting point than fatty acids with *trans* double bonds (Bai ão & Lara, 2007).

The United States Department of Agriculture (USDA) reported the local production of fat and oil has increased from 28,149 million pounds in 2010 to 34,115 million pounds in 2016 and the demand of edible fat and oil in the US increased from 31,498 to 40,116 million pounds for the corresponding year (US Department of Agriculture, 2016). Fat and oil are indispensable parts in our diet not only because they contain different essential fatty acids and provide energy for the human body, but more importantly, they can act as functional ingredients, flavor and vitamin carriers, and structure modifiers (Bhosle & Subramanian, 2005; Dorni et al., 2018; Kostik et al., 2013; Li, Kong, Shi, & Shen, 2016; Ognean, Darie, & Ognean, 2006). When fat and oil are used as food ingredients, they provide several functionalities that will influence the properties of the final food products. For example, fat could affect the flavor, mouthfeel, aroma, and taste of the food (Ognean et al., 2006). Moreover, lipids contribute to the appearance and texture of food,

increases diet palatability, improves the absorption of fat-soluble vitamins, and reduces the rate of food passage through the gastrointestinal tract (Bai ão & Lara, 2007; Ognean et al., 2006; Singh, Auzanneau, & Rogers, 2017).

It is well known that plant oils such as olive, corn, and soybean oils contain more monounsaturated and polyunsaturated fatty acids than solid fats such as butter, shortening, and margarine (Marangoni et al., 2012). According to the WHO and the US Department of Health and Human Service (HHS), a healthy eating pattern should not contain more than 10 percent of calories per day from saturated fats or added sugars, and they recommended to use polyunsaturated fats to replace saturated fats (U.S. Department of Health and Human Services, 2015).

1.2. Lipids used in the bakery industry

Lipid plays an important role in food processing since it provides several functional characteristics to the final products. The major functions of lipid in food include acting as the leavening agent of batters and doughs, modifying flavor and texture of food, improving flakiness and tenderness, lubrication, emulsification, and other properties (Pehlivanođlu et al., 2018). Lipid has different physical states, including solid, semi-solid, and liquid state. The physical state of lipid depends on the composition of the fatty acid chain, the chain length, the saturation level of fatty acids, and the content of cis/trans isomers(Ölütçü & Yilmaz, 2015). In general, fat is a kind of lipid that contains high levels of saturation and causes fat to have a higher melting temperature that results in a solid-like behavior at room temperature (Davidovich-Pinhas et al., 2016). The solid-like lipids are preferred in the processing of some industrial products such as meat patties, frankfurters, cakes and cookies due to some specific characteristics such as better

oxidative stability, entrapment of air, lubrication, and solid lipid functionality (Gómez-Estaca et al., 2019; Pehlivanoglu et al., 2018).

Solid fat-containing food products have specific texture because oil can be physically trapped by the colloidal network of fat, thus form a crystalline network (Patel et al., 2014). Saturated fats are the main components in the fat crystal network, the structure of the liquid oil are mainly depend on the fat crystal, and consequently, without saturated fats, some products would not be formulated (Patel et al., 2014). For example, bakery products such as cookies, pastries, and cakes require a high amount of saturated fats to obtain essential structures. As an edible solid fat, shortening is widely used for bakery products such as pastries, cakes, and cookies for desirable texture and extended shelf-life (Li et al., 2010). Shortening has several functionalities during the production of bakery goods; some of their key roles include improving palatability, assisting entrapment of air, aiding in lubrication, providing a moisture barrier, providing softer texture and desirable flavor, and extending shelf-life of the products (Cheong et al., 2011). Shortening has different forms, including plastic, semi-solid, pourable fluid, flake, and encapsulated powder (Kaur, Jassal, Thind, & Aggarwal, 2012). Shortening is generally produced by solidifying and plasticizing of a fat/oil blend, followed by packaging and tempering (Kaur et al., 2012).

Several fats can be used as shortening in bakery products. Before the vegetable shortening was introduced in the U.S. market decades ago, animal fat such as lard and butter was used as shortening (Knightly, 1981; Mignogna, Fratianni, Niro, & Panfili, 2015). The usage of lard as shortening is due to its low solid fat content at dough mixing temperatures (Knightly, 1981). In addition, lard has excellent shortening characteristic because it is capable of dispersing thoroughly in the dough for bread or other baked goods (Knightly, 1981). Butter and margarine

also are common shortening used in bakery products. During the baking process, butter and margarine melt rapidly at the earlier stage due to their low melting enthalpy and melting temperatures. (Devi & Khatkar, 2016). Later, the development of the hydrogenation technique increases the application of hydrogenated oils in the shortening industry (Li et al., 2010).

1.3. Current challenges in the baking industry

In general, shortening offers the functional characteristics in improving the overall quality of the product, for example, improving the mouthfeel and textural properties of baked products (Pehlivanoğlu et al., 2018). As a solid fat, shortening is usually produced by animal fat or hydrogenation of liquid oil. Unfortunately, most of the shortening has suspected deleterious health effects due to the presence of high-level *trans* and saturated fatty acids (Mert & Demirkesen, 2016a).

In 2003, the United States Food and Drug Administration (FDA) defined *trans* fatty acids as ‘one or more isolated double bonds in the unsaturated fatty acid is in a *trans* configuration (Handa, Goomer, & Siddhu, 2010). In general, *trans* fatty acids can be produced by industrial hydrogenation or natural biohydrogenation in the rumens of animals (Brouwer, Wanders, & Katan, 2010). Partially hydrogenation of liquid oils with hydrogen gas in the presence of metal catalysts is considered as the major source of artificial *trans* fatty acids (Brouwer et al., 2010). For some baked goods such as cookies and cakes, there may be no *trans* fat on the nutritional label. However, based on FDA labeling guide, if a serving of the product contains less than 0.5 gram of *trans* fat, the labeling will be expressed as 0g. So, although the nutritional labeling of products shows 0g *trans* fat, it may still contain trace amounts of *trans* fat.

Since most shortenings are made from the hydrogenation of liquid oil, *trans* fatty acids may be present in the shortenings. It was reported that 23% of *trans* fatty acids were found in

margarine produced from partially hydrogenated oils(Cheong et al., 2011). In addition, other common baking shortenings also contained up to 40–50% *trans* fatty acids (Handa et al., 2010). Although fats containing industrially produced *trans* fatty acids have some beneficial properties, for example, they are solid at room temperature and have a longer shelf life (Handa et al., 2010). However, the usage of *trans* fatty acids containing fat is prohibited due to deleterious health effects.

Several deleterious health effects are associated with the consumption of *trans* fatty acids. There is evidence showing that the usage of shortenings that contain artificial *trans* fatty acids can increase the risk of cardiovascular disease by decreasing of the high-density lipoprotein cholesterol (HDL) and increase the level of low-density lipoprotein cholesterol (LDL) (Li et al., 2010). In addition, the consumption of *trans* fatty acids induces an inflammatory response, and even a low level of intake significantly increases the risk of coronary events (Mozaffarian, Jacobson, & Greenstein, 2010). There are more multiple adverse effects of *trans* fatty acids, for example, the deleterious effects associated with *trans* fat produced by partial hydrogenation include cardiovascular disease, body weight, oxidative stress, insulin sensitivity, endothelial health, and cancer (Handa et al., 2010).

Since the adverse effects of *trans* fatty acids in shortening are obvious and impact public health, several policies recommend avoiding the use of *trans* fatty acids. Many regulations have been published to motivate food manufacturers and restaurants to replace *trans* fatty acids in foods with alternative fats (Mozaffarian et al., 2010). Decades ago, the FDA issued a requirement that the nutrition label of food and dietary supplements need to declare the amount of *trans* fatty acids in the product (Handa et al., 2010; Jang, Jung, & Min, 2005). More recently, FDA (2015) published a notice that partially hydrogenated oils (PHOs), the primary source of

artificial *trans* fatty acids, are no longer GRAS, and all the food manufacturers need to remove all the PHOs from their products before June 18, 2018. However, it is difficult to find an ideal product to replace PHO in the food industry, so FDA is extending the compliance date to January 1, 2020, allowing for an orderly transition of the majority of PHO-contained products made before Jun 18, 2018 (FDA, 2018). Therefore, there is an urgent need to find a PHO replacement in the baking industry.

1.4. Alternatives to shortening

1.4.1. Conventional oil structuring methods

Currently, alternatives to partially hydrogenated shortening can be achieved by different oil structuring methods including fully hydrogenation, interesterification, blending, fractionation of tropical oils such as palm oil and coconut oil, and genetic engineering of oilseeds with high oleic fatty acids (Handa et al., 2010; Ölütcü & Yilmaz, 2015). For decades, hydrogenation has been the process most widely used to structure liquid oil into solid or semi-solid fat by the interaction between hydrogen gas and the double bonds in liquid oil in the presence of a catalyst (Tarrago-Trani, Phillips, Lemar, & Holden, 2006). The fat produced by hydrogenation can be classified as partially or fully hydrogenated depending on the amount of double bonds that are hydrogenated to saturated bonds. When the hydrogenation process is partially complete, which means only a fraction of the double bond is transformed into the saturated bond, the product is referred as PHOs (Tarrago-Trani et al., 2006). These oils contain *trans* fat, whereas, the complete reduction of the double bond results in fat with 100% saturated fatty acids is referred to fully hydrogenated oil (Tarrago-Trani et al., 2006). Since all the double bonds will be reduced to the single saturated bond, fully hydrogenated fat can be considered as zero *trans* fat with a high amount of saturated fatty acids that could be used to produce shortening (Li et al., 2010).

Although there are new studies that question the health problem of saturated fat, there is evidence that replacing saturated fats with polyunsaturated fat at 5% energy substitution could decrease the risk of coronary heart disease about 10% (Micha & Mozaffarian, 2010; Patel & Dewettinck, 2016). Besides, in order to produce fully hydrogenated oil, more selective catalyst, such as expensive platinum, is required which dramatically raises the price of the products.

An interesterification is an approach that can be used as an alternative to partially hydrogenated fat to reduce or eliminate artificial *trans* fatty acids in the diet (Lee, Akoh, & Lee, 2008). Interesterification is a process where the distribution of fatty acids is rearranged within or between triacylglycerols, resulting in new triacylglycerols without changing the profile of individual fatty acid (Dhaka, Gulia, Ahlawat, & Khatkar, 2011). Many plant oils such as palm, sunflower oil, soybean, and corn oil can be used to produce shortening by interesterification (Waheed, Rasool, & Asghar, 2010). Typically, interesterification could modify the melting and crystallization behavior of the original lipid to a desirable level by either chemical or enzymatic process (Dhaka et al., 2011). During chemical interesterification, the bond between fatty acids and the glycerol backbone will be hydrolyzed first, followed by a random re-esterification onto a glycerol backbone under the catalysis of metal alkoxides (Jimenez-Colmenero et al., 2015; Kadhum & Shamma, 2017). However, the lack of specificity of chemical modification of molecules and the high oil loss have limited the application of chemical interesterification in the food industry (Martins, Cerqueira, Fasolin, Cunha, & Vicente, 2016; Tarrago-Trani et al., 2006). Unlike the chemical interesterification, the enzymatic process can target a specific position of the glycerol backbone instead of random esterification by the use of specific microbial lipase (Jimenez-Colmenero et al., 2015; Tarrago-Trani et al., 2006). For example, 1,3-lipase can be used to hydrolyze the ester bond at the position of sn-1 and sn-3 (Rohm, Sch äper, & Zahn, 2018).

However, the enzymatic process requires longer reaction time than the chemical process, and the lipases are expensive and sensitive to reaction conditions including pH, temperature, and moisture (Rohm et al., 2018). The difficulties of using enzymatic interesterification to massively produce fat with desirable properties have limited its use by industry (Rohm et al., 2018; Tarrago-Trani et al., 2006).

In addition to interesterification, fractionation is another common practice to transform the liquid oil into solid fat. Fractionation is a process that is separating the original oil into different fractions that have different melting and textural properties (Hashempour-Baltork, Torbati, Azadmard-Damirchi, & Savage, 2016). Fractionation is useful to separate plant oils that contain a high amount of saturated fatty acids such as palm oil. Since the melting point of fatty acids is different, when the fat is melted at high temperature and cooled slowly to the temperature lower than high-melting point saturated fatty acids, the high-melting fatty acids will form crystalline materials that can be filtered and separated from the remaining liquid part (Wassell & Young, 2007). The crystalline part can be further used in the manufacturing of fat-based products such as margarine, shortening, ice creams, and whipped topping (Omar et al., 2015). Although these present technologies are useful, some adverse effects such as the increase in saturated and *trans* fatty acid content and higher operation cost have led to a demand of new shortening alternatives (Ölütçü & Yilmaz, 2015). Food scientists are seeking approaches that structure liquid oil into solid fat without the formation of *trans* fatty acids while limiting the formation of saturated fatty acids.

1.4.2. Oleogelation

The major challenges for the food industry to find desirable oil structuring method and decrease the *trans* and saturated fats in the food product include the availability, cost, and the

ability to modify the physical properties while retaining desirable functional characteristics such as texture and appearance of the final food products (Dassanayake, Kodali, & Ueno, 2011). Recently, a novel oil structuring method called oleogelation has been investigated as an alternative method to reduce the amount of *trans* and saturated fats in foods. Oleogelation is a technique that forms liquid oils into a gel structure. It has been used in the pharmaceutical and cosmetic area for decades (Pehlivanoglu et al., 2018). Oleogel, the structured oil from oleogelation process, is defined as a thermo-reversible, self-standing, anhydrous, and viscoelastic material with a three-dimensional network (da Pieve, Calligaris, Co, Nicoli, & Marangoni, 2010). Oleogel is formed by the addition of gelling agent named gelators into liquid oils to transform the liquid oil into a gel-like structure that has similar properties as solid fats (da Pieve et al., 2010; Pehlivanoglu et al., 2018).

One of the most significant advantages of oleogel is that it has solid-like properties without altering the fatty acid profile of liquid oil (Kim, Lim, Lee, Hwang, & Lee, 2017). Another promising advantage of oleogel is that the physical properties of oleogel can be modified by changing the type or concentration of gelators, which means it is easy to produce oleogel with varying physical properties that can satisfy the wide spectrum of requirements for different food products (da Pieve et al., 2010). The application of oleogels in food products can decrease the level of saturated fat and eliminate *trans* fat in food, thus avoiding some adverse effects that are caused by high consumption of saturated and *trans* fatty acids (Pehlivanoglu et al., 2018).

Among manufacturing factors, both oil and gelator types are crucial to determine the physical properties of oleogel. Edible oleogels should be prepared by edible oils such as plant oils. Plant oils are usually extracted from oilseeds, and they are used as the raw materials to

produce fat-based products such as frying oil, shortening, and margarine (Ghazani & Marangoni, 2013). The commercially available plant oils are usually refined oils. Historically, olive oil, linseed oil, and other expeller-pressed oil were used without refining process (Vaisali, Charanyaa, Belur, & Regupathi, 2015). However, the development of the food oil industry has led to plant oils being refined before sale to the consumers. Unrefined plant oils, also called crude oil, contain minor components such as phospholipids, free fatty acids, polyphenols, carotenes, waxes, and vitamins in addition to the predominant triacylglycerols (Ghazani & Marangoni, 2013; Pudel et al., 2011). The refining process is used to remove undesirable minor components such as phospholipid, free fatty acids, and color pigments that may have adverse effects on the appearance and stability of oil (Pudel et al., 2011). Unfortunately, the refining process will cause oil loss and it not only removes undesirable minor components but also remove components that have benefits such as tocopherols, polyphenols, and phytosterols (Ghazani, Garc á-Llatas, & Marangoni, 2013; Tasan & Demirci, 2005).

Moreover, refined edible oil may contain 3-monochloropropane diol (3-MCPD) and glycidyl esters (GE), both of which are processing contaminants produced during oil refining, specifically during high temperature deodorization. Refined edible oil, particularly palm oil, is frequently blamed as the culprit responsible for the presence of 3-MCPD in baked goods (Custodio-Mendoza et al., 2019). Meanwhile, several studies reported that a synergistic effect existed when adding lecithin to the oleogel made with fruit wax or stearic acid at a certain ratio (Gaudino, Ghazani, Clark, Marangoni, & Acevedo, 2019; Okuro et al., 2018). Furthermore, it has been reported that addition of β -carotene enhances the rheological property and oil-binding capacity of beeswax oleogels (Martins, Cerqueira, Cunha, & Vicente, 2017). Since edible crude oil has not undergone a thermal process, the level of 3-MCPD is anticipated to be scarce. The

enrichment of minor functional constituents such as lecithin and carotene may strengthen the oleogel with the added gelators when using edible crude oil to fabricate oleogel.

In addition to oil processing, the type or the source of oil also influence the properties of oleogel. It has been reported that the type of oil is important when preparing oleogel because it is associated with the appearance, thermal, rheological, strength, and oil-binding capacity of the oleogel (Doan, Tavernier, Okuro, & Dewettinck, 2018; Fayaz, Goli, & Kadivar, 2017; Pehlivanoglu et al., 2018; S. Yang et al., 2018). Several plant oils such as soybean oil, corn oil, sunflower oil, olive oil, and rice bran oil have been used to prepare oleogels. These plant oils contain different fatty acid profiles or composition, thus affecting the properties of oleogels. For example, Yang and coworkers investigated the influence of oil type by preparing oleogel with β -Sitosterol (BS) and stearic acid in four different oils including olive oil, corn oil, sunflower oil, and flaxseed oil (Yang et al., 2018). Their results indicated that the oleogel prepared with different oils have varying color and microstructures (Yang et al., 2018). Moreover, the flaxseed oil oleogel have relative lower oil loss and higher firmness compared to oleogel prepared with other liquid oils (Yang et al., 2018). Fayaz and coworkers also reported that oleogel prepared with canola oil had lower oil-binding capacity than oleogel prepared with sesame, sunflower, and flaxseed oils (Fayaz et al., 2017). The viscosity and textural properties of oleogel made with rice bran wax (RBX) were highly related to oil types since oils containing saturated and high melting fatty acids have been found to produce oleogel with higher viscosity and harder texture (Pehlivanoglu et al., 2018). Besides textural properties, the composition of oil also should be considered. From the health aspect, plant oils such as soybean oil, sunflower oil, corn oils, rice bran oil, and safflower oil can be used to prepare oleogels because they contain high levels of polyunsaturated fatty acids (Dorni et al., 2018; Pehlivanoglu et al., 2018).

