PHYSICOCHEMICAL PROPERTIES OF RICE BRAN OIL-BASED OLEOGEL SYSTEM

By

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ABSTRACT

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Oleogels are emerging novel alternatives for conventional solid fats in the food industry. However, the physicochemical properties of oleogels are still not yet fully established such that they can fully acquire the physiochemical properties of conventional solid fats. Moreover, due to the vast number of possible combinations and concentrations of organic solvents and structuring agents to produce oleogels which have yet to be fully explored, there is still no conclusive findings on the best formulation of oleogel as of today. Therefore, this study aims to evaluate the physicochemical properties of a particular combination of rice bran oil-based oleogels which are structured with beeswax and palm wax at different concentrations, along with the synergistic effects of both structuring agents. In this study, a total of 13 variations of oleogels were prepared at different wax concentration and combinations and their physicochemical properties were assessed: (i) 2% BW; (ii) 4% BW; (iii) 6% BW; (iv) 8% BW; (v) 10% BW; (vi) 14% BW; (vii) 1% BW 1% PW; (viii) 2% BW 2% PW; (ix) 4% BW 4% PW; (x) 6% BW 6% PW; (xi) 8% PW; (xii) 10% PW and (xiii) 14% PW. Synergistic effects from PW was observed in both thermal analysis (differential scanning calorimetry and slip melting point). All RBO oleogel samples exhibited shear-thinning behavior at increasing shear rate and were found to portray more elastic

than viscous properties. The type of wax and concentration of wax were also the major determining factors of the intensity and magnitudes of most of the parameters measured in the analysis carried out. The oxidative stability of the RBO oleogel samples were also found to be incompatible with that of commercial margarine used as a control, due to the presence of high amounts of unsaturated fatty acids in RBO oleogels. Microscopic studies showed that the microstructure of PW (small spherulitic crystals) was more dominant in hybrid wax oleogels than BW (needle-like crystals). From the colorimetry analysis, the color of the margarine control sample was also found to be significantly different from the RBO oleogel samples. OBC test results on the other hand indicated that there was no significant difference in the OBC values among all the RBO oleogel samples except for 1% BW 1% PW. Synergistic effects from both waxes were observed in textural analysis where harder hybrid wax RBO oleogels were produced in comparison to RBO oleogels gelled with a single oleogelator at the same concentration. Overall, two particular oleogels, namely BW4PW4 and BW6PW6 showed potential for further study to scrutinize the synergistic potentials of BW and PW in other physicochemical properties not done in the present study as synergism in RBO oleogels have shown to be a promising solution to complement the limitations of each type of wax when they are used to gel RBO alone.

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DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions

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APPROVAL SHEET

This final year project report entitled "**PHYSICOCHEMICAL PROPERTIES OF RICE BRAN OIL-BASED OLEOGEL SYSTEM"** was prepared by LORRAINE NG RUO YUEN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **LORRAINE NG RUO YUEN** (ID No: **20ADB04759**) has completed this ~~final year project~~ / ~~dissertation~~ / thesis* entitled "PHYSICOCHEMICAL PROPERTIES OF RICE BRAN OIL-BASED OLEOGEL SYSTEM" under the supervision of Dr Ali Yassoralipour (Supervisor) from the Department of Agriculture and Food Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year ~~project report~~ / ~~dissertation~~ / thesis in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(LORRAINE NG RUO YUEN)

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LIST OF ABBREVIATIONS

ALA	Alpha-Linolenic Acid
ANOVA	One-Way Analysis of Variance
BW	Beeswax
CLA	Conjugated Linoleic Acid
DHA	Docosahexaenoic Acid
DSC	Differential Scanning Calorimetry
EHA	Eicosapentaenoic Acid
FAMEs	Fatty Acid Methyl Esters
FFA	Free Fatty Acid
FID	Flame Ionization Detector
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GRAS	Generally Recognised as Safe
HMWOs	High-Molecular Weight Oleogelators
LDL	Low-Density Lipoprotein
LVR	Linear Viscoelastic Region
Linoleic Acid	LA
LMWOs	Low-Molecular Weight Oleogelators
OBC	Oil Binding Capacity
PUFAs	Polyunsaturated Fatty Acids
PW	Palm Wax
RBO	Rice Bran Oil

SPSS	Statistical Package for Social Sciences
Triacylglycerols	TAG
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research Background

Oils and fats are indispensable cooking condiments in many restaurants, eateries and even used on a household scale. Besides being used as a cooking medium in majority of the food preparation methods such as frying and baking, oils and fats are also consumed as they are directly as salad dressings and spreads respectively. Besides being used as a food condiment and for cooking, oils and fats also impart valuable attributes such as mouthfeel, texture and aroma into a huge array of foods (Drewnowski and Almiron-Roig, 2010). Favourite foods like cheese, butter and ice cream to name a few confer creamy, soft and light texture particularly due to the presence of trans and saturated fats.