In addition to oil types, the gelator also plays an important role in the formation of oleogels. An ideal gelator should be food-grade, economical, efficient at a low concentration, and have the ability to imitate the property of solid fat (Marangoni, 2012). Several gelators have been studied including ethylcellulose, plant waxes, fatty acids and alcohols, monoacylglycerides, phytosterol mixtures with γ -oryzanol, ceramide, lecithin, sorbitan monostearate, proteins, and so on (Davidovich-Pinhas et al., 2016). The gelators can be classified into different categories based on different chemistry or functionality. For example, based on the chemical composition of gelators, they can be classified as lipid-based or non-lipid gelator while based on the molecular characteristics, they can be classified into low molecular weight and polymeric gelators (Patel & Dewettinck, 2016). Moreover, regarding to the number of gelators used to prepare oleogel, the gelators can be a mono-component or mixed system (Patel & Dewettinck, 2016).

A number of food-grade gelators, including monoacylglycerides, lecithin, plant/insect waxes, fatty acids/ fatty alcohols, phytosterol and oryzanol, ceramides, and ethylcellulose, have been successfully used to form oleogels with variable physical properties (Hwang, Kim, Singh, Winkler-Moser, & Liu, 2012). Among the different gelators, natural waxes are considered to be the most promising gelators because they are inexpensive, commercially available, and have the ability to form a three-dimensional network at a concentration as low as 1-4 wt% (Wang, Gravelle, Blake, & Marangoni, 2016). Based on the different origin, the wax gelators are comprised of varying fractions of n-alkanes, fatty alcohols, and fatty acids, and the proportions of these constituents have a critical role on gelation (Singh et al., 2017). Several wax gelators such as rice bran wax (RBX), sunflower wax (SW), beeswax, candelilla wax, and carnauba wax have been reported in the literature (Davidovich-Pinhas et al., 2016). For instance, rice bran wax, a byproduct of rice milling, has been reported to structure rice bran oil and olive oil into oleogels

at a critical concentration as low as 0.5 and 1 wt%, respectively (Dassanayake, Kodali, Ueno, & Sato, 2009; Wijarnprecha, Aryusuk, Santiwattana, Sonwai, & Rousseau, 2018). Also, candelilla wax can structure safflower oil and canola oil at the concentration of 1 and 2 wt%, respectively (Singh et al., 2017). Sunflower wax (SW), consisting of wax esters, fatty acids, fatty alcohols, and hydrocarbons, has been reported as a good gelator for structuring soybean oil at concentrations as low as 0.3–0.5 wt% (Hwang, Kim, Evans, Koga, & Lee, 2015). Moreover, beeswax, an approved food additive, also has been reported to be a good gelator to prepare oleogels with olive oil (Öğütçü & Yılmaz, 2014).

In addition, monoacylglycerides (MAG) has been extensively highlighted as a potential gelator because it has been commonly used as an emulsifier in the food industry, and more importantly, it can form an elastic gel immediately after crystallization (da Silva, Arellano, & Martini, 2018). Moreover, a recent study suggested that MAG may have positive health effects by increasing the absorption of n-3 long-chain PUFAs compared with triacylglycerol (Cruz-Hernandez et al., 2016). The oleogelation with MAG occurs in two steps: (1) an α -crystal or a mixed inverse lamellar phase is formed and (2) further cooling result in the formation of a sub- α -crystal at a temperature around 13 °C (Sintang, Rimaux, Van de Walle, Dewettinck, & Patel, 2017; D. X. Yang, Chen, & Yang, 2018). The morphology of MAG oleogels has been reported as needle-like in olive oil and spherulitic in corn oil, which indicated the type of oil would also affect the crystal structure of MAG oleogels (Marangoni, 2012).

Phytosterols are another category of gelators that have been well documented (Matheson, Dalkas, Clegg, & Euston, 2018). Phytosterols such as BS, cholesterol, and dehydrocholesterol are usually used together with γ -oryzanol to form oleogel since they could not form oleogel efficiently when used individually (Pernetti, van Malssen, Flöter, & Bot, 2007; Singh et al.,

2017). β -sitosterol is one of the phytosterols that naturally presents in crude plant oils that have potential health benefits such as anticancer possibility and the reduction in LDL-cholesterol (Lei et al., 2017; Mahaddalkar, Suri, Naik, & Lopus, 2015). When combined BS with γ -oryzanol at a ratio of 1:1, they can form a strong gel that has high firmness and melting point (Marangoni, 2012). β -sitosterol and γ -oryzanol formed a tubular microstructure in sunflower oil that has a diameter of 6.7-8.0 nm, which result in a transparent oleogel (Bot, den Adel, Roijers, & Regkos, 2009). The tubular microstructure immobilized the sunflower oil both inside and outside the tube (Bot et al., 2009). The transparency, firmness, and melting behavior of BS and γ -oryzanol oleogel are affected by the ratio of BS and γ -oryzanol present (Sawalha et al., 2015).

The overall goal of this research is to develop a novel and healthy shortening by fabricating oleogels using crude edible oils (solvent extracted soybean oil and expeller-pressed corn germ oil). The physical properties of cookies prepared by different oleogels were also determined.

CHAPTER 2. SOYBEAN OLEOGELS PREPARED WITH MONOACYLGLYCERIDES (MAG) AND/OR BETA- SITOSTEROL (BS)

2.1. Abstract

Both crude and refined soybean oil oleogel was formed with β -sitosterol (BS) or monoacylglycerides (MAG), or the combination of both (1:1) at a fixed concentration of 10 wt%. The thermal behavior of oleogels was measured using differential scanning calorimetry (DSC). The crystal structure and morphology of oleogels was characterized using X-ray diffraction (XRD) and polarized light microscopy (PLM). The hardness of oleogel and commercial vegetable shortening was compared using a texture analyzer. The characteristics of cookies made with oleogels were compared with cookies made with commercial vegetable shortening. Overall, the incorporation of BS and/or MAG into crude and refined soybean oil can produce oleogel with solid-like properties. Refined soybean oil formed stronger and firmer oleogels as compared to crude soybean oil. Refined soybean oil (RSO) structured by BS presented branched fiber-like, elongated plate-like, and needle-like crystals while it gelled by MAG contained spherulite crystals. RSO made with the combination of BS and MAG adapted crystal morphologies from both BS and MAG. The same crystal morphologies were observed in crude soybean oil (CSO) with fewer quantities. Comparing the quality of cookies made with oleogels and commercial vegetable shortening, equal or better performance of both RSO and CSO on weight, thickness, width, spread ratio, and hardness of cookies than that of commercial vegetable shortening was observed. By combining the results of the physical characterization and cookie making performance, it can be concluded that both crude and refined soybean oleogels could resemble

commercial shortening, which offers the possibility of using oleogel to replace shortening in the baking industry.

2.2. Introduction

Under the new regulation from the FDA, partially hydrogenated oils (PHOs), the core source to manufacture shortening, are no longer “Generally Recognized as Safe” (GRAS) and cannot be added to foods after June 18, 2018 (FDA, 2018). However, it is difficult to find an ideal product to fully replace PHO in the food industry, so FDA is extending the compliance date to January 1, 2020 to allow for an orderly transition of the majority of PHO-contained products made before Jun 18, 2018 (FDA, 2018). In order to comply with the new regulation, the baking industry replaced PHOs with palm and coconut oil. However, the Dietary Guidelines for Americans (2015-2020) and the new Scientific Report of the Dietary Guidelines committee (2015) both recommend that consumers increase consumption of polyunsaturated fats and decrease consumption of saturated fats to decrease risk for coronary heart disease (CHD) (U.S. Department of Health and Human Services, 2015). The association of high dietary saturated fat with increased risk of CHD suggests that improving the nutritional profiles of foods by substituting their saturated fat with unsaturated fat could have an important and positive impact on consumer health (Lucan, 2016). Certainly, palm or coconut oil is not a promising answer for PHO replacement since both oils contain extremely high amounts of saturated fats. As a matter of course, food manufacturers and scientists are seeking alternative approaches that can replace PHOs and produce shortening that can mimic the physical properties of PHOs-based shortening while maintaining the balanced fatty acid profile.

Currently, it has become possible to transform liquid oil into semi-solid oleogels in the presence of gelling agents, named gelators. Although oleogels show solid-like properties similar

to PHOs, they contain high amounts of unsaturated fatty acids and low amounts of saturated fatty acids, which is considered as a significant advantage by contributing to the decrease of saturated fat in the diet (Kim et al., 2017). The essence to produce oleogels is the selection of appropriate gelators with a low amount, typically less than 10%. In recent years, a handful of gelators, including fatty acids, fatty alcohols, triacylglycerol, diacylglycerol, monoacylglycerides, waxes, lecithin, and phytosterols, have been applied to fabricate oleogels with variable functionalities (Wang et al., 2016). Among them, monoacylglycerides (MAG) has been extensively highlighted as a potential gelator because it has been commonly used as an emulsifier in the food industries, and more importantly, it can form an elastic gel immediately after crystallization (da Silva et al., 2018). Moreover, MAG may have positive health effects by increasing the absorption of n-3 long-chain PUFAs compared with triacylglycerol alone (Cruz-Hernandez et al., 2016). Phytosterols are another category of gelators that have been well documented (Matheson et al., 2018). β -sitosterol is one of the phytosterols that naturally exists in crude plant oils to offer potential health benefits such as anticancer possibility and the reduction in LDL-cholesterol (Lei et al., 2017; Mahaddalkar et al., 2015).

Although several studies have used MAG or BS as gelator to produce oleogels, there is limited research combine MAG with BS to form oleogel (Lupi et al., 2018; Truong, Prakash, & Bhandari, 2019; Zychowski et al., 2018). Moreover, the research dealing with oleogel formation for food applications used refined edible oils with no exceptions. Refined edible oil may contain 3-monochloropropane diol (3-MCPD) and glycidyl esters (GE), both of which are processing contaminants produced during oil refining. Refined edible oil, particularly palm oil, is frequently blamed as the culprit responsible for the presence of 3-MCPD in baked goods (Custodio-Mendoza et al., 2019). Meanwhile, several researchers reported that a synergistic effect existed

when adding lecithin to the oleogel made with fruit wax or stearic acid at a certain ratio (Gaudino et al., 2019; Okuro et al., 2018). Since edible crude oil has not undergone significant thermal processing, the level of 3-MCPD is anticipated to be scarce. The enrichment of functional minor constituents, such as lecithin, may exert a synergistic effect with the added gelators when using edible crude oil to fabricate oleogel.

The aim of this study is to fabricate crude soybean oil (CSO) oleogels using BS and/or MAG and comparing their physical properties and cookie-making performance with the oleogels prepared using refined soybean oil (RSO). In particular, the thermal and physical characteristics of oleogels were investigated using differential scanning calorimetry (DSC), rheometer, x-ray diffraction (XRD), polarized light microscopy (PLM), and texture analysis. As a common aerated baked good, cookies were made with oleogels and the physical properties of which were compared with that made with commercial vegetable shortening.

2.3. Materials and methods

2.3.1. Chemicals

Commercial soybean oil, all-purpose flour, Crisco[®] all-vegetable shortening, sugar, salt, baking soda, and dextrose were purchased locally. To prepare crude soybean oil, soybean seeds were cracked using a hammer mill, followed by hexane extraction using a pilot scale explosive-proof solvent extraction unit (Armfield model FT29; Ringwood Hampshire, England). The solvent in the crude soybean oil was removed by nitrogen sparging under vacuum. Distilled monoacylglycerides of vegetable oil (MAG, Dimodan[®] HP K-A) was a gift from DuPont Nutrition and Health (New Century, KS, USA). Beta-sitosterol was purchased from TCI America (Portland, OR, USA). All other chemicals were purchased from VWR International (West Chester, PA, USA).

2.3.2. Preparation of soybean oil oleogels

Oleogels were prepared by using either BS or MAG, or the combination of both at an equal ratio. The final concentration of gelator in oleogels was 10 wt%. The gelator was added into glass beakers containing crude or refined soybean oil, and the mixture was heated above the melting temperature (140 °C) under continuous stirring (300 rpm) to ensure they were fully melted. The homogenous liquid oil mixture was cooled at room temperature for 20 min and stored at 5 °C for 7 days before characterization. Samples containing 10 wt% BS, 5 wt% BS and 5 wt% MAG, and 10 wt% MAG in crude soybean oil were labeled as CSO-1, CSO-2, and CSO-3, respectively; while in refined soybean oil were labeled as RSO-1, RSO-2, and RSO-3, respectively. To measure the physical properties of oleogel, the oleogel (20g) was prepared in duplicates for further analysis. To prepare cookies, the oleogel (64g) were made in three separate batches, which constituted a replication. Each of these batches was then used to make separate batches of cookies which were then analyzed.

2.3.3. Thermal properties of soybean oil oleogels

The thermal properties of oleogels were measured by using differential scanning calorimeter (DSC Q-2000, TA Instruments Ltd., New Castle, DE, USA). Briefly, 8-10 mg sample was loaded into an aluminum pan and hermetically sealed. The reference was an empty aluminum pan. The temperature was increased to 120 °C and maintained for 10 min, then cooled to 0 °C using a cooling rate of 10 °C/min. After 5 min at 0 °C, the system was again heated at 10 °C/min up to 100 °C. Peak crystallization and melting temperatures were determined from the cooling and heating thermogram. These parameters were analyzed by Advantage/Universal Analysis (UA) Software (TA Instruments Ltd., New Castle, DE, USA).

2.3.4. Rheological properties of soybean oil oleogels

Rheological measurements were carried out on a Discovery Hybrid Rheometer-2 rheometer (TA Instruments Ltd., New Castle, DE, USA) with a 2° cone plate (40 mm diameter, 57 μm gap). Strain sweep tests at a frequency of 1 Hz were performed to determine the linear viscoelastic region (LVR). LVR shows the region where the structure of sample is undisrupted. Small-amplitude oscillatory shear (SAOS) measurements were performed inside the LVR regime in a frequency range between 10⁻¹ and 10² Hz at 25 °C with 9 points per decade. Oleogels were tested immediately after they were taken out from the refrigerator, and at least two replications were performed. The elastic (G') and viscous (G'') modules were recorded by the software TRIOS (TA Instruments Ltd., New Castle, DE, USA).

2.3.5. Crystalline structure of soybean oil oleogels

The molecular organization of the oleogels was determined by XRD spectroscopy using a Rigaku Ultima IV X-ray diffractometer (Austin, Texas, USA) with Cu-Kα operating at 40 kV and 44 mA. Samples were scanned at 2θ range from 2 to 30° at a rate of 0.6 °/min and a step size of 0.05 °.

2.3.6. Morphology of soybean oil oleogels

Crystal morphology was observed under polarized light using an Olympus BX51 polarized light microscope (PLM) (Olympus Optical Co., Ltd., Japan). A small amount of the oleogel was placed on a glass slide and covered with a cover slide. The slide was then mounted under the microscope and visualized using a 20 × objective lens under the crossed polarizer and analyzer.

2.3.7. Firmness of soybean oil oleogels

A puncture test was applied to investigate the firmness of oleogels, which was compared with that of commercial vegetable shortening. Based on the method of Hwang et al. (2014), the oleogel (14 g) in vial was placed on the platform of a texture analyzer (TA.XT plus, Stable Microsystems, Surrey, UK) with a 2 kg compression load cell. A ball probe (TA-18A, ¾ inch diameter stainless steel ball) was then lowered 10 mm into the oleogel samples at a crosshead speed of 1 mm/sec at room temperature. The maximum force (N) that is a measure of firmness was recorded from the plot of force versus penetration distance.

2.3.8. Preparation of cookie using soybean oil oleogels

Cookie making was carried out according to the AACC method (AACC 10-50.05, 2000) and prepared using a cookie kit (National Mfg., Lincoln, NE, USA). The baking formula was comprised of flour 225g (14% moisture basis), sugar 130 g, salt 2.1 g, sodium bicarbonate 2.5 g, dextrose solution 33 g, distilled water 25 g, and shortening/soybean oleogel 64 g. The dough was divided into six portions, placed on a greased aluminum cookie sheet (2 mm thickness) and lightly flattened. Two metal gauge strips (7 mm thickness) were anchored on each side of the cookie sheet and the dough was rolled to thickness using one forward and one return stroke with a rolling pin. The dough was then cut using an aluminum cutter (60 mm inside diameter) and placed onto an aluminum baking sheet. Excess dough was removed and discarded. Cookies were baked in a Baxter mini rotating rack oven (OV 300E, Baxter Corp, Orting, WA, USA) at 205 °C (400 °F) for 10 min. Upon removal from the oven, the cookies were lifted from baking sheets and cooled for 30 min on nonstick racks, after which were wrapped with polypropylene bags and kept at room temperature until further analysis.

2.3.9. Characterization of cookies

The width (W) and thickness (T) of cookies were measured with a measuring grid (National Mfg., Lincoln, NE, USA) 1 h after baking and spread ratio (W/T) was then calculated. The width of cookies was measured by place three cookies side by side on the grid and calculate the mean. The thickness of cookie was measured by piling up six cookies together and then lying the piled cookies down on the measuring grid. The mean thickness was calculated. Cookies were stored at room temperature (20 °C) in a sealed plastic bag for further measurements. Cookie hardness was measured 24 h after baking using a texture analyzer (TA.XT plus, Stable Microsystems, Surrey, UK) equipped with a 2 kg load cell, using a knife blade 3mm thick probe. A compression was applied at a speed of 1 mm/s until the cookie was broken. The maximum force required to break the cookie was recorded as hardness and measured as Newtons (N).

2.3.10. Statistical analysis

Two batches of oleogel (20g) were made to evaluate the physical properties of oleogels. The results for physical properties of oleogel include DSC, rheological, XRD, and PLM were performed in duplicate. Three batches of oleogel (64g) were prepared to make three separate batches of cookies, and each batch was constituted a replication. The hardness of these three oleogel replications and shortening and all the cookie characteristics were measured in triplicate using fresh samples. The values were expressed as means of duplicates or triplicates from each of independent experiments, and \pm SD was also calculated for triplicate experiments. The data were statistically analyzed using SAS version 9.4 (SAS Institute Inc. Cary, NC). One-way analysis of variance (ANOVA) was conducted, and a significant difference was defined at $p < 0.05$ by Tukey's test.

2.4. Results and discussion

2.4.1. Formation of oleogels

After storage at 5 °C for 7 days, the oleogel samples were taken from the incubator and placed upside down at room temperature to observe the gel formation and visual appearance (Figure 1A&B). Initially, self-standing solid-like structures were formed in all 6 soybean oleogels (Figure 1A). The evenness of the oleogel samples was different. The oleogel samples showed consistent structure except for RSO-1 and CSO-1 which gelled with 10 wt% BS and spotted clusters were present. In general, the oleogel is formed from the bottom of the tube. However, we observed that soybean oil oleogels (RSO-1 and CSO-1) made with 10 wt% BS solidified initially as small clusters and then aggregated together after the formation of several clusters. Yang et al. (2018) reported similar results for crystals of oleogel formed by BS alone tended to agglomerate and settle, resulting in an unstable structure and leading to a release of liquid oil even after a short storage period. After storage at room temperature for 4 hours, crude soybean oil oleogel (CSO-2) made with the mixture of BS and MAG displayed phase separation and started to flow (Figure 1B). As refined soybean oil (RSO-2) made with the same combination of gelators was stable at room temperature, the additional minor constituents in CSO that typically are removed during the refining process of RSO likely diminished the gelling capacity of either BS or MAG at this concentration and accelerated the collapse of the gel network. A physically stable crude soybean oil oleogel can be formed when 10 wt% of gelator (BS or MAG) was used.

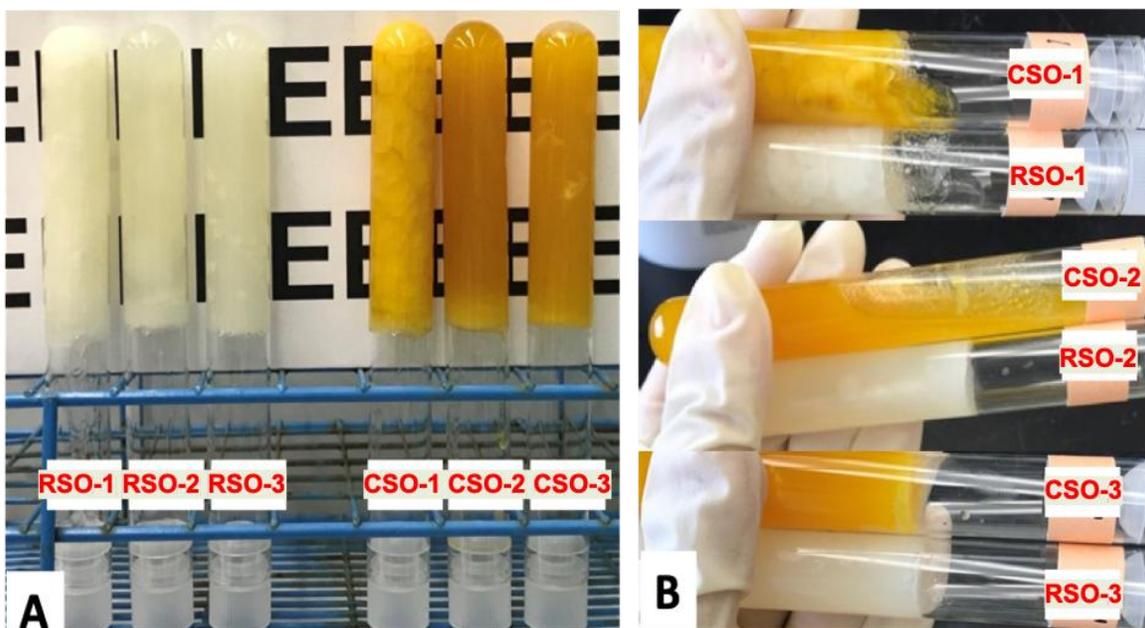


Figure 1. Oleogel samples prepared with crude (CSO) and refined soybean oil (RSO) after storage at 5 °C for 7 days and set at room temperature for (A) 30 mins, and (B) 4 hrs after removal from the incubator (1, 2, and 3 denote 10 wt% β -sitosterol+ 5 wt% monoacylglycerides, and 10 wt% monoacylglycerides, respectively)

In addition to the appearance, the color of oleogels was also different among the 6 samples. Crude soybean oil oleogels (CSO-1, CSO-2, and CSO-3) was an orange color while refined soybean oil oleogels (RSO-1, RSO-2, and RSO-3) was an opaque white gel. The color difference is caused by the presence of carotenoids in crude soybean oil that are depleted in refined soybean oil during oil refining/bleaching process. The naturally existing orange color oleogel may provide unique applications in certain foods.

2.4.2. Thermal characterization

To investigate the impact of crude and refined soybean oil on the thermal behavior of oleogels, DSC was conducted to obtain the peak temperature of oleogels during crystallization and melting process (Figure 2). Pure β -sitosterol and MAG showed a single exothermic peak at 119.2 °C and 51.0 °C during crystallization, and an endothermic peak at 138.9 °C and 53.6 °C during melting, respectively (Figure 2A). In a previous study, the melting temperature of pure

MAG from the same company was 71.5 °C (Wang, Decker, Rao, & Chen, 2019). Such discrepancy might be caused by different sources to produce MAG.

There was a significant difference between crude and refined soybean oil oleogels in terms of melting and crystallization temperatures and enthalpy (Figure 2A&B). In general, RSO oleogels had relative higher onset temperature, peak temperature, and enthalpy as compared to CSO oleogels during both crystallization and melting processes. RSO-1 and CSO-1, which was made with 10 wt% BS, only had a broad peak area during crystallization. It was reported in several previous studies that no exothermic peaks existed for oleogels made with sitosterol (Yang et al., 2017, 2018). RSO-3 and CSO-3 that containing 10 wt% of pure MAG displayed two thermal behaviors during crystallization and melting. Particularly, CSO-3 had major peaks at 10.2 °C and 48.4 °C during crystallization, and 11.7 °C and 57.6 °C during melting; while RSO-3 also had two structures crystallizing at 12.3 °C and 51.2 °C and melting at 13.2 °C and 56.1 °C. It is widely recognized that MAG initially forms α -crystal or a mixed inverse lamellar structure and then converts to sub- α -crystal at a temperature around 13 °C, which is close to the crystallization of aliphatic chains (Sintang, Danthine, et al., 2017; Sintang, Rimaux, et al., 2017; Yang et al., 2018). In this study, both oleogels made with 10 wt% MAG had one peak at a lower temperature close to 13 °C, which corroborated the presence of sub- α -crystals.

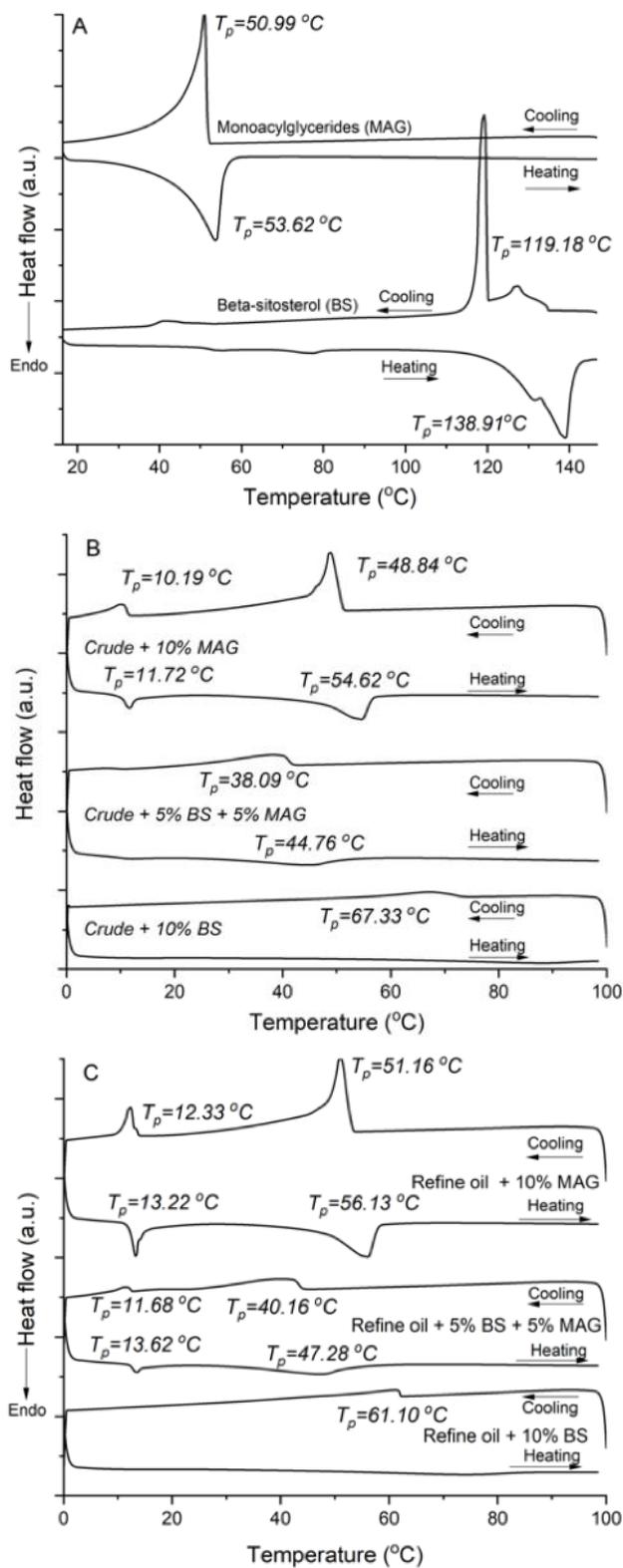


Figure 2. Differential Scanning Calorimetry heating and cooling flow curves for (A) β -sitosterol and monoacylglycerides; (B) oleogels made with crude soybean oil, and (C) oleogels made with refined soybean oil

When BS was mixed with MAG, the peak crystallization and melting temperature for both systems were decreased, presumably because of the addition of BS antagonized the crystal structure of MAG. This was consistent with the appearance study that the oleogel made with MAG crystallized faster than that made with the mixture of MAG and BS (Sintang, Danthine, et al., 2017). Even more surprising, the disappearance of peak at the lower temperature in CSO-2 corresponding to sub- α -crystal was registered on thermograph (Figure 2B), suggesting the minor constituents in crude soybean oil could potentially abrogate this structure. The commercial shortening we used for comparison has a melting onset temperature around 34 °C and a peak temperature around 42 °C. Oleogels prepared by MAG had at least one parameter close to the thermal behavior of the commercial shortening, which indicates four oleogels have the potential to be used as a replacement for commercial shortening.

2.4.3. Rheological measurements

A frequency sweep test was conducted to measure the elastic modulus (G') and the viscous modulus (G'') of soybean oil oleogels (Figure 3). Overall, G' was higher than the G'' for all the oleogels and both increased slightly but gradually over the entire frequency range. This result implied that all soybean oleogels were viscoelastic semi-solids with good tolerance to deformation. A similar result was reported by Yang and coworkers that BS and steric acid-based gels were true gels because they have higher G' than G'' (Yang et al., 2017). Moreover, they also reported that oleogel with a positive slope of elastic modulus and loss modulus with the increase in frequency had solid-like behavior and characteristics.

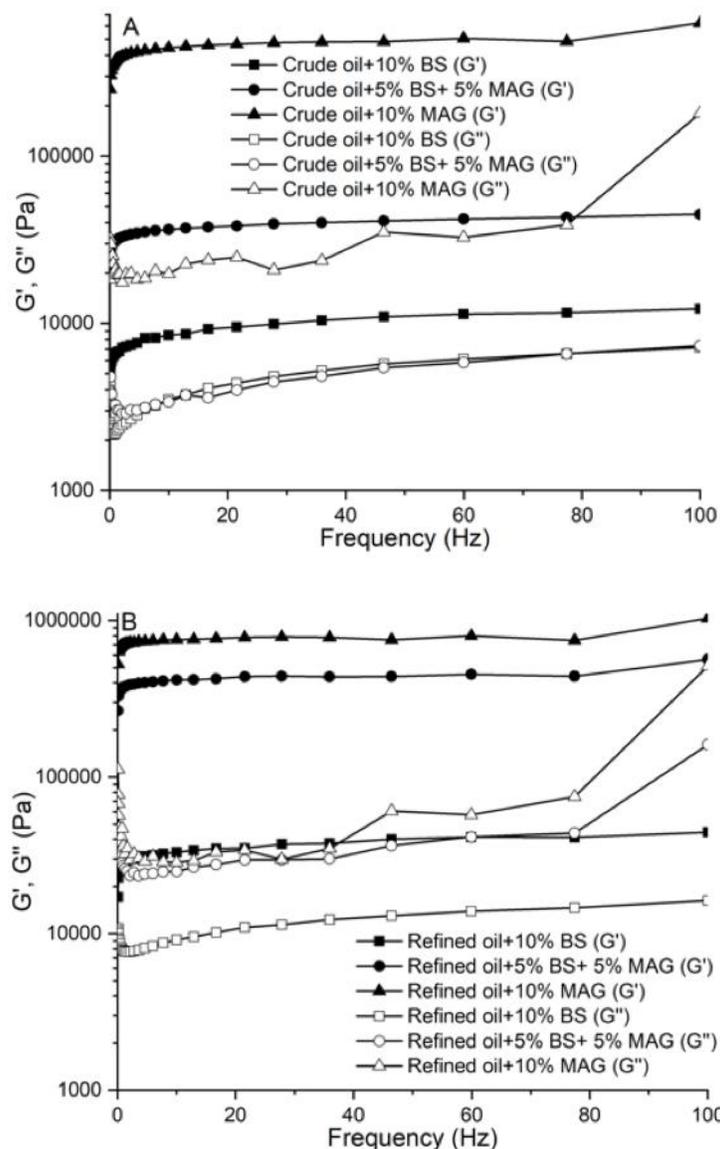


Figure 3. Frequency dependence of elastic modulus (G') and viscous modulus (G'') for oleogels made with (A) crude soybean oil, and (B) refined soybean oil at 25 °C

When comparing the viscoelastic properties of CSO oleogels with RSO oleogels, all RSO oleogels had higher G' and G'' than the oleogels made with CSO, indicating that the same type and concentration of gelator build up stronger and firmer gel networks in RSO rather than CSO. This result countered our hypothesis that synergistic effect might exist when using crude oil to form oleogel but explained the reason why CSO-2 melted after storage at room temperature in a short period of time. Contrary to our hypothesis, CSO produced a weaker or looser gel in which

the structure was unstable as compared with that of RSO oleogel. This was supported by the collapsed CSO-2 structure leading to liquid in the test tube while RSO-2 structure remained after the oleogels were removed from the incubator. The hardness of oleogel, an indicator for gel strength, also confirmed this result (Figure 4). Among all the samples, oleogel formed using refined soybean oil were harder than oleogels made by crude soybean oil. The hardness of RSO-3 was 6.35 N, three times higher than commercial vegetable shortening. In order to form a stable crude soybean oil oleogel, higher gelator concentration was required.

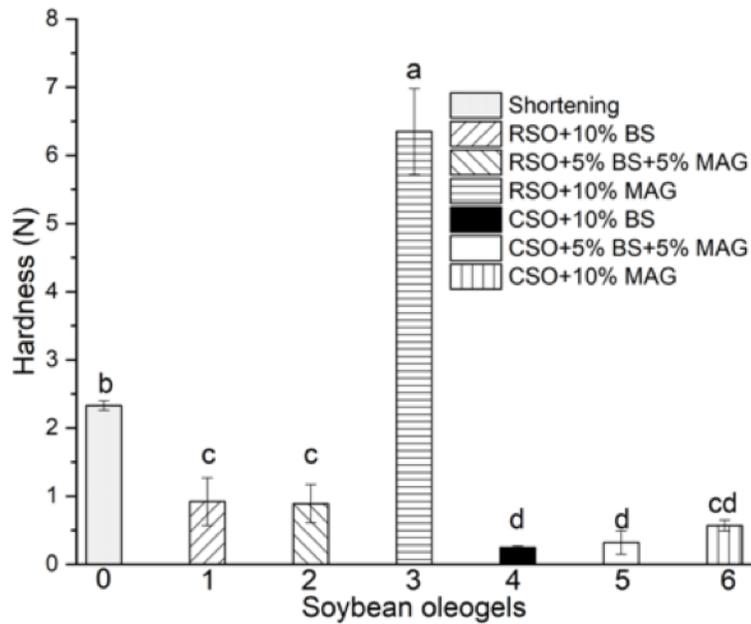


Figure 4. Mechanical property, hardness (N), of oleogels made with crude and refined soybean oil

In addition to soybean oil type, gelator also imparted the viscoelastic properties to oleogels. For instance, soybean oleogel made with MAG as the sole gelator had the highest G' and G'' , which indicated MAG in single gelator system formed the strongest gel compared to the oleogel made with either BS alone or the combination of both MAG and BS at an equivalent concentration (10 wt%). The hardness of oleogels made with MAG was also higher than their counterparts in both types of oils (Figure 4). Typically, crystal structures that have higher G' tend

to form a large amount of crystals with small sizes which might be the case in RSO-3 (da Silva et al., 2018). Moreover, a higher G' is also associated with the increased firmness and compactness of the oleogel and likely caused the higher hardness of oleogel made with MAG only compared to those made with combined gelators or BS alone.