Oils and fats are mainly composed of monounsaturated, polyunsaturated and saturated fatty acids while process such as partial hydrogenation of oil forms additional components, specifically trans fats which are known to be detrimental to consumers' health (Puscus et al, 2020). Commercially produced solid fats from partial hydrogenation include shortening and margarine which are commonly used in baked goods to produce a crispy and flaky texture. Even though saturated and trans fats provide the desirable texture and mouthfeel in foods, researches have shown that diets high in saturated fat content will place

an individual at risk of developing chronic coronary heart disease whereas the situation is even more dire for trans fats where individuals are more at risk of developing preliminary coronary heart disease as trans fats are not as easily digested by the human body (Dhaka et al, 2011). As such, the difficulty in digesting trans fats will lead to the accumulation of trans fats in human body, increasing the level of low-density lipoprotein (LDL). The World Health Organization (WHO) suggests the intake of trans fats to be kept at less than 1% of an individual's total energy intake per day (World Health Organization, 2020). However, the reason why conventional solid fats are widely utilized today is partly due to its long shelf stability on top of the aforementioned sensory attributes that they provide. Comparative to liquid oil, solid fats contain higher saturated fat content which will result in a more compact chemical structure due to the formation of linear fatty acid chain and this gives them the innate properties of being more shelf-stable as the compact structure will reduce the susceptibility of solid fats to oxidative rancidity (Vu et al, 2022).

1.2 Problem Statement

In recent years, concerns encompassing the health impact of saturated and trans fats from consumers and governmental authorities have led scientists and researchers to experiment and look for functional alternatives to conventional solid fats which can possibly mimic the properties of conventional solid fats but with a more promising and healthy lipid profile (Zetzl and Marangoni, 2011). This has then led to the novel creation of edible oleogels through physical structuring of liquid oil rich in unsaturated and polyunsaturated fatty acids

without altering their initial chemical composition so as to eliminate the harmful trans fats present in conventional solid fats and lowering the amount of saturated fats while producing stable and rigid semi-solid structure similar to that of conventional solid fats (Frolova et al, 2022).

Oleogelation, known otherwise as oil structuring utilizes an organic solvent which is none other than the liquid oil used, along with an oleogelator which serves and provides a three-dimensional network to entrap the oil molecules, thus immobilizing them and forming a semi-solid oil structure, known as oleogels (da Silva, Arellano and Martini, 2019). The structural integrity of oleogels is characterized by the interactions formed between the oil and oleogelator molecules, including hydrogen bonding and various types of van der waals interactions and all these are dependent on the combinations and concentrations of the organic solvent and oleogelator used (Manzoor et al, 2022). Oleogels have been critically studied in recent years as they have a high potential of replacing the use of conventional solid fats in the market. Besides the food science fields, oleogels have also been studied extensively in biochemical fields in nutraceutical delivery (Dhal et al. 2023).

To ensure that oleogels can fully replace conventional solid fats in the market, not only must the oleogels produced be healthier in terms of their lipid profile, they also must attain physicochemical properties that are compatible to that of conventional solid fats to gain consumers' acceptance in terms of various sensory attributes and characteristics when used in foods. The physicochemical

properties of oleogels in general are affected by several factors, which include the type of oleogelator, type of vegetable liquid oil, the ratio of oleogelator used in relation to the amount of liquid oil as well as the interactions between the oleogelator and oil molecules (Scholten, 2018). As of today, the physicochemical properties of oleogels that can entirely mimic the properties of conventional solid fats have yet to be well-understood and established due to the complexity and numerous possible oleogelator-oil combinations and concentrations which require efforts in optimization as well as multiple trials and errors. Even though there are current researches that focus on oleogels made with different types of vegetable oils and oleogelators, there are still no conclusive findings on the formula which would produce an oleogel that can have the highest proximity to the properties of conventional solid fats available in the market.

1.3 Objectives

The objectives of this study are to:

1. Investigate the physicochemical properties of rice bran oil (RBO)-based oleogel system made with beeswax (BW) and palm wax (PW), including the synergism of both waxes as a potential substitute for commercial margarine.
2. Evaluate the oxidative stability and fatty acid composition of the rice bran oil-based oleogel in comparison to a commercial margarine used as a control.

CHAPTER 2

LITERATURE REVIEW

2.1 Roles of Dietary Lipids

Other than the provision of energy, the main function of dietary lipids in foods include the formation of cellular structure such as cell surface membrane, function as a thermal insulator in human body, involvement in the cell signalling pathway and for fat-soluble micronutrients like Vitamins A, D, E and K to dissolve and be transported to target organs to carry out their respective functions (Bordoni et al, 2021). Lipids can vary in forms and functions as they are biological molecules that form structure with different monomers and under the broad category of lipids consists of compounds like triglycerides, phospholipids, fatty acids, cholesterol and monoglycerides to name a few (Field and Robinson, 2019).

2.1.2 Sources of Dietary Lipids

Animal-based dietary lipids are commonly classified as milk solid fats, meat fats as well as fish oil and all of them varies in terms of their fatty acid composition. Animal fats from milk and meat-based products mostly comprises of saturated fatty acids as well as cholesterol which can be found in food products like cheese,

sausage, butter and others (Mudge, 2022). On top of that, small amounts of trans fats can also be found naturally in animal milk and meat products and they are termed as conjugated linoleic acid (CLA) (Warwick, 2023). These naturally obtained trans fats from animal-based products pose equally high level of health risk as those of artificial ones, thus they should also be consumed in moderation to prevent the development of chronic heart diseases (Greger, 2014). Nevertheless, it is not necessary that all animal-derived dietary lipids must be associated with chronic heart diseases. Certain types of dietary lipids cannot be synthesized internally in our body and therefore they must be obtained from our diet. Polyunsaturated fatty acids (PUFAs) including derivatives like eicosapentaenoic acid (EHA) and docosahexaenoic acid (DHA) are essential fatty acids obtainable mostly from animal sources which cannot be synthesized by our body but are required to perform important functions in our bodies (Karsli, 2021). Major animal-derived sources of EHA and DHA include oily fish like salmon, mackerel, sardines and anchovies which can be absorbed directly by human body (Richter, 2023).