2.4.4. X-Ray diffraction

X-ray diffraction (XRD) was performed to study the polymorphism of oleogel samples, and the d-spacing of the crystal was calculated based on 2θ (Figure 5). For all the peaks resolved in the XRD pattern, the intensity of each individual peak in oleogels made with refined soybean oil was higher than that with crude soybean oils. Higher intensity of the long spacing peaks for oleogel prepared with refined soybean oil implied higher self-sorting and rearrangement of the gelator molecules, which resulted in more crystalline formation (Yang et al., 2017). The XRD results strengthened the evidence for the antagonistic role of crude soybean oil on oleogel formation, presumably resulting from certain minor constituents it contains. All the oleogel samples had a broad peak at angles in the region of 2.3 - 19.7° (2θ), corresponding to d-spacing of 4.5 - 38.4 \AA . The broadness of the peak was associated with the amorphous scattering of liquid-state soybean oil triacylglycerol molecules that comprised 90 wt% of the oleogel. For oleogel made by 10 wt% MAG, a single diffraction peak at 2.1° was noticed, pinpointing the inter-planar distance of MAG bilayers with an approximate width of 42 \AA . A main peak corresponding to a distance of 4.5 \AA with a number of other peaks (3.9 and 4.36 \AA) along the shoulder of the amorphous band at short spacing was also observed. These peaks resembled a β polymorphic form by the in-plane ordering of MAG aliphatic chains, which was in agreement with the reports of previous studies (da Pieve et al., 2010; Dassanayake et al., 2011; Sintang, Danthine, et al., 2017).

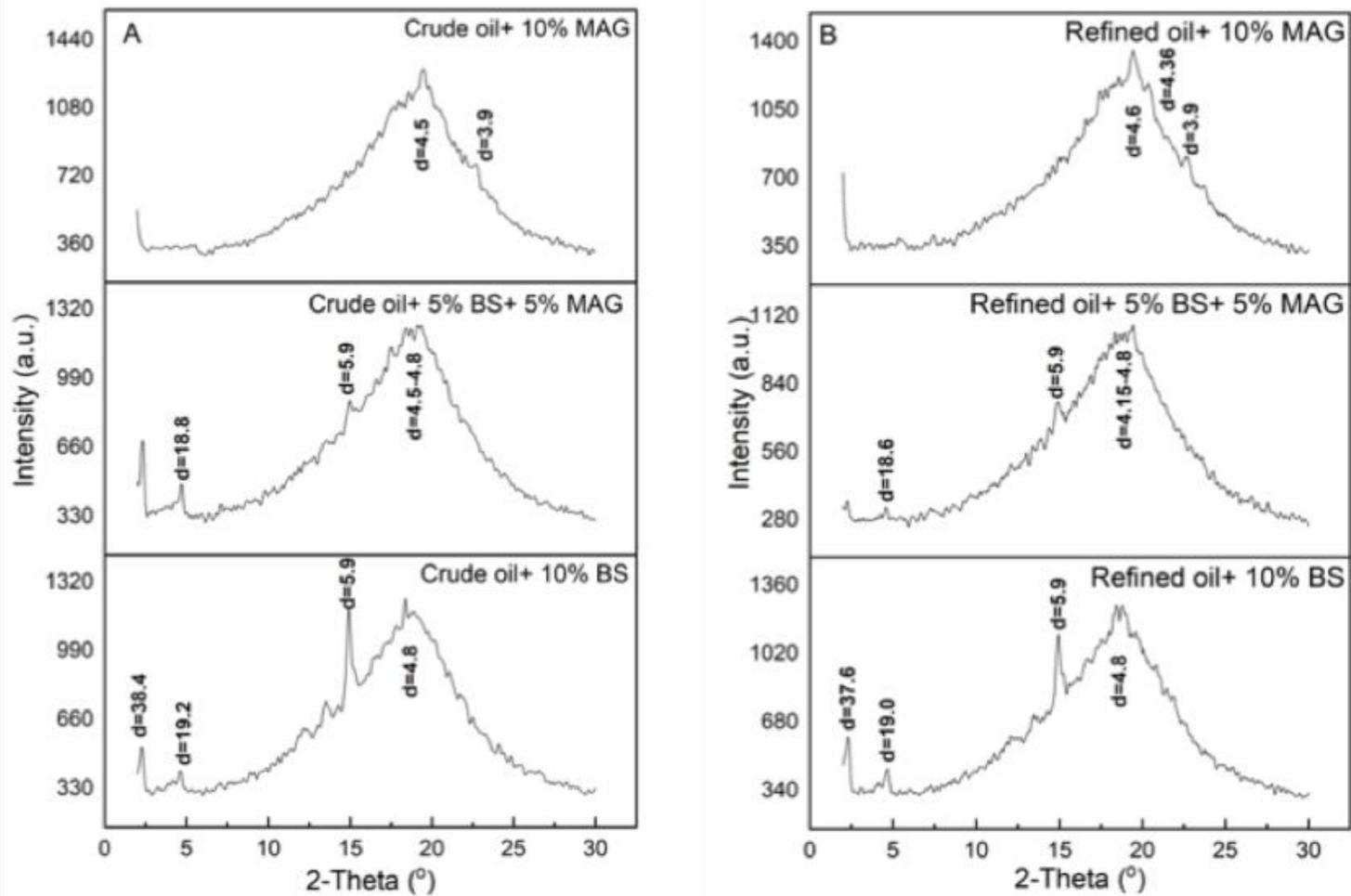


Figure 5. X-Ray Diffraction patterns of oleogels made with (A) crude soybean oil, and (B) refined soybean oil

For the oleogel samples made with 10 wt% BS, two additional peaks occurred around 4.7° and 15° , corresponding to d-spacing of 18.8 and 5.9 Å, respectively. These two peaks were exclusively assigned to BS as both are the representative reflections of pure BS crystal forms in x-ray pattern. Pure BS exists in three different crystal forms (i.e., anhydrous, hemi-hydrated, and monohydrated crystals), and their x-ray diffraction patterns are very similar, including the two peaks found in our study (Christiansen, Rantanen, von Bonsdorff, Karjalainen, & Yliruusi, 2002). Pure BS crystals form a double layer structure, where two opposing molecules face each other with the hydroxyl groups (Ribeiro et al., 2016). In the oleogels made with the mixture of 5 wt% BS and 5 wt% MAG, the distinct peaks for BS were still retained, although the intensity was reduced. On the other hand, the small peaks corresponding to β -polymorphic in both CSO-3 and RSO-3 were dissipated. Unlike other multi-components gelator system, our findings confirmed that the rearrangement of soybean oil oleogel molecules and polymorph transition was not formed by the combination of both BS and MAG. Instead, they could interrupt the preexisted crystal structures, thus reducing the stability of oleogels.

2.4.5. Crystal morphology

To gain better visualization of crystalline structure in both crude and refined soybean oil oleogels, the polarized light microscopy (PLM) was used to visualize the microstructure of oleogels (Figure 6). Although both RSO-1 and CSO-1 were prepared using 10 wt% BS as a sole gelator, more than one morphology was recorded, including needle-like and irregular lath crystals (labeled arrows in Figure 6). This was in accordance with previous reports that thinner and smaller sized needle-like crystals were obtained in BS-based oleogels (Vaikousi, Lazaridou, Biliaderis, & Zawistowski, 2007). It was reported that three different morphologies consisting of needlelike, plate, and spherulite crystals appeared in oleogels that were made with phytosterol

only (Sintang, Danthine, et al., 2017). The irregular lath or plate crystals might be due to the quick crystallization of pure BS, thus maintaining its double-layer structure as evidenced by XRD pattern (Figure 5). The possible reason for forming other morphology could be the different orientation of crystalline platelets. Unlike oleogels made with 10 wt% BS, the crystals in samples made with 10 wt% MAG only densely aggregated as a cluster together and showed spherulite morphology. This result was consistent with previous studies that spherulites and clustered crystal-aggregates were observed for MAG-based gels (da Silva et al., 2018). Based on these results, the microstructure of oleogel is clearly affected by the type of gelator.

For oleogel made with two gelators (5 wt% BS and 5wt% MAG), the morphologies that appeared in the single gelator-based oleogel also remained in the oleogel made by the combined gelators. Nevertheless, some obvious morphology differences were noticed. In the combined gelators system, a large amount of spherulite crystals that formed by MAG intermingled with lath or plate crystals originated from BS. Interestingly, needle-like crystals formed by BS in the single gelator systems disappeared. Moreover, both RSO-2 and CSO-2 contained higher amounts of spherulite crystals than elongated plate-like crystals, albeit the same mass of gelator was applied (5 wt%). This result indicated that BS has weaker crystalline-forming capability than MAG. It was expected that the crystal shape from both gelators would be present in oleogel made with the combined gelator, but the disappearance of needle-like crystals formed by BS revealed that MAG plays a dominating role when crystalline together with the same amount of BS. Such role could potentially restrain BS from forming crystalline, which consolidated our understanding of why the combined gelator system had poorer viscoelastic properties and hardness (Figures 3-4). Again, such effect is gelator dependent and some gelator combination had no impact on the crystalline structures in the oleogels. For instance, when BS is combined

with other gelators, such as stearic acid, both gelators assembled into an independent structure without disrupting their original crystal structure (Yang et al., 2017). The maintaining of the crystalline shape from each gelator was believed to be independent self-organization of the individual gelators (Yang et al., 2017).

When comparing the crystal structure of oleogels with regard to oil types (crude and refined soybean oil), it was precisely at this moment that gelator possessed greater ability to build up crystal networks in refined rather than crude soybean oils. On the one hand, more dark areas present in oleogels made with CSO, except for 10 wt% MAG oleogels (RSO-3 and CSO-3), signifies the presence of unsolidified liquid oil that cannot be retained by the gelator. On the other hand, the amount of crystal formed in crude soybean oil was smaller than in its counterpart particularly when BS was present. Such observations were corroborated with the findings of oleogel formation and DSC results, which again supported that the additional minor compounds in crude soybean oil might have disrupted the crystal structures.

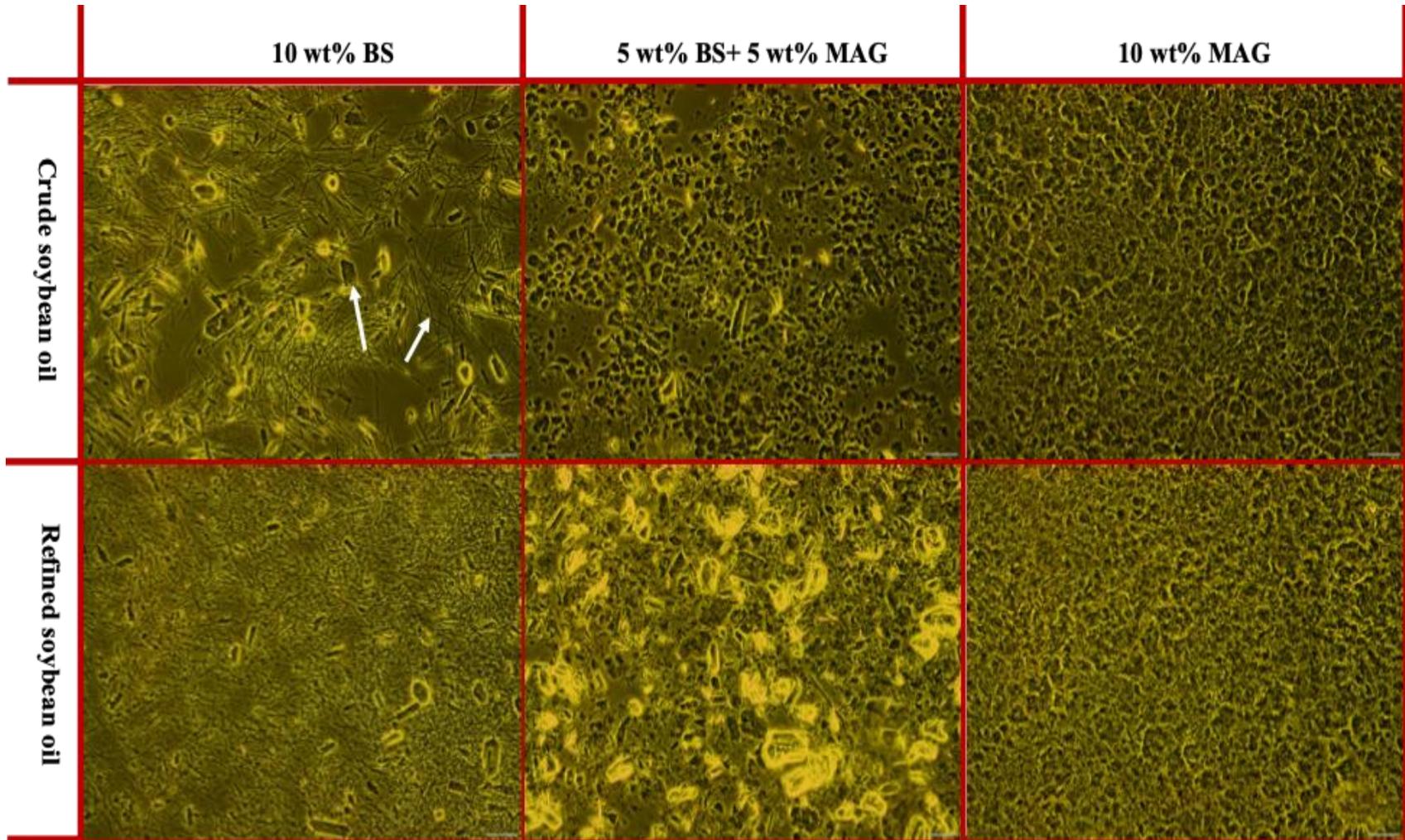


Figure 6. Polarized light microscopy images of crude and refined soybean oil oleogels (scale bar = 20 μm)

2.4.6. Properties of cookies

Another purpose of this study was to prepare cookies using six soybean oleogels using official AACC formula and compare the appearance and quality with the control cookies made with commercial vegetable shortening. The surface color of cookies made with RSO oleogels was not different from the control cookies made with commercial vegetable shortening, except CSO oleogels cookies that had a slightly yellower color (Figure 7). This result was reasonable since the color of RSO oleogels was white while CSO oleogels had an orange color, which can lead to the yellower color of the final cookie products.

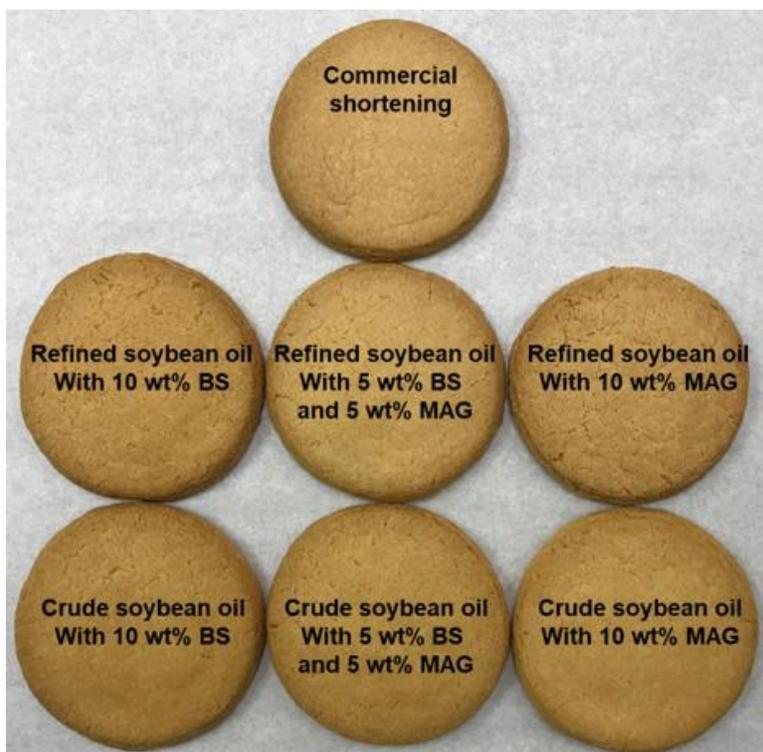


Figure 7. Appearance of cookies made with commercial shortening and soybean oil oleogels

In addition to the appearance of cookies, the weight, width, and thickness of seven cookies samples were measured, and the spread ratio of cookies was calculated (Table 1). Overall, the weight of cookies made with oleogel was similar to the weight of the control cookies ($p > 0.05$), including the dough density which did not differ from each other. The thinnest (1.1

cm) was found in cookies made with RSO-2 ($p < 0.05$) and the rest had a similar thickness as the control cookies. The width and spread ratio of cookies made with either RSO-1 or CSO-1 were significantly higher than the control. The lowest diameter of 7.19 cm was observed in the control cookie while the highest diameters of 7.60 and 7.55 cm were cookies made with CSO-1 and RSO-1, respectively. Consequently, the lowest spread ratio of 6.13 was observed in the control cookies while the highest spread ratios of 6.80 and 6.84 were observed in cookies made with CSO-1 and RSO-1, respectively. This result suggested that both RSO-1 and CSO-1 performed superior aeration than commercial shortening in dough mixing. It can also be concluded that the soybean oil source did not have a remarkable effect on the property of cookies since similar characteristics were observed in the cookies made with both sources of oleogels.

Table 1. The physical properties of cookies prepared by soybean oil oleogels

Sample name	Weight (g)	Thickness (cm)	Width (cm)	Spread Ratio	Hardness (N)
Shortening	28.41 ± 0.15 a	1.17 ± 0.02 a	7.19 ± 0.18 c	6.13 ± 0.15 c	63.05 ± 23.48 ab
RSO + 10% BS	28.41 ± 0.32 a	1.1 ± 0.04 b	7.55 ± 0.15 ab	6.84 ± 0.38 a	69.45 ± 26.22 ab
RSO + 5% BS+ 5% MAG	28.80 ± 0.96 a	1.17 ± 0.03 a	7.26 ± 0.21 bc	6.18 ± 0.30 c	76.67 ± 12.89 a
RSO + 10% MAG	28.53 ± 0.76 a	1.13 ± 0.05 ab	7.33 ± 0.12 abc	6.51 ± 0.39 abc	71.54 ± 8.55 ab
CSO + 10% BS	29.17 ± 0.45 a	1.12 ± 0.06 ab	7.6 ± 0.14 a	6.8 ± 0.45 ab	67.21 ± 2.15 ab
CSO + 5% BS+ 5% MAG	28.90 ± 0.80 a	1.16 ± 0.02 ab	7.29 ± 0.20 abc	6.27 ± 0.27 bc	59.83 ± 7.39 ab
CSO + 10% MAG	28.39 ± 0.57 a	1.14 ± 0.01 ab	7.40 ± 0.24 abc	6.48 ± 0.24 abc	48.95 ± 9.65 b

* Results followed by different letters are significantly different from each other ($p < 0.05$).