In plant-based diet, unsaturated fatty acids are more prominent as compared to saturated fatty acids found in animal-derived lipids (Coulston, 1999). Plant-derived lipids are rich in linoleic acid (LA) and alpha-linolenic acid (ALA) but unlike animal-derived lipids, they do not directly supply either EHA or DHA (Napier et al, 2014). Nevertheless, through a series of metabolism, LA can be converted into arachidonic acid whereas ALA is converted first into EHA then finally into DHA for absorption into the body to carry out their vital functions (Anderson and Ma, 2009). Linoleic acid can be obtained in vast amounts from

plant-based sources like nuts and most type of liquid vegetable oils like canola oil, sunflower oil and soybean oil (Whelan and Fritsche, 2013). Plant-derived sources of ALA include walnuts, flaxseed, legumes and leafy greens (Murdoch, 2020). However, not all things come good. Certain plant-derived dietary lipids do contain saturated fatty acids where the more prominent sources are from coconut oil as well as palm oil. The commonly known form of saturated fatty acid in palm oil is none other than palmitic acid and in coconut oil, the main form of saturated fatty acids are lauric and myristic acids (Lim, 2021). As evident from their respective states at ambient temperature, coconut oil, which is normally in solid state contains a comparatively higher amount of saturated fatty acids of about 90% than palm oil of about 50%, which is normally in liquid state at room temperature (Boateng et al, 2016).

2.1.3 Lipid Oxidation

Unlike fresh produces such as meat, seafood and even fruits and vegetables which are commonly linked to spoilage mechanisms by microorganisms, the spoilage of fats, oils and product derivatives of fats and oils are often associated with non-microbial spoilage in which lipid oxidation caused the highest incidents of food spoilage. Lipid oxidation occurs often as a result of exposure of the fats and oils to environmental factors such as oxygen, temperature fluctuations and light from the surrounding during storage (Halvorsen and Blomhoff, 2011). Intrinsic factors include the degree of unsaturation as well as the amount of free fatty acids available (Dominguez et al, 2019). Generally, the

higher the level of unsaturation and availability of free fatty acids, the faster the rate of lipid oxidation (Ahmed et al, 2016). Food products, which contain fats and oils, be it in their natural or processed forms are also bound to spoilage by lipid oxidation, which greatly reduces the organoleptic properties of the product.

Sensory attributes such as the texture, color, flavor and aroma of the food product can be altered as a result of lipid oxidation (Bayram and Decker, 2022). Off-odors formed as a result of lipid oxidation are usually the main organoleptic aspects that deter consumers from buying or using the food product. Besides influencing the flavor profile of food products and compromising on their nutritional content, oxidated lipids are also known to be harmful and even detrimental to human health (Vieira, McClements and Decker, 2015).

Lipid oxidation is a self-sufficient mechanism where the primary products formed are used in the subsequent stages of the mechanism until the reactants are fully depleted (Nam, 2011). There are various sophisticated mechanisms associated with lipid oxidation, which include enzymatic and non-enzymatic lipid oxidation. Autoxidation is the most prevalent type of non-enzymatic lipid oxidation involving free radicals which not only produces off-odors in food products but also the production of by-products which are carcinogenic (Huang and Ahn, 2019).

As a result, in the production and development of oleogels, lipid oxidation is an important aspect for consideration, especially in monitoring its shelf-life since oleogels are mostly made with polyunsaturated vegetable oils which are more

susceptible to lipid oxidation due to their higher degree of unsaturation as compared to solid fats which are high in saturated fatty acids. Nevertheless, oleogels are said to have higher oxidative stability than liquid vegetable oils owing to the immobilization of the oil molecules by the oloegelators which reduces the exposure of the oil molecules to oxygen and other potential external factors that accelerates lipid oxidation. As such, the effectiveness of the oleogelators used at different concentrations in prolonging the shelf-stability of oleogels are evaluated through peroxide value test in this research.

2.2 Types of Oleogelators

Oleogelators are essential structuring agents in the formation of oleogels as they provide a structural network which will hold the oil molecules in place, allowing the formation of bonds and interactions between the oleogelator and oil molecules to achieve a gel-like structure of oil. Generally Recognized as Safe (GRAS) oleogelators which are typically food-grade are used in the formulation of oleogels. Different types of oleogelators used at differing concentrations were being explored by many researchers to investigate on their gelation properties and efficiency as they are vital in influencing the resulting consistency and stability of the oleogel (Huang et al, 2023).

There are two main categories of oleogelators used to form oleogels, namely the low-molecular weight oleogelators (LMWOs) and high-molecular weight oleogelators (HMWOs) (Babu et al, 2022). At present, more research effort encompassing the use of LMWOs is observed compared to the use of HMWOs

in oleogels as majority of the LMWOs available are hydrophobic in nature which can dissolve in the organic solvent used in oleogel formulations to form gel (Sivakanthan, 2022). However, research conducted by Banas and Harasym (2021) suggested that the polymeric activities displayed by HMWOs are likely to confer a more stable semi-solid structure in terms of its viscoelastic properties. Various types of HMWOs include polysaccharides, proteins as well as polymers whereas commonly used LMGOs include fatty acids, waxes, lecithin, monoglycerides and phytosterols (Perta-Crisan et al, 2023). Oleogelators are typically added in minute amounts and their mode of entrapping the oil molecules can be classified either as a self-organization system where the oleogelator molecules form molecular interactions with the solvent molecules or a crystal particles system where the oleogelator molecules will form crystal microstructures via the process of nucleation (Dassanayake, Kodali and Ueno, 2011).

2.2.1 Natural Waxes

Natural waxes mostly obtained from plant sources are LMWOs which offers great advantages in the formulation of oleogels and they are mostly made up of varying composition of fatty acids and fatty alcohols (Gao et al, 2021). Since the composition of organic compounds varies between each type of wax from various sources, this will render differences in the resulting properties of oleogels made with different types of wax. However, one common point that they all have in common is that they are thermoreversible. The waxes will melt in oil when they are heated beyond their melting point and then crystallizes when

they start to cool, enabling the formation of a three-dimensional crystal network containing the condensed oil molecules within (Sahu et al, 2021). Natural waxes are mostly food-grade, cost-friendly, offers good gelation properties and is widely available (Hwang et al, 2018). Oleogels made with natural waxes only required a very small amount of wax, ranging from 1% to 4% weight by weight (w/w) relative to the weight of the organic solvent used (Yang et al, 2020). This makes the production of oleogels more feasible since only a small amount of wax-based oleogelator is required for gelation to occur. In addition, the melting point of natural waxes ranges between 50℃ to 80℃ which allows them to form stable solid gels at room temperature just like conventional solid fats (Scharfe and Floter, 2020).

However, on the downside, even though natural waxes are only added in small quantities in the making of oleogels, they pose certain limitations too. The use of natural waxes in oleogels for food application may potentially cause a coarse and waxy mouthfeel which could be unpleasant to the palate as it might hinder the perception of other flavors and aromas form the food consumed (Till. 2022).

Natural waxes including rice bran wax, carnauba wax or known otherwise as PW, candelilla wax, BW and sunflower wax are mostly GRAS certified for food applications and they are some of the more popular options of natural waxes as oleogelators (Ghazani, Dobson and Marangoni, 2022). In this research, BW and carnauba wax were used as the choice of oleogelators.

The use of BW in oleogel formulations has been extensively studied by many researchers. Besides being a food-grade oleogelator, BW also confers exceptional properties including hydrophobic properties, antioxidant properties as well as its viscoelastic properties (Chen et al, 2022). The hydrophobic properties of BW is attributed to the vast amount of organic compounds present in BW, which include hydrocarbon chains, linear and complex esters as well as fatty acids (Buchwald et al, 2009). This allows BW to be efficiently dispersed in the organic continuous phase or the liquid vegetable oil to form a homogenous and stable gel. Research done by Penagos et al (2023) suggests that BW, in the absence of surfactants can function as effective stabilizers alone without displaying the Pickering stabilization effect as the extend of nucleation of BW in the continuous phase was extensive. As a result, BW can be used as a single oleogelator in the formation of oleogel without the need for synergism with a separate oleogelator. On top of that, BW has also shown to portray remarkable gelling capabilities at wax concentration as low as 3% (w/w) to gel liquid oil (Ropciuc et al, 2023). Alternative research done by Han et al (2022) shown that BW can gel liquid oil even to as low as 1% wax concentration owing to its needle-like crystal morphology.

Unlike most waxes, the melting point of carnauba wax is one of the highest, ranging between 65℃ to 89℃ (Thakur et al, 2022). It is mostly made up of about 80% of aromatic esters and about 20% consisting of fatty acids, fatty alcohols as well as hydrocarbons which confers carnauba wax its elastic properties (Liu et al, 2019). A study conducted by Noonim and coworkers (2022) stated that oleogels formulated with carnauba wax displayed a higher

crystallization enthalpy, resilience at elevating temperature and resistance against oxidation in comparison to BW. Carnauba wax were also found to portray spherulitic crystal morphology which produced very subtle amount of crystals at low concentrations of carnauba wax at 0.5% and 1% (Norazura Aila et al, 2022). The scarce amount of crystal formation at low concentration of carnauba wax suggests that carnauba wax can only possibly gel liquid oil at a higher concentration. This can be supported by a research done by Blake (2015) which stated that the crystal morphology has a significant impact on the wax concentration threshold required to gel a liquid oil where non needle-like crystal morphology such as that of carnauba wax would have a higher wax concentration threshold which would in turn require higher concentration of carnauba wax for gelation to occur.