The hardness of seven cookies was also characterized using a texture analyzer (Table 1). Cookies made with RSO-2 were the firmest (76.67 N) while cookie prepared with CSO-3 had the lowest firmness or hardness (48.95 N). When compared to the control cookies, the hardness of all oleogel cookies was not significantly different from that of the control. In comparison between cookies made with different gelator (BS or MAG), there was no significant difference in hardness except for the cookie with RSO-2 and CSO-3 were different from each other. The

hardness of oleogel and commercial vegetable shortening showed that RSO-3 had the highest hardness while CSO-1 has the lowest hardness (Figure 4). It can be deduced that the hardness of oleogel is not directly related to the hardness of cookies. In summary, the quality of cookies made with soybean oleogels was superior or equivalent to the control cookies made with commercial vegetable shortening. From a functional perspective, these results show that soybean oleogels have the possibility to replace the commercial vegetable shortening in cookie preparation.

2.5. Conclusion

This study, for the first time, demonstrated the fabrication of oleogels using crude soybean oils. The structural properties and cookie making performance of crude soybean oil oleogel were systematically characterized and compared with the oleogels made with refined soybean oil. Overall, both BS and MAG can form crude and refined soybean oil oleogel in a single or a combined gelator system at a fixed gelator concentration of 10 wt%. However, the crude soybean oil oleogel was unstable when the combination of BS and MAG was used as a gelator. The physical characteristics of crude soybean oil oleogel were poorer than that of refined soybean oil oleogel, as reflected by the lower viscoelastic modulus and hardness. Such striking results indicate the antagonistic effect between crude soybean oil and gelators in forming oleogels. However, such effects did not impact the cookie-making performance of crude soybean oil oleogels since the cookies made with oleogels exhibited superior or equivalent quality to those prepared using commercial vegetable shortening. Altogether, it can be assumed that both crude and refined soybean oil oleogels made with BS and/or MAG have the potential to replace commercial shortening. More extensive applications of crude oil oleogels can be conducted in the future to the baking industry to develop foods without reducing food quality.

CHAPTER 3. CORN OIL OLEOGELS PREPARED WITH RICE BRAN WAX

3.1. Abstract

The expeller-pressed corn germ oil oleogels was prepared using rice bran wax (RBX) at different concentrations (3, 5, 7, and 9 wt%). Their structural properties were evaluated and compared with the refined corn oil oleogels. The performance of oleogels for potential food application was tested by incorporating oleogels into cookies as a replacement for commercial shortening. Colorimeter, differential scanning calorimetry (DSC), rheology, X-ray diffraction (XRD), and polarized light microscopy were used to characterize the color, thermal behavior, rheological property, and crystal structure of the oleogels, respectively. The hardness of oleogel was measured by a texture analyzer and compared to the commercial vegetable shortening. All the oleogels prepared with different concentrations of wax were incorporated into a cookie formula to fully replace commercial shortening, and the characteristics of cookies made with oleogels were tested and compared to the commercial cookies made with commercial shortening. Overall, it was found that RBX could form oleogels in both refined and expeller-pressed corn germ oil at a wax concentration ≥ 3 wt%. Although expeller-pressed corn germ oil oleogels had a comparable critical gelling concentration and thermal behavior, the lower storage modulus (G') and loss modulus (G''), the weaker hardness, the disappearance of peaks in XRD, and the fewer crystals supported that refined corn oil produced stronger gel than expeller-pressed corn germ oil. As the concentration of gelator increased, the strength of gel increased, which was represented by higher melting and crystallization temperatures, higher G' and G'' , greater hardness, and more tightly connected crystal structure. Both refined and expeller-pressed corn germ oil produced oleogel with similar morphology, exhibiting irregular platelet structure with different

crystal sizes. Although the type of oil did not influence the shape of crystals, refined corn oil produced oleogel with the strong network while expeller-pressed corn germ oil oleogels exhibited relative less crystal network. Cookies made with oleogels had similar appearance, weight, width, thickness, and spread factor to those made with commercial shortening. This result indicated that oleogels made by refined and expeller-pressed corn germ oil together with waxes had the potential to imitate the functionality of commercial shortening; thus, the replacement of shortening using oleogels could be accepted to reduce the PHOs and the saturated fats in commercial bakery products.

3.2. Introduction

The FDA determined that PHO is no longer generally recognized as safe (GRAS), and cannot be used during food processing after June 18, 2018 (FDA, 2018). In order to comply with FDA's new policy, food manufacturers, mainly bakery manufactures who use PHO as their major ingredients to produce shortening, are seeking alternatives to replace PHO in their products. Currently, several processes including full hydrogenation, chemical or enzymatic interesterification, or simply blending of liquid oil with tropical oils with high saturation, have been utilized to replace PHO in food products such as cookies and cakes (Doan, Tavernier, Danthine, Rimaux, & Dewettinck, 2018).

Among most of the techniques to structure lipids with desirable physical properties, oleogelation is a promising means as it does not change the unsaturated fatty acid profile of liquid oils as do other processing methods. Moreover, it uses only food-grade gelators to convert the liquid oil into a self-standing, thermo-reversible and three-dimensional gel network that have solid-like properties (Hughes, Marangoni, Wright, Rogers, & Rush, 2009). The production of food oleogels is fairly simple and is low cost. Depending on the application, the structural and

physical properties of oleogels can be modulated by several factors, for instance, the type and concentration of gelators, the type of oil, and processing conditions. Both gelator and oil types are extremely important to determine the crystal structures of oleogels and the physical properties thereof. Many food-grade gelators, including monoacylglycerides, lecithin, plant/insect waxes, fatty acids/ fatty alcohols, phytosterol and oryzanol, ceramides, and ethylcellulose, have been successfully used to form oleogels with variable physical properties (Hwang et al., 2012). Among them, plant-based waxes are considered as tremendous potential gelators due to their availability on a massive scale, low cost, and high oil binding and structuring properties (Doan, Tavernier, Danthine, et al., 2018; Hwang et al., 2012). The low polarity and long chain length of plant wax allow them to structure liquid oil at a low critical concentration, and thus is favorable for manufacturers and consumers (Mert & Demirkesen, 2016b). For instance, rice bran wax (RBX), a byproduct of rice milling, has been reported to structure rice bran oil and olive into oleogels at a critical concentration as low as 0.5 and 1 wt%, respectively (Dassanayake et al., 2009; Wijarnprecha et al., 2018). Additionally, oil type also exerts a profound role on the properties of oleogels as it determines the critical concentration of a gelator which is attributed to the solubility differences of a certain gelator in different oils (Dassanayake, Kodali, Ueno, & Sato, 2012).

Recently, expeller-pressed plant oils have been commercially available because of the consumers' desire for natural and solvent-free culinary oils. The production of expeller-pressed oils introduces minimal to the raw material prior to the mechanical crush. This gives rise to the final products with desirable organoleptic flavor and retaining the natural beneficial minor components (e.g., carotenes, tocopherols, and polyphenols) (Ghazani & Marangoni, 2013). In addition, expeller pressing involves no organic solvent and low temperature, which results in the

oil with no chemical contaminant, such as the notorious 3-mono-chloropropane-1, 2-diol (3-MCPD) found in refined oils. Moreover, expeller-pressed corn germ oil contains a high amount of phospholipids that may contribute to the synergistic effect with gelators to stability of the oleogel (Gaudino et al., 2019; Yilmaz & Özütcü, 2015). Therefore, expeller-pressed oil might be an excellent source of oils to produce healthier oleogels for food applications.

In this study, oleogels were prepared by mixing different concentrations of RBX with either expeller-pressed corn germ oil or refined corn oil. Physical properties of expeller-pressed corn germ oil oleogel including color, hardness, thermal behavior, rheological property, and crystal morphology were characterized and compared with that of refined corn oil oleogels. To imitate the potential application of oleogel in replacing PHOs, the RBX-based expeller-pressed corn germ oil and refined corn oil oleogels are incorporated into cookie making. The characteristics of cookies include weight, width, thickness, spread factor, and hardness were evaluated and compared to cookies made with commercial shortening.

3.3. Materials and methods

3.3.1. Chemicals

Commercial corn oil, all-purpose flour, Crisco[®] all-vegetable shortening, sugar, salt, baking soda, and dextrose were purchased locally. Corn germ was kindly provided by Bunge North America (Chesterfield, MO). Rice bran wax (RBX) was gifted from KahlWax (Kahl GmbH & Co., Trittau, Germany) and is a white solid that has soft/characteristic odor and is insoluble in water with a flash point over 150 °C. The density of RBX is between 0.85-1.05 g/cm³ at 20 °C.

3.3.2. Preparation of expeller-pressed corn germ oil

Expeller-pressed corn germ oil was prepared using a low-shear screw oil expeller (Model DD 85 G-1, Komet IBG Monoforts, Mönchengladbach, Germany). This expeller was equipped with an electrical-resistance heating ring attached around the outer surface of the press head and was powered by a 3 kilowatt driver with a speed-control gearbox. Corn germ was fed by gravity into the hopper and pressed with an R8 compression screw at a screw speed of 18 rpm and an 8-mm diameter restriction die. Temperature of the expeller was maintained at 75 °C using a Störk-Tronic temperature controller (Störk GmbH & Co KG, Stuttgart, Germany). The expeller-pressed corn germ oil was collected, filtered, and centrifuged (3,250 g for 20 min) to remove any impurities.

3.3.3. Preparation of corn oil oleogels

Oleogels were prepared by using RB with either commercial corn oil or expeller-pressed corn germ oil at different concentration (3, 5, 7, and 9 wt%). The gelator was added into glass beakers containing corn oil, and the mixture was heated above melting temperature (about 120 °C) under continuous stirring (300 rpm) to ensure the fully mixed together. The homogenous liquid-oil mixture was cooled at room temperature for 20 min. The samples were finally stored at 5 °C for 7 days before characterization. Samples containing 3, 5, 7, and 9 wt% RB with commercial corn oil were named RB-1, RB-2, RB-3, and RB-4, respectively; while the expeller-pressed corn germ oil samples were named CRB-1, CRB-2, CRB-3, and CRB-4, respectively. To measure the physical properties of oleogel, the oleogel (20g) was prepared in duplicates for further analysis. To prepare cookies, the oleogel (64g) were made to incorporate into cookie-making which were then analyzed.

3.3.4. Color determination

The color measurement of oleogels and cookies was performed with a Minolta Chroma Meter (Model CR-310, Japan). The colorimeter was configured with a diffuse illumination/0-degree viewing geometry. The lightness L , a ($-a$ greenness and $+a$ redness) and b ($-b$ blueness and $+b$ yellowness) HunterLab color parameters were measured. The colorimeter was calibrated with a standard white tile ($Y = 92.2$, $x = 0.3162$, and $y = 0.3324$).

3.3.5. Thermal properties of corn oil oleogels

The thermal properties of oleogels were measured by using differential scanning calorimeter (DSC Q-2000, TA Instruments Ltd., New Castle, DE, USA). In general, 6-8 mg sample was placed into an aluminum pan and sealed with a hermetical lid. An empty aluminum pan sealed with hermetical lid was used as a reference. To measure the thermal behavior of wax-based oleogel, the temperature was initially increased to 120 °C and maintained for 10 min to ensure that the sample was fully melted, then cooled to 0 °C using a cooling rate of 10 °C/min. After 5 min at 0 °C, the system was heated to 120 °C at a heating rate of 10 °C/min. The onset and peak crystallization and melting temperatures, and the enthalpy during heating and cooling were analyzed by Advantage/Universal Analysis (UA) Software (TA Instruments Ltd., New Castle, DE, USA).

3.3.6. Rheological properties of corn oil oleogels

Rheological measurements were carried out on a Discovery Hybrid Rheometer-2 rheometer (TA Instruments Ltd., New Castle, DE, USA) with a 2° cone plate (40 mm diameter, 57 μm gap). Strain sweep tests at a frequency of 1 Hz were performed to determine the linear viscoelastic region (LVR). LVR shows the region where the structure of sample is undisrupted. Small-amplitude oscillatory shear (SAOS) measurements were performed inside the LVR regime

in a frequency range between 10^{-1} and 10^2 Hz at 25 °C with 9 points per decade. Oleogels were tested immediately after they were taken out from the refrigerator, and at least two replications were performed. The elastic (G') and viscous (G'') modules were recorded by the software TRIOS (TA Instruments Ltd., New Castle, DE, USA).

3.3.7. Crystalline structure of corn oil oleogels

The molecular organization of the oleogels was determined by XRD spectroscopy using a Rigaku Ultima IV X-ray diffractometer (Austin, Texas, USA) with Cu-K α operating at 40 kV and 44 mA. Samples were scanned at a rate of 1.2 °/min and a step size of 0.05 ° through a 2θ range from 2 to 30 °

3.3.8. Morphology of corn oil oleogels

Crystal morphology was observed under polarized light using an Olympus BX51 polarized light microscope (PLM) (Olympus Optical Co., Ltd., Japan). A small amount of the oleogel was placed on a glass slide and covered with a cover slide. The slide was then mounted under the microscope and visualized using a 20 × objective lens under the crossed polarizer and analyzer.

3.3.9. Firmness of corn oil oleogels

A puncture test was applied to investigate the firmness of oleogels, which was compared with that of commercial vegetable shortening. Based on the method of Hwang et al. (2014) the oleogel (10 g) in vial was placed on the platform of a texture analyzer (TA.XT plus, Stable Microsystems, Surrey, UK) with a 2 kg compression load cell. A ball probe (TA-18A, 3/4 inch diameter stainless steel ball) was then lowered 10 mm into the oleogel samples at a test speed of 1 mm/sec at room temperature. The maximum force (i.e., a measure of firmness) that is a

measure of firmness was recorded from the plot of force versus penetration distance. The puncture test was done in triplicate and recorded in Newtons (N).

3.3.10. Preparation of cookies using corn oil oleogels

Cookie making was carried out according to the AACC method (AACC 10-50.05, 2000) and prepared using a cookie kit (National Mfg., Lincoln, NE, USA). The ingredients used to prepare cookies include flour 225 g (14% moisture basis), sugar 130 g, salt 2.1 g, sodium bicarbonate 2.5 g, 33 g of dextrose solution (prepared by dissolve 8.9 g dextrose in 150 ml distilled water), distilled water 25 g, and shortening/oleogel 64 g. The dough was divided into six portions, placed on a greased aluminum cookie sheet (2 mm thickness) and lightly flattened. Two metal gauge strips (7 mm thickness) were anchored on each side of the cookie sheet and the dough was rolled to thickness using one forward and one return stroke with a rolling pin. The dough was then cut using an aluminum cutter (60 mm inside diameter) and placed onto an aluminum baking sheet. Excess dough was removed and discarded. Cookies were baked in a Baxter mini rotating rack oven (OV 300E, Baxter Corp, Orting, WA, USA) at 205 °C (400 °F) for 10 min. After baking, the cookies were removed from baking sheets and cooled for 30 min on nonstick racks, after which were placed in with polypropylene bags and kept at room temperature until further analysis. Each batch could prepare six cookies, and every two cookies were considered as a replication.

3.3.11. Characterization of cookies

Cookie characteristics include width (W) and thickness (T) were measured with a measuring grid (National Mfg., Lincoln, NE, USA) 1 h after baking. The width of cookies was measured by place three cookies side by side on the grid and calculate the mean. The thickness of cookie was measured by piling up six cookies together and then lying the piled cookies down

on the measuring grid. The mean thickness was calculated. The spread ratio (W/T) was calculated based on the average width and thickness. Cookies were stored at room temperature (20 °C) in a sealed plastic bag for further measurements. Cookie hardness was evaluated using a texture analyzer (TA.XT plus, Stable Microsystems, Surrey, UK) 24 h after baking. The texture analyzer was equipped with a 2 kg load cell and a knife blade 3mm thick probe was used to measure the hardness of cookie. Compression was applied until breaking at a speed of 1 mm/s. The maximum force required to break the cookie was considered as a hardness parameter. The experiment was done in triplicate and measured as Newtons (N).

3.3.12. Statistical analysis

Two batches of oleogel (20g) were made to evaluate the physical properties of oleogels. The results for physical properties of oleogel include DSC, rheological, XRD, and PLM were performed in duplicate. One batch of oleogel (64g) were prepared to make one batch of cookies. Each batch could prepare six cookies, and every two cookies were considered as a replication. The hardness of oleogel and shortening, and all the cookie characteristics were measured in triplicate with fresh samples. The values were expressed as means of duplicates or triplicates from each of independent experiments, and \pm SD was also calculated for triplicate experiments. The data were statistically analyzed using SAS version 9.4 (SAS Institute Inc. Cary, NC). One-way analysis of variance (ANOVA) was conducted, and a significant difference was defined at $p < 0.05$ by Tukey's test.

3.4. Results and discussion

3.4.1. Color and texture analysis

Before analysis, all the oleogel samples were stored refrigerated (5 °C) for seven days. The appearance, color parameters (L^* , a^* , and b^*), and texture (hardness) of oleogels were

prepared using refined corn oil and expeller-pressed corn germ oil with different concentrations (3, 5, 7, and 9 wt%) of RBX were significantly different (Figure 8A, B). To visualize the formation of oleogel, all the samples were placed upside down and were considered as oleogel if no oil flows down. All the samples successfully formed oleogels (Figure 8A) denoting that both refined corn oil and expeller-pressed corn germ oil have the capability to form oleogel with RBX at a concentration of $\geq 3\%$. Oleogel could be formed with 0.5 and 1 wt% RBX in rice bran oil at a temperature lower than 10 °C and 80 °C, respectively (Wijarnprecha et al., 2018).

However, our preliminary experiment showed that no oleogel was formed when the concentration of RBX was less than 3 wt%. Similarly, Doan and coworkers documented that rice bran oil required a critical minimum gelling concentration of 5 wt% RBX to form oleogel (Doan, Van De Walle, Dewettinck, & Patel, 2015). Moreover, Hwang et al. (2012) evaluated three RBX from different suppliers and found two of them could form oleogels at a concentration of 0.5 and 1wt% while the other required at least 5 wt%. In other words, the different components such as minor constituents and impurities may have greatly affected the gelling property of RBX.