2.3 Synergistic Effects of Oleogelators

Synergism involves the combination of two or more oleogelators to either enhance the properties of each of the oleogelators used or simply to complement the limiting properties of an oleogelator when it is used alone. Considering that different oleogelators are made up of differing composition of organic compounds, the properties that a single oleogelator can offer is very limited. Therefore, researchers have further looked into the possible combinations of oleogelators to produce a functional synergistic effect that can be beneficial to the resulting oleogel produced. Based on a research done by Xia and coworkers (2022) on the combination of carnauba wax and 1-docosanol as oleogel-based

nanoencapsulation, it was found that the combination of carnauba wax and 1-dososanol produced oleogel strength which was way higher than when either of the component was used alone. In another study conducted by Jeong and coworkers (2021) on the synergistic effects of bioactive compounds like β-carotene and glycerol monostearate with candelilla wax-sunflower oil based oleogel, it was found that the synergistic effects of the bioactive compounds contributed to an increase in hardness and antioxidant properties of the oleogel where both indicated lower values when the oleogel was not fortified with bioactive compounds or when only one of the bioactive compounds was used.

In this study, a combination of BW and carnauba wax was used to produce a hybrid oleogelator oleogel. This is particularly due to the fact that BW can gel liquid oil at low concentration but conversely, carnauba wax can only gel liquid oil at much higher wax concentration due to the differences in their crystal morphology. As a result, the synergistic effects of the combination of the two oleogelators were studied to find out if carnauba wax, when used in low concentration with BW can potentially gel liquid oil to reduce its wax concentration threshold for gelation.

2.4 Types of Liquid Vegetable Oil

The processing conditions as well as the raw materials used in the making of the oil can impact the fatty acids composition of the oil produced which also means that for the same type of oil, the fatty acid compositions could differ between

different brands, origin of the raw materials and manufacturers and this can result in differing gelation and other related physiochemical properties of the oleogels (Borriello et al, 2022). Besides, the type of oil used in gelation can also affect the visual aspect of the resulting oleogel, particularly in terms of the color and surface gloss (Yang et al, 2018). At present, majority of the researches focuses on edible plant-based oils which are the more abundant, cost-efficient and are popular household oils used. The use of RBO-based oleogels is still not as vastly investigated by most researchers and therefore there are still opportunity in figuring the potential of rice bran oil in oleogel production since rice bran oils are not considered a common household oil.

Rice bran oil is known to be rich in polyunsaturated fatty acids which are good sources of omega 6 fatty acids essential for brain function, growth and development as well as a source of bioactive compounds that has free radicals scavenging and anti-inflammatory properties (Pang et al, 2023). Rice bran oil is also significantly richer in unsaturated fatty acids, with about 47% monounsaturated fatty acids, 33% polyunsaturated fatty acids and 20% saturated fatty acids which makes rice bran oil a good choice of organic solvent to replace the trans fats and reduce the saturated fatty acids content in conventional solid fats (Wisetkomolmat et al, 2022).

2.5 Applications of Oleogels

In recent researches, scientists have embarked on various ventures on the possible applications of oleogels including the use of oleogel in food, pharmaceutical for delivery of medicinal drugs as well as the perfumery and cosmetics industry (Tan et al, 2023). In the food industry, oleogels are mainly developed in search of a better alternative against conventional solid fats which contain high amounts of trans and saturated fatty acids, provide encapsulation in food to prevent moisture or oil migration and to increase the shelf-life of liquid oils (Ghan et al, 2022). Although there are aims to make oleogels commercially available in the market for usage in food products, the properties of each oleogel system developed is still in a premature stage and require further optimization in terms of their physicochemical properties.

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

3.1.1 Preparation of Oleogel Samples

Refined, bleached and deodorized 1 litre of King Brand rice bran oil was purchased from Lotus' to be used as the choice of organic solvent. Food grade PW and BW were acquired from Shopee store (Take it Global Sdn Bhd) to be used as the oleogelators. The equipment required include metal pot, spatula, plastic storage containers, induction cooker, stopwatch, glass thermometer and analytical balance.

3.1.2 Differential Scanning Calorimetry (DSC)

To analyze the thermal behavior of RBO oleogels, a Mettler Toledo^{TM} Differential Scanning Calorimeter was used. The materials needed for this analysis comprised of the prepared oleogel samples, a total of 10 aluminum crucibles with lids and a hermetically sealed empty aluminum crucible whereas equipment and instrument involved were microbalance, forceps, punching needle and a crucible sealing press.

3.1.3 Microscopic Studies

ECLIPSE Ti2 inverted microscope was used for the analysis of the microstructure of the oleogel samples. The material required were the prepared oleogel samples and a total of 10 microscopic slides and cover slips. The apparatus and instrument needed for this analysis were 20 mL beakers and a hotplate respectively.

3.1.4 Rheological Analysis

A stress-controlled single head rheometer equipped with a Peltier plate temperature controller and a 40-mm parallel plate was used to perform various rheological tests on the oleogel samples. The materials needed were isopropanol for washing and cleaning between each sample, the prepared oleogel samples, 2-3 plastic spoons and some laboratory tissue paper.

3.1.5 Texture Analysis

To determine the hardness, adhesiveness and cohesiveness of the oleogel samples, a texture analyzer (TA-XT Plus) equipped with a 35-mm cylindrical probe. The materials required were the prepared oleogel samples, laboratory tissue paper, ruler as well as plastic spoons.