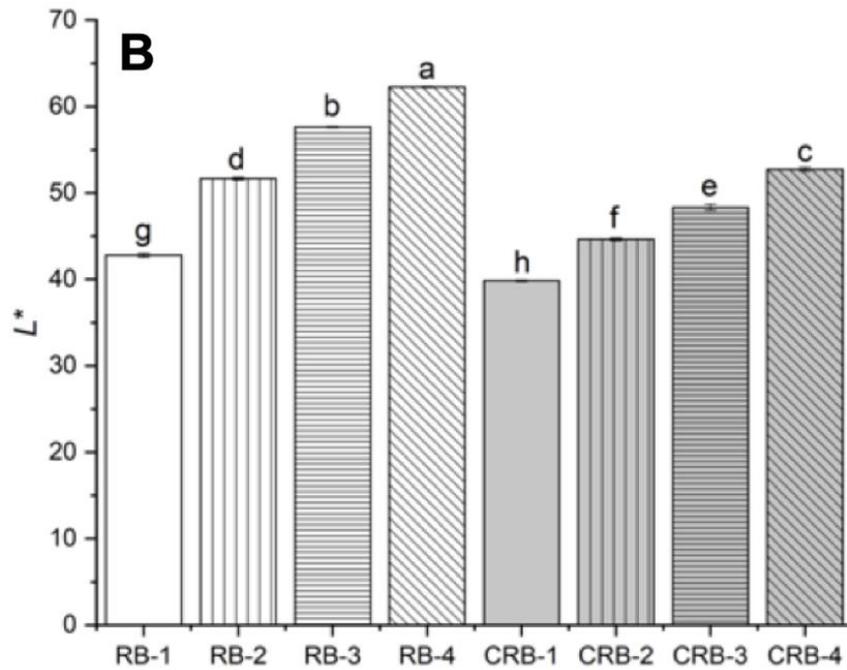
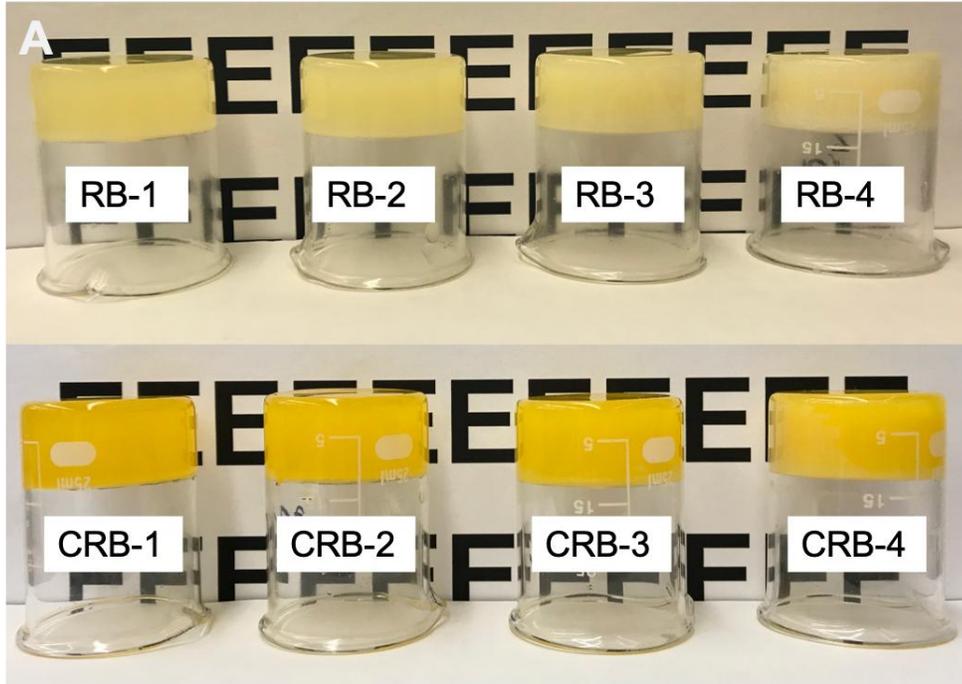


Figure 8. Appearance (A) and color values (B) lightness (L^*); (C) a^* and b^* of refined corn oil oleogels and expeller-pressed corn germ oil oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%)

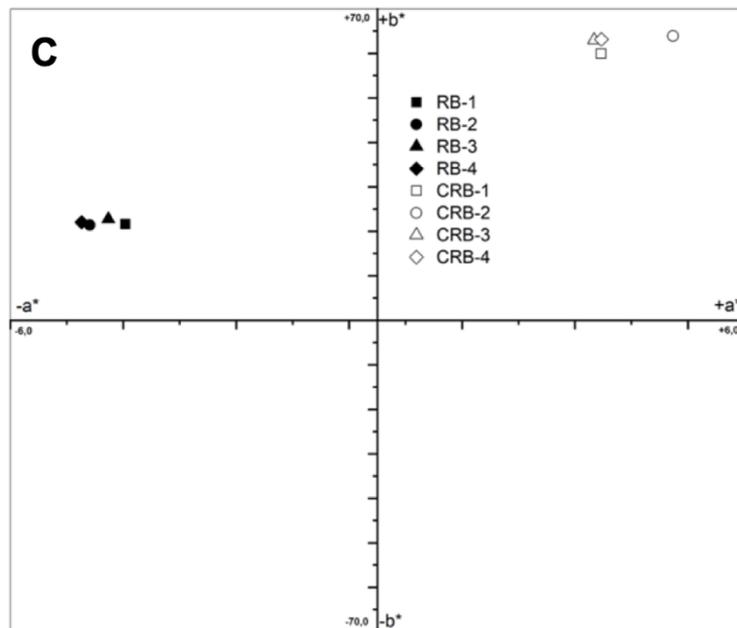


Figure 8. Appearance (A) and color values (B) lightness (L^*); (C) a^* and b^* of refined corn oil oleogels and expeller-pressed corn germ oil oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%) (continued)

According to the visual observation, the color of the oleogels was different. Neat RBX is a white solid flake. It was obvious that refined corn oil produced whiter oleogels while expeller-pressed corn germ oil produced yellower oleogels. This result is expected since the crude oils usually contain natural colorants that contribute to the darker color of expeller-pressed corn germ oil which is then retained by the oleogels. Similarly, a whiter color was observed in the expeller-pressed corn germ oil oleogels as the concentration of RBX increased. In order to further confirm the visual observation, the color of the samples was measured (Figure 8B&C). Refined corn oil oleogels showed higher lightness (L^*) than expeller-pressed corn germ oil ones at a same concentration of RBX (Figure 8B). All refined corn oil oleogels showed a yellow tone ($+b^*$) and green pigment traces ($-a^*$). Expeller-pressed corn germ oil oleogels had similar yellow tone but with greater intensity ($+b^* > 60$); however, it had strong redness ($+a^*$), differing drastically from that of refined corn oil oleogels (Figure 8C). Increasing the concentration of RBX significantly increased the lightness of oleogels independent of oil type but had weak impact on

a^* and b^* . Therefore, it was clear that the oil type can affect the oleogel color, which can be attributed to the particular minor constituents in expeller-pressed corn germ oils such as lutein and zeaxanthin (yellow) and carotenoids (red) (Moreau, Johnston, & Hicks, 2007).

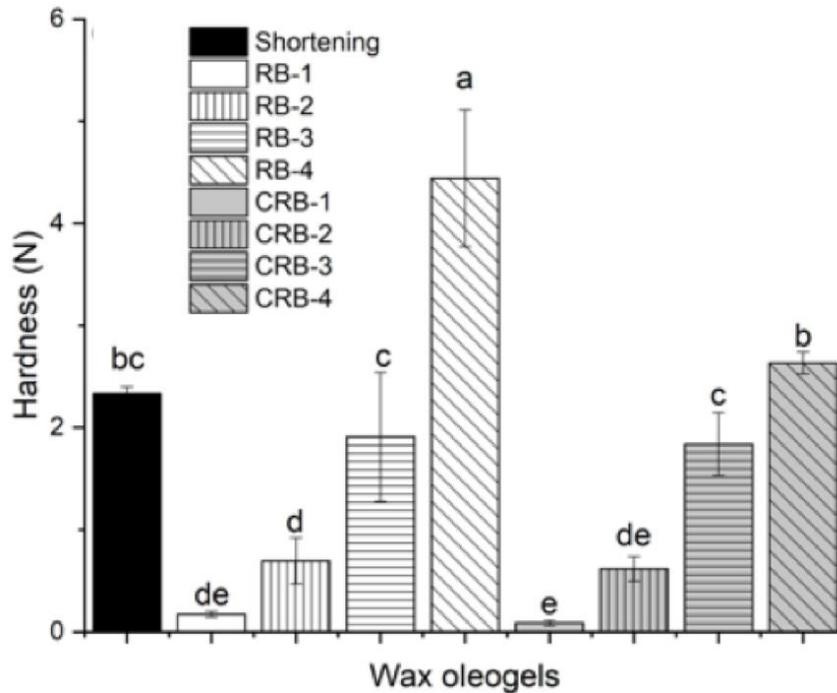


Figure 9. The hardness (N) of refined corn oil oleogels and expeller-pressed corn germ oil oleogels made with rice bran wax at various concentration (3, 5, 7, and 9 wt%)

The mechanical property of oleogels was characterized by measuring their hardness (Figure 9). In general, higher RBX concentration yielded harder and stronger oleogels in both oil types, which was in agreement with other studies (Hwang et al., 2012; Ölütcü & Yilmaz, 2015). The hardness of oleogels formed by refined corn oil was nearly twofold higher than that of expeller-pressed corn germ oil oleogel with the presence of 9 wt% RBX. Interestingly, no significant difference on hardness was recorded among both types of oleogels as RBX concentration was less than 9 wt%. This suggests that the minor constituents in expeller-pressed corn germ oil did not improve the gelling capacity of RBX as compared to refined corn oil.

Moreover, the hardness of commercial vegetable shortening was measured to compare with oleogels (Figure 9). The hardness of commercial vegetable shortening was greater than oleogels made with 3 and 5 wt % of RBX, while similar to the ones with 7 wt% RBX, but softer than the ones made with 9 wt% RBX. No significant difference for hardness was found between RB-3, CRB-3 & 4, and commercial shortening. One of the most important characteristics of oleogels is the hardness that determines their potential applicability to replace PHO in actual industrial products. Our findings herein indicate that 7 wt% of RBX is sufficient to build a gel structure in both refined corn oil and expeller-pressed corn germ oil and that has similar hardness as the commercial shortening.

3.4.2. Thermal characterization

Thermal behaviors include crystallization and melting are important for the technological and functional properties and stability of oleogels. The thermal behaviors of oleogels prepared with varying RBX concentrations and oil type were measured with DSC (Figure 10), and peak temperatures (T_m and T_c) and enthalpy (ΔH_m and ΔH_c) of oleogels and RBX were analyzed.

Pure RBX exhibited two thermal events as revealed by two exothermic peaks at 73.76 °C and 59.84 °C, and two endothermic peaks at 76.73 °C and 63.93 °C, respectively, manifesting its multicomponent nature (Figure 10B). This result was consistent with Doan et al. (2015) who found all waxes, including neat RBX, beeswax, candelilla wax, fruit wax, and berry wax had multi-peak thermal profiles. Meanwhile, few studies also reported a single thermal event with crystallization and melting peak for RBX at 70.1 °C and 78.2 °C, respectively (Blake, Co, & Marangoni, 2014; Dassanayake et al., 2009). Such discordance might be caused by the source of RBX since the chemical composition of wax can be varied from different suppliers and processing.

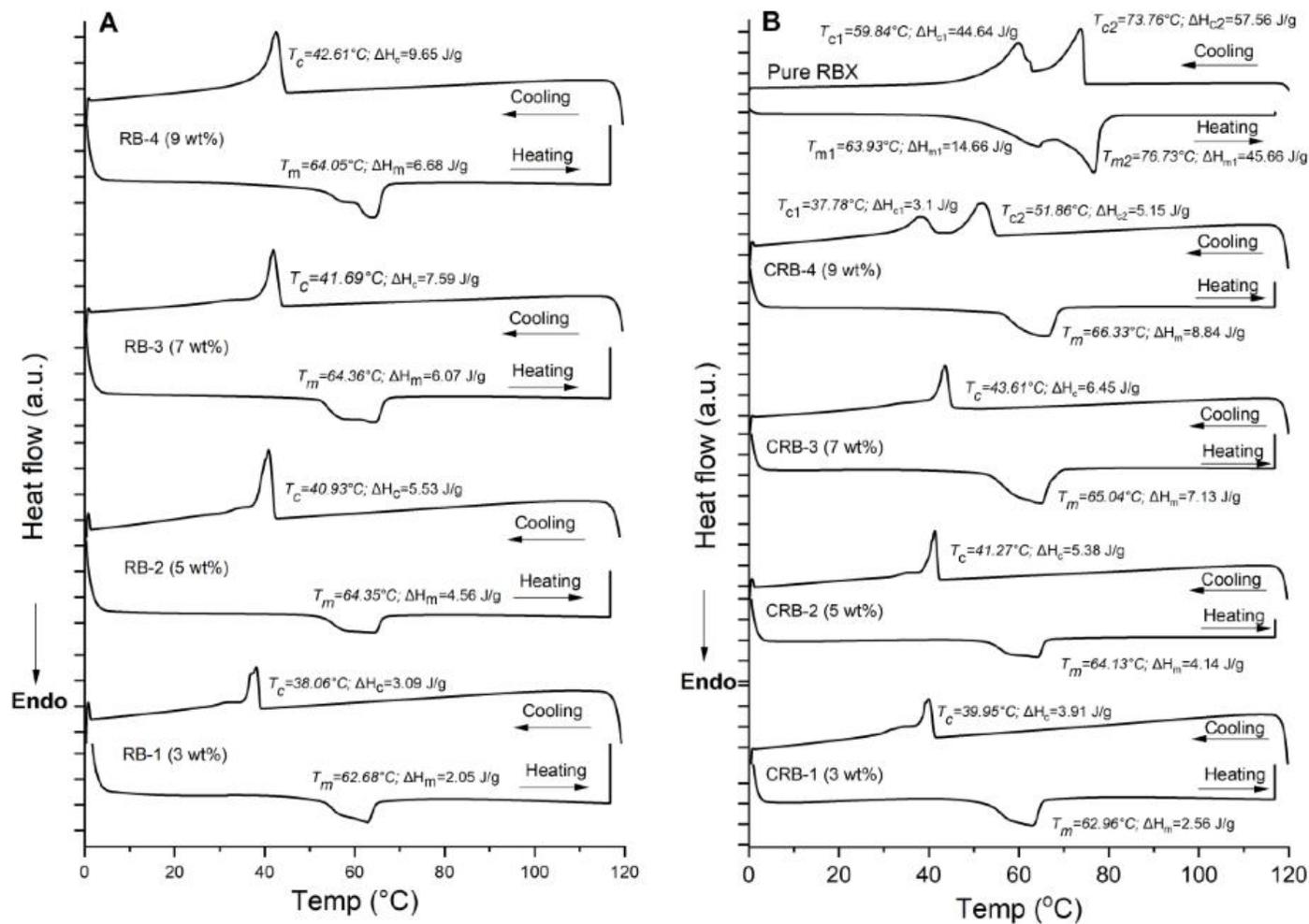


Figure 10. Differential Scanning Calorimeter heating and cooling flow curves for (A) refined corn oil oleogels; (B) expeller-pressed corn germ oil oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%)

The refined corn oil oleogels showed single exothermic and endothermic peaks at all RBX concentration (3, 5, 7, and 9 wt%) (Figure 10A). One can also note that the thermal behavior of the oleogels was driven by RBX concentration since the rising melting temperature (T_m), crystallization temperature (T_c) and their corresponding enthalpies (ΔH_m and ΔH_c) were observed upon the increase of RBX concentration. In particular, as the concentration of RBX increased from 3 wt% to 9 wt%, the crystallization and melting temperature increased from 38.06 °C to 42.61 °C, and 62.68 °C to 64.05 °C for refined corn oil oleogels (Figure 10A). The lower peak temperature and the reduced enthalpy at a lower gelator concentration were caused by the dilution effect of solvent, corn oil in this case, which was in accordance with several previous studies (Blake et al., 2014; Doan, et al., 2017;2018; Hwang, Singh, & Lee, 2016).

The expeller-pressed corn germ oil shared similar thermal behavior as its counterpart at a lower RBX concentration (3, 5, and 7 wt%) with crystallization and melting temperature increased from 39.95 °C to 43.61 °C, and 62.96 °C to 65.04 °C, respectively (Figure 10B). Interestingly, two exothermic events along with one endothermic event were detected in the expeller-pressed corn germ oil oleogels with 9 wt% RBX (CRB-4). The two exothermic peaks (37.76 °C and 51.86 °C) occurred during the crystallization of CRB-4 was probably caused by minor constituents in expeller-pressed oils which is absence in refined corn oil that facilitates the reorganization and crystallization process of multicomponent in RBX, thus partially compensating the dilution effect of the solvent. The single endothermic peak maybe the murgence of two endothermic peaks in RBX since these two peaks existed at the temperature close to each other.

The melting enthalpy can be used as a parameter to estimate the amount of crystalline mass in the oleogels and the enthalpy could be influenced by the concentration of gelators (Doan

et al., 2017). The enthalpy of crystallization ($\Delta H_c = 1.09 \text{ CRBX} - 0.06$; $r^2 = 0.99$) and melting ($\Delta H_m = 0.77 \text{ CRBX} + 0.22$; $r^2 = 0.93$) in refined corn oil oleogels increased linearly with the increase in RBX concentration, agreeing with our previous study on base algae oil oleogels prepared by monoacylglycerides (Wang et al., 2019). A similar trend also was observed in expeller-pressed corn germ oil oleogels ($\Delta H_c = 0.70 \text{ CRBX} + 1.80$, and $r^2 = 0.99$; $\Delta H_m = 1.09 \text{ CRBX} - 0.88$, and $r^2 = 0.98$). Since all the oleogels exhibited higher exothermic and endothermic enthalpy at higher RBX concentrations, it can be assumed that the oleogel prepared with higher amount of RBX contained a greater amount of crystalline mass (Figure 10). When comparing the thermal behaviors of the oleogels made with two different oils, there was no difference for the corresponding enthalpies except CRB-4, indicating expeller-pressed corn germ oil could produce oleogel that had similar thermal behavior as the ones made with refined corn oil.

3.4.3. Rheological measurements

The strain sweeps (0.01-100%) were conducted before the frequency sweep in order to determine the linear viscoelastic region (LVR) for all the oleogels. The frequency sweep tests were used to measure the solid-like and liquid-like characteristics of all the oleogels which represented as elastic modulus (G') and viscous modulus (G''), respectively (Figure 11).

It was clear that all the oleogels exhibited solid-like property since $G' > G''$ at all RBX concentrations. A higher G' indicated that oleogels had good tolerance to deformation. This result agreed with the previous study that rice bran oil oleogel made with RBX had higher G' than G'' , which explained their ability to resist deformation (Doan et al., 2015). A frequency-dependence in conjunction with the positive slope of elastic and loss modulus with the increase in frequency again suggested solid-like behavior of oleogels. Typically, G' and G'' increased with the increase of RBX concentration. Both G' and G'' of oleogels contained 9 wt% RBX were 100

times higher than the one made with 3 wt% RBX. The increasing of G' and G'' indicated that RBX concentration had a significant influence on the rheological properties of oleogels. A similar result was reported oleogel made with sunflower wax and berry wax where higher G' and G'' with the increasing of gelator concentration (Doan et al., 2017). Moreover, the increasing of the magnitude of slope was more obvious as the concentration of RBX decreased which implied that oleogel made with a lower RBX concentration had less tolerance to deformation compared to oleogel made with higher RBX concentration.

Refined corn oil oleogels exhibited higher G' and G'' than expeller-pressed corn germ oil oleogels which indicated that refined corn oil actually forms a stronger gel network with a same amount of RBX than expeller-pressed corn germ oil. This result was in line with our previous study that oleogels made with refined soybean oil had higher elastic and viscous modulus than the ones made with hexane-extracted crude soybean oil (unpublished work). The different viscoelastic properties of oleogels prepared by refined and expeller-pressed/crude oil can be ascribed to the existence of minor constituents as the latter oil is composed of endogenous lecithin, tocopherol, polar colorants, and certain phenolics (Chen, McClements, & Decker, 2011). Those minor constituents may act as a crystal habit modifier of RBX and interfere with crystal network formation resulting in the formation of oleogels with less modulus and hardness, especially at higher concentration (9 wt%).

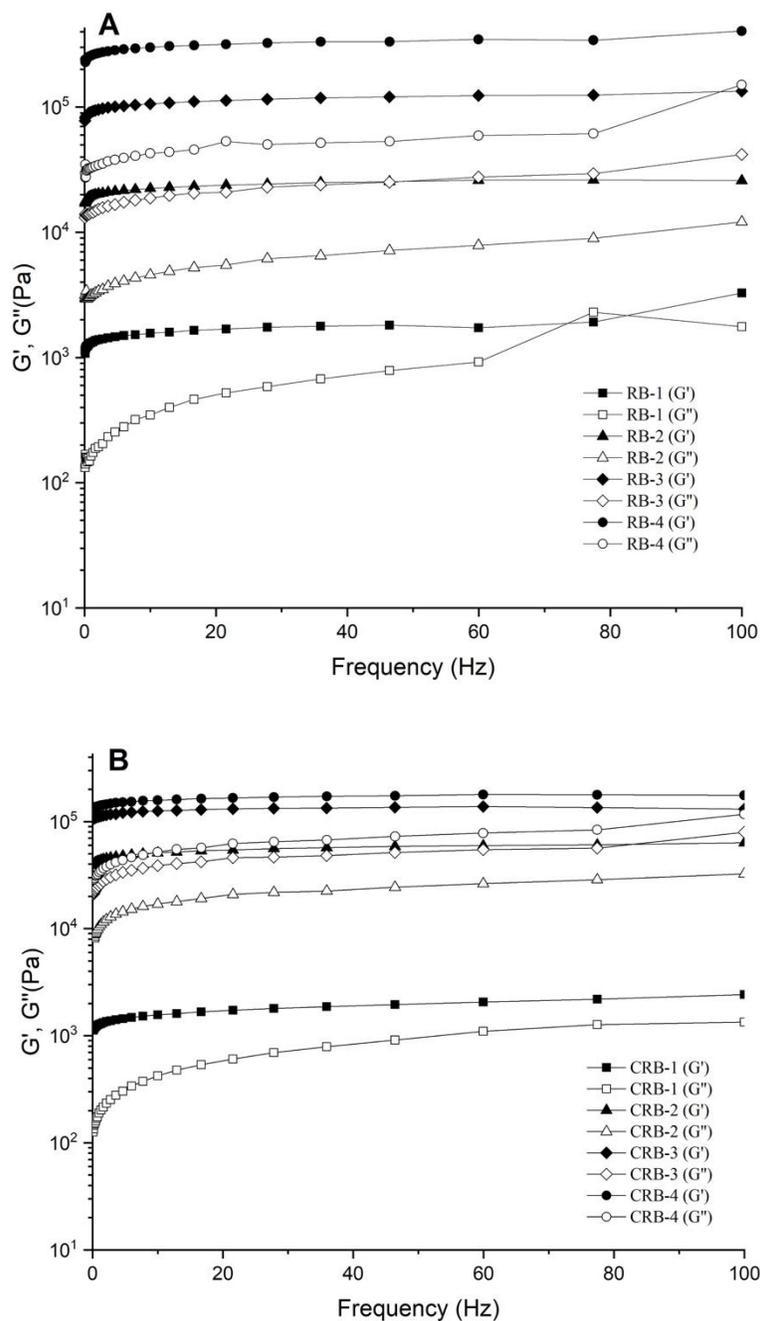


Figure 11. Frequency dependence of elastic modulus (G') and viscous modulus (G'') for (A) refined corn oil oleogels; (B) expeller-pressed corn germ oil oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%) at 25 °C

Similar antagonistic effect was reported in some binary gelator systems. For instance, the presence of lecithin decreased the strength of oleogel made by 12-hydroxystearic acid (Tamura & Ichikawa, 1997). Okuro et al. (2018) also found that the role of lecithin on the formation of

natural wax-based sunflower oil oleogel was dependent on the type of waxes. In contrast, lecithin only exerted a synergistic effect with a low-melting fruit wax resulting in weakened crystal network of the oleogels formed by high- and mid-melting point waxes (Okuro et al., 2018). RBX used in this study is considered as high-melting point wax as the T_m is 76.73 °C (Patel, Babaahmadi, Lesaffer, & Dewettinck, 2015). Still, further research on how gelators could impact the properties of expeller-pressed oil oleogels is required.

3.4.4. X-Ray diffraction

The polymorphism of oleogel samples was characterized by X-ray diffraction (XRD), and the d-spacing of the crystal was calculated on the basis of 2θ . Figure 12A&B displayed the XRD patterns of RBX-based oleogels prepared with refined corn oil and expeller-pressed corn germ oil, and the main peaks corresponded to the d-spacing of the crystal.

All refined corn oil oleogels showed three major peaks at 4.60 Å, 4.16 Å, and 3.74 Å in the XRD pattern with varying intensity as the concentration of RBX changes (Figure 12A). In the wide-angle region, a peak around 4.6 Å is the evidence of β -polymorphic form while a peak between 3.8-4.2 Å is evidence of β' -polymorphic form. In other words, RBX based refined corn oil oleogels contained both β and β' polymorphic crystals that have triclinic (T//) and orthorhombic (O \perp) sub-cell structures, similar to the wax-based canola/soybean oil blend (Dassanayake et al., 2009).

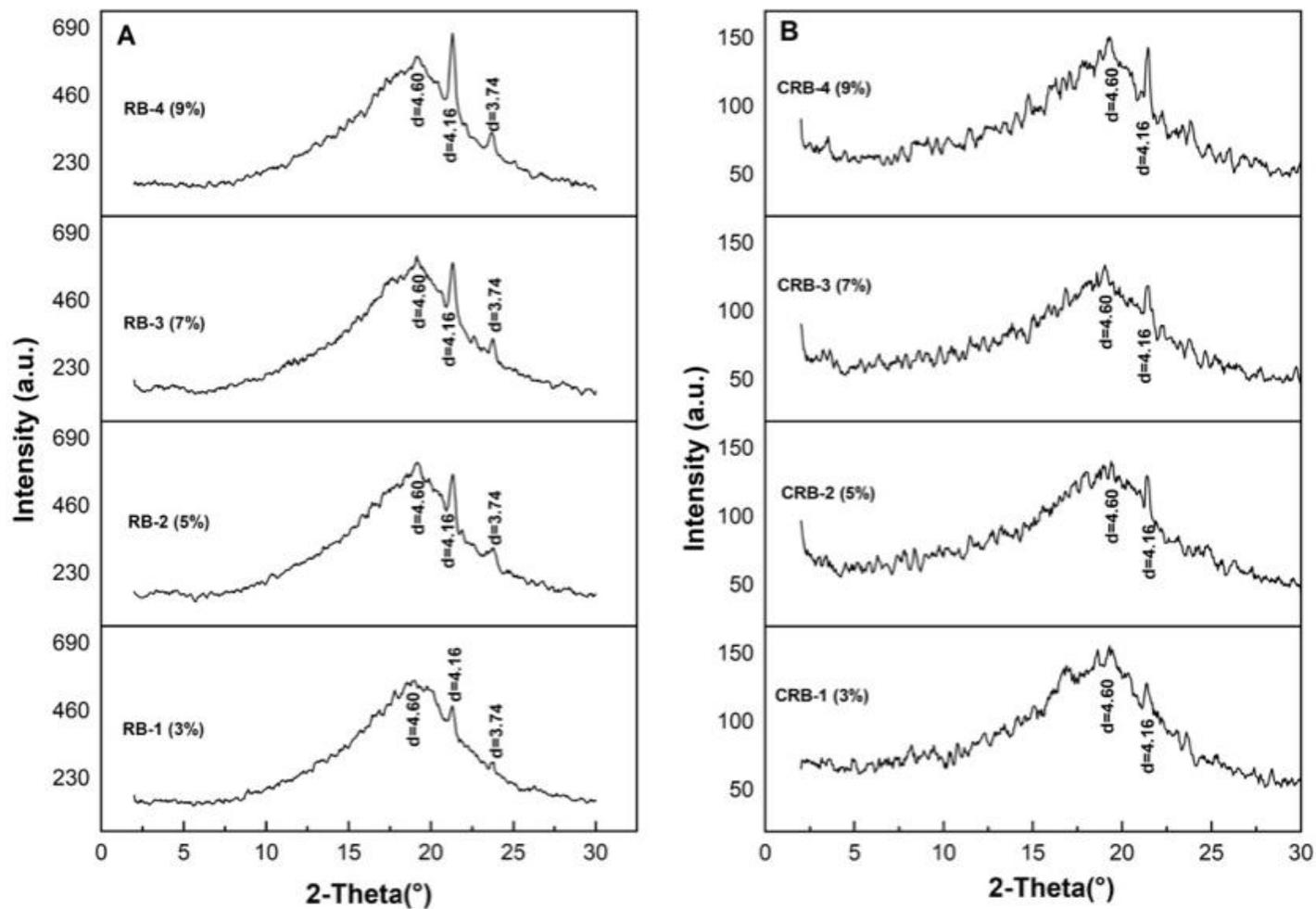


Figure 12. X-Ray Diffraction patterns of (A) refined corn oil oleogels; (B) expeller-pressed corn germ oil oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%) at 25 °C

The intensity of each peak increased as the concentration of RBX increased. This is anticipated as the increased wax concentration was reported to facilitate self-sorting and rearrangement and to generate more crystalline structure in the wax-based oleogels, which would be represented as greater intensity on the XRD pattern (Yang et al., 2017). This also supports the greater hardness and higher modulus of oleogel made with a higher wax concentration in aforementioned results. Unlike refined corn oil oleogels, only two peaks at d-spacing of 4.60 Å and 4.16 Å were registered in all expeller-pressed corn germ oil oleogels (Figure 12B). The disappearance of the peak was direct evidence that RBX in expeller-pressed corn germ oil produced weaker gel network, which again corroborated our previous study (Chapter 2).

3.4.5. Crystal morphology

Polarized light microscopy (PLM) was used to further identify microstructure and evaluate crystal morphology of the oleogels (Figure 13). Various crystal morphologies of RBX based oleogels were previously reported, including needle-like (Blake et al., 2014; Dassanayake et al., 2009; Wijarnprecha et al., 2018), or dendritic crystals (Doan et al., 2015; Mert & Demirkesen, 2016b), all of which was claimed as a desirable feature for gel formation since the greater surface area of needle-like crystals enable the gelator to have better interaction with liquid oils. RBX oleogels exhibited irregular blocky/platelet structure (Figure 13) with different crystal sizes, which is in agreement with previous observation on RBX, sunflower wax, and candelilla wax oleogels (Blake & Marangoni, 2015). They also explained that the needle-like morphology of wax-based oleogels was actually either due to the edge effect of the platelet (the case in our study) or the folded part of crystal, which could be formed during the slide preparation for the microscope.

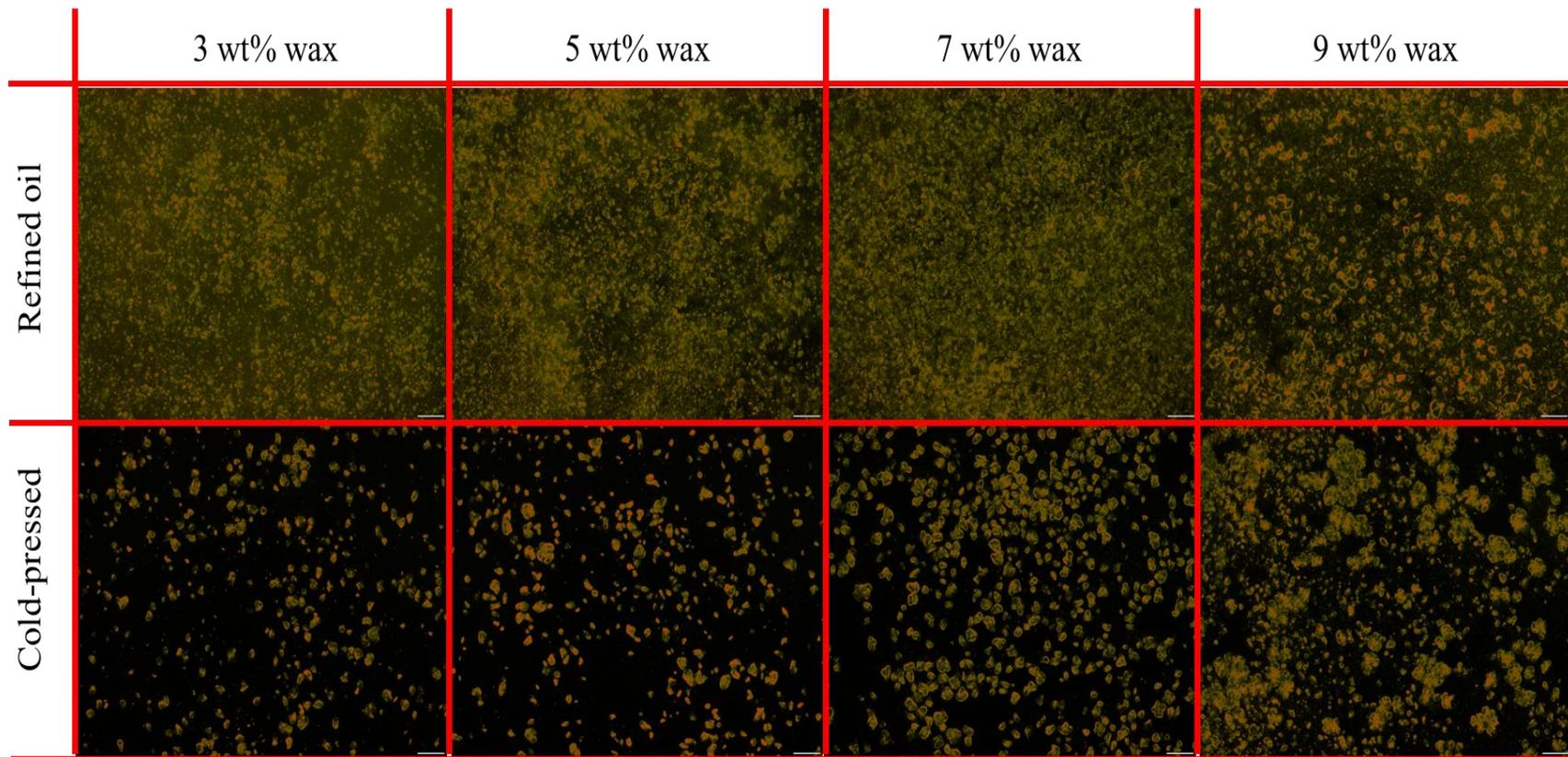


Figure 13. Polarized light microscopy images of oleogels made with rice bran wax at various concentrations (3, 5, 7, and 9 wt%) at 25 °C

In addition, we noticed that most of the dark area shown in RBX oleogels still contained crystals that were too small and dark to be seen clearly in the image. This is because RBX formed numerous crystals that piled up together resulting in multi-layer crystals that could not be observed clearly at the same exposure time and focus. When the oleogel was exposed to more light, the crystals in the dark lower-layer could be viewed, but crystals in up-layer (Figure 13) were too bright to be visualized but shown as bright spots.

The amount and the size of crystal increased with RBX concentration, which was in a good agreement with previous studies (Blake et al., 2014; Wijarnprecha et al., 2018). The greater size of the crystal could be formed by the growth of RBX crystals on the available small crystalline materials and clusters. Moreover, the difference in the size and amount of crystals also supported our previous statement that the color difference of the oleogels might be caused by the reduction in the amount of crystals since as the amount and the size of crystals increased, the color of the oleogel could be less transparent. Oil type also influenced morphology of the oleogels to certain degree (Figure. 12). Overall, less amount of RBX crystals was formed in expeller-pressed corn germ oil oleogels than in refined ones at the same concentration of RBX. This result further supported our rheology and XRD result that refined corn oil showed a better capability to produce higher quantities of crystals, making the oleogels stronger and firmer.