3.1.6 Oil Binding Capacity (OBC)

Hettich Universal 320 centrifuge machine was used to centrifuge the oleogel samples. The materials used include the prepared oleogel samples, Eppendorf tubes, 10 mL disposable plastic syringes and some laboratory tissue paper while the apparatus and instrument required for this analysis include 10 mL beakers, hotplate and analytical balance.

3.1.7 Slip Melting Point

The equipment and apparatus used in this analysis include a hotplate, retort stand, glass thermometer and 500 mL glass beaker. The materials used for this analysis include distilled water, ice, capillary tubes and the prepared oleogel samples.

3.1.8 Oxidative Stability Test (Peroxide Value)

The reagents and materials required for the test include chloroform, glacial acetic acid, saturated potassium iodide (KI) solution, 0.01 N of sodium thiosulfate solution, 1% of starch solution, distilled water, Whatman filter paper and the prepared oleogel samples. Equipment and apparatus used for the test were incubator, analytical balance, Erlenmeyer flasks, 100 mL measuring cylinder, 10 mL syringe, retort stand and burette.

3.1.9 Colorimetry

Minolta CR-400 colorimeter was utilized to measure the color of the oleogel samples. Material and equipment needed are the prepared oleogel samples and mini petri dishes respectively.

3.1.10 Gas Chromatography (GC)

To quantify the free fatty acids composition in the oleogel samples, control sample (commercial margarine) and the organic solvent (RBO), a Gas Chromatography-Flame Ionization Detector (GC-FID) was used. The reagents required include GC-grade hexane, 0.5 M sodium methoxide solution, saturated sodium chloride solution and distilled water. The materials and apparatus needed include the prepared oleogel samples, a 200 μL micropipette, micropipette tips, glass vials with cap, 5 mL measuring cylinders, glass test tubes with cap, Pasteur pipette, 1 μL manual GC syringe and test tube holder. Other equipment used include a vortex mixer and an analytical balance.

3.2 Methodology

3.2.1 Preparation of Oleogel Samples

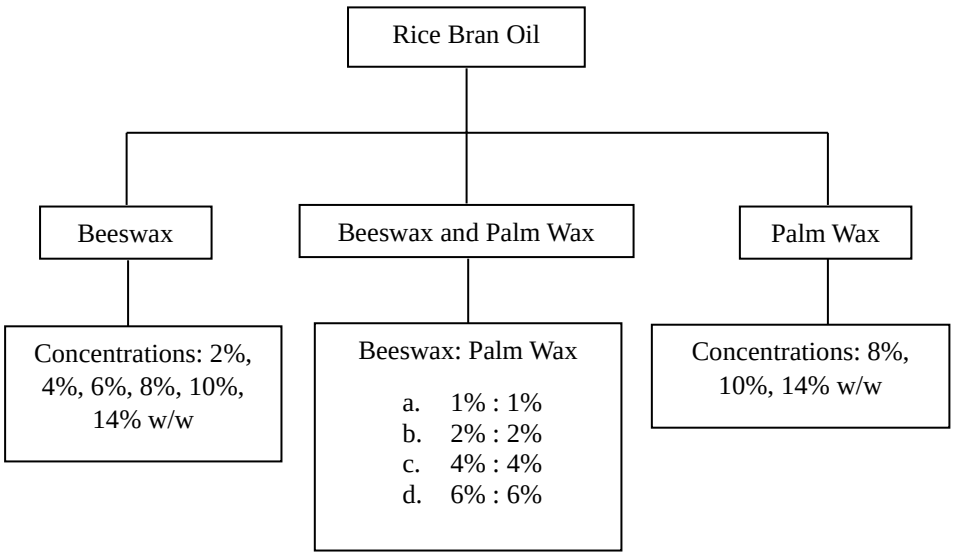


Figure 1: Preparation of oleogel samples based on the required combinations of RBO-oleogelator(s) and their respective % w/w concentrations.

Table 1: Weight of the respective wax and RBO required for each concentration of oleogel samples (100 g).

Concentration of Oleogel Samples (% w/w)	Weight of Wax (g)	Weight of RBO (g)
BW2	2.00	98.00
BW4	4.00	96.00
BW6	6.00	94.00
BW8	8.00	92.00
BW10	10.00	90.00
BW14	14.00	86.00
BW1PW1	1.00 BW + 1.00 PW	98.00
BW2PW2	2.00 BW + 1.00 PW	96.00
BW4PW4	4.00 BW + 4.00 PW	92.00
BW6PW6	6.00 BW + 6.00 PW	88.00
PW8	8.00	92.00
PW10	10.00	90.00
PW14	14.00	86.00

A total of 100 g of each concentration and combination of oleogels samples were prepared. Both the RBO and oleogelator(s) were weighed separately according to the % w/w of the oleogel sample needed. The RBO was first heated to 80℃ under stirring. Upon reaching the desired temperature, the weighed PW and BW were added slowly alone or in combination and mixed with the RBO to its complete dissolution. After the wax has completely dissolved in the RBO, the mixture was stirred continuously with a spatula without heating for a total of 3

minutes. The mixture was then transferred into labelled microwavable plastic storage containers and then stored at ambient temperature (25 ± 3℃) for 24 hours for gelation to occur until the samples are used to perform subsequent analysis.

3.2.2 Differential Scanning Calorimetry

To determine the thermal behavior of the oleogel samples, approximately 5 mg of oleogel sample was first weighed using a microbalance and loaded into aluminum crucible and hermetically sealed using a crucible sealing press. A reference sample comprised of an empty aluminum pas was used. The two aluminum pans (sample and reference) were then loaded into the furnace. The temperature was first increased to 80℃ from ambient temperature (25℃) and held for 5 minutes, then cooled to -40℃ at a cooling rate of 5℃/minute (Contreras-Ramirez et al, 2022). It was then held for 5 minutes at -40℃. After holding for 5 minutes at -40℃, the sample was then heated again at 5℃/minute, up to 80℃. Both the crystallization and melting curves were then obtained based on the thermogram generated for each oleogel sample. The crystallization and melting profiles of the RBO oleogels were then generated using the STARe Excellence Thermal Analysis Software.

3.2.3 Microscopic Studies

The oleogel samples were first melted using a hotplate. Upon melting, a thin smear of each oleogel sample was prepared on individual preheated microscopic

slides and the smear was then covered with a cover slip and labelled accordingly. Upon crystallization, the prepared samples were then viewed under the ECLIPSE Ti2 inverted microscope at 40× magnification to observe the microstructure of the crystals formed (Ghan et al, 2020).

3.2.4 Rheological Analysis

Shear Viscosity Test

The oleogel samples were first loaded onto the Peltier plate and the rheometer head was lowered slowly to compress the sample. Excess sample was scraped off using a plastic spoon carefully. The shear viscosity was then measured at 20℃ at an increasing shear rate of 0 to 100 s^{-1} (Fayaz et al, 2017). After each analysis, the oleogel samples were cleaned off from both the Peltier plate and the 40-mm parallel plate and then washed with isopropanol before loading the next sample. The graph of shear stress vs shear rate was obtained for all the samples for subsequent data analysis with a total of 3 repeats for each sample.

Temperature Sweep Test

The oleogel samples were first loaded onto the Peltier plate and the rheometer head was lowered slowly to compress the sample. Excess sample was scraped off using a plastic spoon carefully. The viscosity of the oleogel samples was then measured at a constant shear rate of 100 s^{-1} by increasing the temperature from 20℃ to 80℃ at a heating rate of 5℃/minute (Fayaz et al, 2017). After each analysis, the oleogel samples were cleaned off from both the Peltier plate and

the 40-mm parallel plate and then washed with isopropanol. Before loading the next sample, the temperature of the Peltier plate was made sure to have cooled down to 20℃. The graph of viscosity vs temperature was then obtained for all samples for subsequent data analysis with a total of 3 repeats for each sample.

Frequency Sweep Test

The oleogel samples were first loaded onto the Peltier plate and the rheometer head was lowered slowly to compress the sample. Excess sample was scraped off with a plastic spoon carefully. The viscosity of the oleogel samples was then measured at a strain of 0.1%, constant temperature of 20℃ and by increasing the angular frequency from 1 Hz to 100 Hz which was within the linear viscoelastic limit (Fayaz et al, 2017). After each analysis, the oleogel samples were cleaned off from both the Peltier plate and the 40-mm parallel plate and then washed with isopropanol before loading the next sample. Graphs plotting various moduli of viscoelastic properties of RBO oleogels vs angular frequency were then obtained for all samples for subsequent data analysis by conducting a total of 3 repeats.

Dynamic Temperature Ramp

The oleogel samples were first loaded onto the Peltier plate and the rheometer head was lowered slowly to compress the sample. Excess sample was scraped off with a plastic spoon carefully. The viscosity of the oleogel samples was measured at a strain of 0.1%, constant angular frequency of 1 Hz which was within the linear viscoelastic limit and by increasing the temperature from 20℃ to 80℃ at a heating rate of 5℃/minute (Fayaz et al, 2017). After each analysis,

the oleogel samples were cleaned off from both the Peltier plate and the 40-mm parallel plate and then washed with isopropanol. Before loading the next sample, the temperature of the Peltier plate was made sure to have cooled down to 20℃. Graphs plotting various moduli of viscoelastic properties of RBO oleogels vs temperature were then obtained for all samples for subsequent data analysis by conducting a total of 3 repeats.

3.2.5 Texture Analysis

Semi-solid and solid oleogel samples were analyzed for various characteristics including hardness, adhesiveness and cohesiveness. The oleogel samples (shaped into a 2 cm × 2 cm × 2 cm size using a plastic spoon) were loaded onto the loading platform. A 35-mm cylindrical probe was used for the compression test. Prior to analysis, the height and force of the instrument was calibrated using a 5 kg calibration weight. Each RBO sample was compressed using a 35 mm cylindrical probe twice at a penetration rate of 2 mm/s into a 12 mm depth to obtain the parameters of hardness, adhesiveness and cohesiveness and following that, the probe was removed at a returning speed of 2 mm/s (Ghan et al, 2020). A total of 3 repeats were carried out for each sample.