3.4.6. Properties of cookies made with oleogels

The major goal of fabricating oleogels is to replace other high saturated fat products in food processing. Thus, in this section, all the oleogels were used as a commercial shortening replacer to prepare cookies and their performance on cookie quality was evaluated. There were no obvious differences for the color of cookies made with all RBX oleogels compared to cookies made with commercial vegetable shortening (control) (Figure 14). Only the center of the cookies

made by expeller-pressed corn germ oil oleogel showed a slightly darker surface color than the control which may cause by the yellowish color of the oil. The color analysis supported the observation in the way that all the cookies had no technical difference on lightness (L^*) (Figure 14A). Moreover, the number of yellowness and redness, with a combination of yellow ($+b^* \sim 32$) and red tone ($+a^* \sim 10$), grouped all cookies together suggesting the similarity on the color of cookies (Figure 14B).

In addition to the color, the characteristics including weight, width, thickness, and hardness of cookies were measured, and the spread factor was calculated in order to compare with control (Table 2). No significant differences were found for weight, thickness, and spread ratio of cookies made with refined corn oil oleogels and commercial shortening. For width, cookies made with RB-1 and RB-2 were similar to the control; while other cookies had a significantly bigger width, which was technically considered as close to the control as the difference was less than 3%. However, the hardness of cookies varied for all the samples and no statistical difference was found between all the cookies except RB-1 when compared to the control. Interestingly, the hardness of oleogel seemed to be inversely related to the hardness of cookies. The highest hardness of cookies was found in the cookies made with RB-1 oleogel, which had the lowest hardness among all the refined corn oil oleogels.

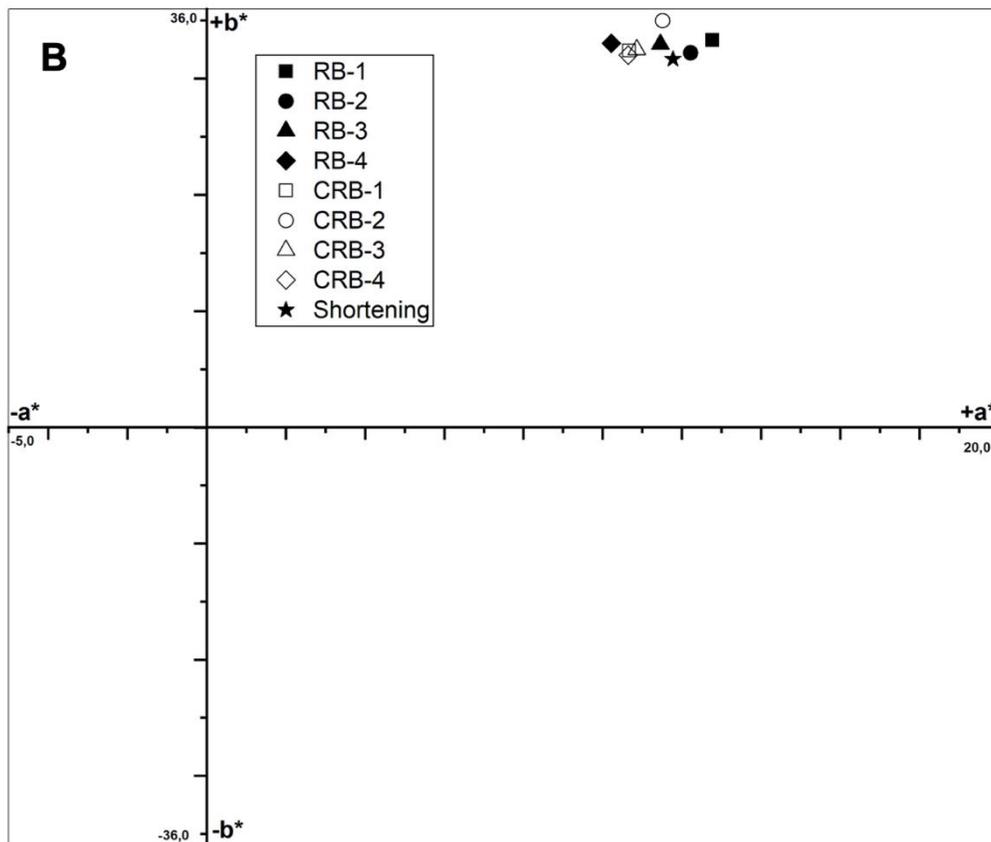
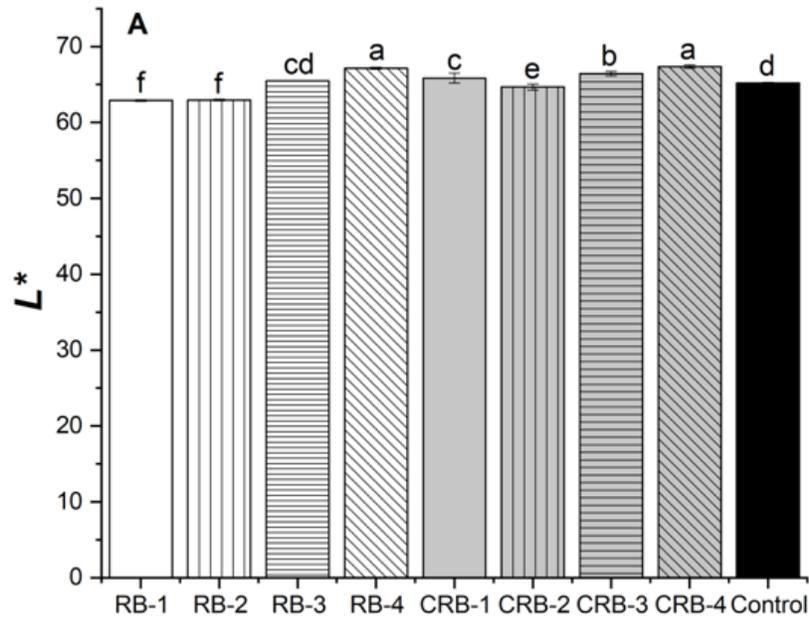


Figure 14. Color values (A) lightness (L^*); and (B) a^* and b^* (the inserted pictures are the visual observation of cookies) of cookies made with commercial shortening, expeller-pressed corn germ oil oleogels, and refined corn oil oleogels at various rice bran wax concentrations (3, 5, 7, and 9 wt%)

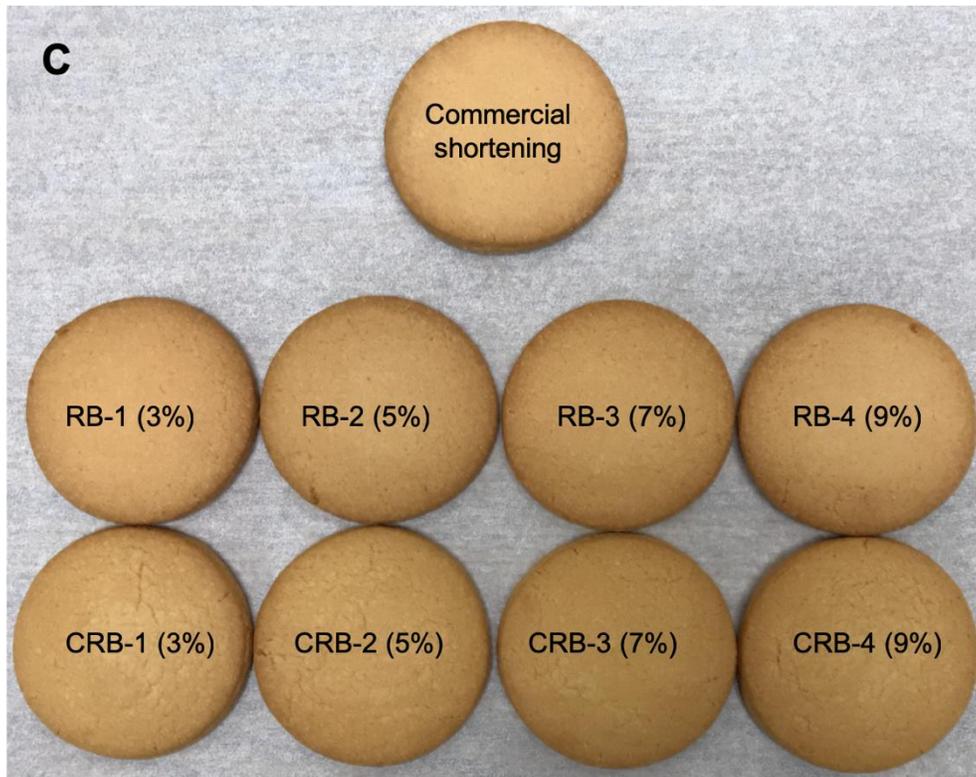


Figure 14. Color values (A) lightness (L^*); and (B) a^* and b^* (the inserted pictures are the visual observation of cookies) of cookies made with commercial shortening, expeller-pressed corn germ oil oleogels, and refined corn oil oleogels at various rice bran wax concentrations (3, 5, 7, and 9 wt%) (continued)

As the concentration of RBX increased from 3 wt% to 9 wt%, the hardness of refined corn oil oleogel increased from 0.17 N to 4.44N; but the hardness of cookies decreased from 114.93 N to 76.08 N. The type of fat used to prepare cookie could influence the hardness of cookie. It was reported that the breaking strength (hardness) of cookie made with liquid sunflower oil was higher than cookies made with bakery fat, margarine, or hydrogenated fat (Jacob & Leelavathi, 2007). One possible reason was that solid fats could incorporate a large amount of air during dough mixing whereas the oil could not retain the air in the dough resulting in a harder texture (Jacob & Leelavathi, 2007). As the concentration of RBX increased, the hardness of cookies made with refined corn oil oleogels decreased, which indicated oleogel prepared with less RBX might have lower air retainability resulting in the higher cookie hardness.

Table 2. The physical properties of cookies prepared with commercial shortening, refined corn oil oleogels and expeller-pressed corn germ oil oleogels

Sample	Weight (g)	Width (cm)	Thickness (cm)	Spread ratio	Hardness (N)
Shortening	29.36±1.27 a	7.05±0.06 a	1.20±0.02 abc	5.87±0.11 abc	81.75±7.08 c
RB-1	29.70±1.21 a	6.96±0.07 abc	1.24±0.03 ab	5.62±0.13 bcd	114.93±21.80 b
RB-2	29.31±1.14 a	6.92±0.04 bcd	1.22±0.04 ab	5.70±0.19 abcd	91.17±16.34 c
RB-3	31.08±3.22 a	6.84±0.10 d	1.24±0.10 ab	5.55±0.50 cd	87.26±8.39 c
RB-4	30.34±2.07 a	6.88±0.04 cd	1.17±0.03 bc	5.90±0.17 abc	76.08±10.71 c
CRB-1	32.95	6.98±0.08 ab	1.12±0.12 c	6.30±0.64 a	140.92±16.19 a
CRB-2	29.5	6.99±0.04 ab	1.17±0.05 bc	6.01±0.25 ab	123.25±7.40 ab
CRB-3	30.15	6.92±0.01 bcd	1.21±0.04 abc	5.73±0.17 bcd	134.54±1.35 ab
CRB-4	31.22	6.89±0.05 bcd	1.29±0.01 a	5.34±0.06 d	133.12±2.48 ab

* Results followed by different letters are significantly different from each other ($p < 0.05$).

When compared the cookies made with expeller-pressed corn germ oil oleogel to the control the result was not as consistent as the cookies made with refined corn oil oleogels. For instance, only the cookies made with CRB-4 was different from control cookies with regard to both width and spread ratio. All other cookies had a similar width, thickness, and spread ratio when compared to control cookies. Unlike the hardness of cookies made with refined corn oil oleogels, no noticeable relationship was found for CRB cookies. Moreover, all the cookies prepared with expeller-pressed corn germ oil had greater hardness than those made with refined corn oil oleogels cookies, even though the oleogels themselves showed reversed rank for hardness. Although the hardness of CRB cookies differed from the control, other similar characteristics (i.e., width, thickness, and spread ratio) still allowed CRB oleogels to have the potential to use as shortening replacement in cookie-making.

3.5. Conclusion

Although the properties of oleogel made with different gelators and oils had been well documented, this is the first study on the incorporation of expeller-pressed corn germ oil in the preparation of oleogel. The physical properties and the potential food application of oleogel

made with both refined corn oil and expeller-pressed corn germ oil cross a wide range of gelator (RBX) concentration were determined in this study. Overall, RBX exhibited good gelling properties to produce oleogel with both refined corn oil and expeller-pressed corn germ oil at a gelator concentration of 3 wt%. RBX oleogel showed good gelling properties represented by the higher crystallization temperature, stronger rheological properties, greater hardness, and a more massive amount of crystals that formed oleogels with a tighter and stronger network. As the wax concentration increased, the physical properties of oleogels including thermal behavior, rheological properties, hardness, and crystal morphology were enhanced. Similar to our unpublished study on soybean oil, the oleogels made with expeller-pressed corn germ oil were weaker than refined corn oil at same RBX concentration. When using oleogels to replace commercial shortening to prepare cookies, all the oleogel cookies exhibited comparable characteristics to the control. Therefore, it could be assumed that both refined corn oil and expeller-pressed corn germ oil oleogels gelled by RBX have the potential to replace commercial shortening to prepare products with high unsaturated fats. More extensive applications of both types of oleogels could be exploited in other fat-based products.

CHAPTER 4. OVERALL CONCLUSION

This study, for the first time, investigated the physical properties of oleogel prepared with crude plant oils. The primary purpose of this study is to examine the effects of oil type, gelator type, and gelator concentration on the physical properties of oleogels, and investigate the potential application of crude and refined oil oleogels as an alternative to hydrogenated vegetable shortening in bakery products. Overall, this study showed the possibility of using crude plant oils to form the oleogel with the three-dimensional network that could imitate the property of solid fat, thus, having the potential to replace the solid fat high in saturated fats to produce food with a healthier profile.

In the first part of this project, the structural properties of solvent extracted crude soybean oil oleogel were systematically characterized and compared with the oleogels made with refined soybean oil. Two gelators, β -sitosterol and monoacylglycerides, are used either individually or in combined to study their influence on oleogel property. Overall, both crude and refined soybean has the capability to form oleogel when β -sitosterol and monoacylglycerides are used individually at a concentration of 10 wt%. Crude soybean oil oleogel made by the combination of β -sitosterol and monoacylglycerides was unstable and separated in a few days, which indicated that crude soybean oil showed an antagonistic effect on the formation of oleogel. The smaller hardness, weaker rheological properties, and less crystal network also illustrated the weaker gelling property of crude soybean oil. Although the crude soybean oil oleogels produce relative softer gel than the refined soybean oil, it does not mean the crude oleogel will dissatisfy the performance when it is incorporated into the food system. Cookie was selected to investigate the display of oleogel in real food system because cookie requires a high amount of shortening (14 wt%). Interestingly, the antagonistic effect in oleogel did not impact the cookie making

performance of crude soybean oil oleogels since the cookies made by them exhibited superior or equivalent quality to those prepared using commercial vegetable shortening.

Although we concluded that crude soybean oil has weak gelling property, it could not represent the property of all the crude plant oils. So, in the second part of this study, we investigate and compare the physical properties and the cookie making performance of oleogel made with solvent free expeller-pressed corn germ oil and commercial refined corn oil. Instead of studying the influence of gelator type, we use rice bran wax as the single gelator but at a varying concentration to examine the effects of concentration of gelator on the characteristics of oleogel. Similar to the previous result, expeller-pressed corn germ oil oleogel was weaker than refined corn oil oleogel at the same gelator concentration. However, the cookie making performance of expeller-pressed corn germ oil oleogel did not differ as large as the oleogel properties, which was in agreement with our previous result for soybean oil oleogel. Furthermore, the concentration of gelator, in this case, rice bran wax, showed a more significant influence than the oil type. As the concentration of rice bran wax increased from 3 to 9 wt%, the hardness and the rheological property of the oleogel increased about 26 and 100 times stronger, respectively. When comparing the difference between oil type, the hardness and rheological property of refined corn oil oleogel were less than ten times greater than expeller-pressed corn germ oil oleogel. This result indicated that in order to prepare oleogel with desirable physical property, changing the concentration of gelator is more effective than changing the type of oil.

In conclusion, although crude oils showed weaker gelling property than refined oil, it has the capacity to prepare oleogel that can be used to replace hydrogenated vegetable shortening. Altogether, it could be assumed that both refined and crude oils including corn and soybean oil

gelled by β -sitosterol, monoacylglycerides, or rice bran wax have the potential to replace commercial hydrogenated shortening to prepare products with high unsaturated fats.

CHAPTER 5. FUTURE WORK

Although this study investigated the physical properties of refined and crude oleogel and their potential application in cookie-making, more works can be done in the future to understand the role of oleogel. More extensive applications of crude oil oleogels can be conducted in the future. For example, more food grade gelators could be incorporated into crude oil to prepare oleogels. A fatty acid profile test can be conducted to study what fatty acids exist in refined and crude plant oils, and oleogels. The fatty acid profile of oleogel can be further compared to the fatty acid profile in commercial fats (i.e., shortening, margarine) to see whether a healthier fatty acid profile can be found in oleogel. Moreover, the influence of minor component in the crude oil could be further studied since we still do not know the exact reason why minor components such as lecithin in the crude oil reduce the strength of oleogel while the addition of extra lecithin in refined oil could enhance the formation of oleogel.

Another area for further exploration is the stability of oleogel and products made with oleogel. For example, the oxidative stability of oleogels and cookies made with oleogels should be assessed to further understand the role of oleogel on the quality of final products. The storage stability of oleogel and products made with oleogel can be measured since it is important to know the shelf-life of products. The impact of crude oil oleogels on the properties of dough can also be investigated to understand their role on cookie formation better. Moreover, the performance of oleogel in other fat-based products such as cakes, cheeses, and chocolate can be investigated to study the potential of using oleogels to replace fat in the different food system.

Finally, to ensure the possibility of using oleogel in industry, several extensive works could be done. For example, a cost analysis could be conducted to compare the cost of using oleogel with that of using traditional structured oil (i.e., interesterified fat). Moreover, the

sensory test can be performed to examine whether consumers will accept products made with oleogel.

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