3.2.6 Oil Binding Capacity

The oleogel samples were first melted using a hotplate. After fully melting the samples, 1 mL of each oleogel sample was transferred into a tared transparent 15 mL Eppendorf conical tube, then capped and stored in the refrigerator for 1 hour. After gel formation, each tube was weighed again and the initial weight of each tube were recorded. The tubes were then centrifuged using the Dynamica Velocity 18R Pro centrifuge machine at 10000 rpm for 15 minutes. After centrifuging, the liquid oil released was drained off by inverting the Eppendorf tubes on a paper cloth. Finally, the tubes were weighed again and the final weight of the tubes were recorded. Based on the initial and final weight readings, the OBC values were calculated gravimetrically. A total of 3 repeats were carried out for each sample (Ogutcu and Yilmaz, 2014).

$$\textbf{Mass of released oil (g)} = \text{Initial weight of tube (g)} - \text{Final weight of tube (g)}$$

$$\textbf{Total mass of sample (g)} = \text{Inital weight of tube with containing sample (g)} - \text{Weight of empty tube (g)}$$

$$\%\ \textbf{Oil Released} = \frac{\text{Weight of released oil (g)}}{\text{Total Sample Weight (g)}} \times 100\%$$

$$\textbf{OBC} = 100 - \%\ \text{Oil released}$$

3.2.7 Slip Melting Point

The melted oleogel samples were each filled into a capillary tube to a 1 cm mark. After filling the capillary tubes, they were then stored in the freezer for 24 hours. After 24 hours, the tubes were taken out from the freezer for analysis. The capillary tube was attached to the bottom tip of a glass thermometer by tying up with a rubber band. The top of the thermometer was clamped using the retort stand and the bottom tip which is attached to the capillary tube was suspended into a beaker of ice distilled water bath that is placed on a hotplate. The water bath was subsequently heated and the temperature at which the oleogel column rises was recorded as the slip melting point. A total of 3 repeats were carried out for each sample (Latib et al, 2013).

3.2.8 Oxidative Stability Test (Peroxide Value)

The prepared oleogel samples as well as a control sample (margarine) were incubated under accelerated conditions at 60℃ in an incubator for a total of 20 days and titrations to measure the peroxide value of each oleogel sample was carried out every 4 days. Acetic acid-chloroform mixture was first prepared by mixing glacial acetic acid solution and chloroform solution in a ratio of 3:2. A total weight of 5 g of oleogel sample was weighed in an Erlenmeyer flask and the sample weight was recorded. After which, 30 mL of the acetic acid-chloroform mixture was added into the Erlenmeyer flask and the flask was swirled to mix and dissolve the sample. Once the sample has fully dissolved, 1 mL of saturated potassium iodide solution was added into the flask and the

mixture was then swirled for 1 minute. A resulting yellow solution is obtained. After 1 minute of swirling, 30 mL distilled water was added and the flask was swirled to homogenize the contents. A total volume of 0.5 mL of 1% starch solution was added to the mixture as indicator, forming an Emerald green solution. Before titration, the initial volume of the burette was recorded. The final solution was then titrated with 0.01 N sodium thiosulfate solution till the point where the solution turned from green to a milky white color (Horwitz & Official Methods of Analysis of AOAC International, 2002). The final burette reading was then recorded and the peroxide value for each oleogel sample was calculated using the formula shown below. Titration for each sample was conducted with 3 repeats.

$$\text{Peroxide Value} = \frac{\text{V} \times \text{N} \times 1000}{\text{Ws}}$$

where:

Peroxide Value = milliequivalents (mEq) peroxide per kg of sample

Ws: sample weight (g)

N: normality of sodium thiosulfate ($Na_2S_2O_3$) solution

V: volume of sodium thiosulfate ($Na_2S_2O_3$) solution used (mL)

3.2.9 Colorimetry

The instrument was first calibrated and zeroed before taking measurements. Liquid or semi-solid oleogel samples were filled into mini petri dishes whereas measurements for solid samples were taken from an even and flat surface

directly from the sample container. The color of the RBO oleogels were measured using Minolta CR-400 colorimeter and all measurements were done in three repeats (Kim et al, 2022).

3.2.10 Gas Chromatography

Preparation of Methyl Esters

A total of 100 mg (± 5 mg) of oleogel sample was weighed into a glass test tube and capped. A total volume of 5 mL of hexane was then added to the test tube, capped and the sample was then vortexed or swirled to fully dissolve the sample. Following that, 250 μL of sodium methoxide was transferred into the test tube and capped. The resulting mixture in the test tube was then vortexed for 1 minutes while pausing every 10 seconds to allow the vortex to collapse. Next, 5 mL of saturated sodium chloride solution was added into the test tube. The test tube was then capped and shaken vigorously for 15 seconds. The mixture was allowed to sit for 10 minutes for the formation of an interphase. After 10 minutes, 2 mL of the upper hexane layer was removed and transferred into a vial containing 2 mL of hexane solution to dilute the methyl esters. The vial was capped and inverted to mix the contained mixture. The resulting mixture was then used for subsequent GC analysis (Salimon, Omar and Salih, 2014).

Injection of Derivatized Sample into GC

The GC was equipped with a flame ionization detector (FID) and a BPX 70 column (60 m × 0.25 mm × 0.25 μm) to obtain the fatty acid profile of the oleogel samples, RBO and control sample (margarine). The injector port was set at 220℃

while the FID temperature was set at 250℃. Helium was used as the carrier gas with a flow rate of 23 mL/minute and nitrogen was used as the makeup gas with a flow rate of 20 mL/minute. The starting column temperature was set at 115℃ which was programmed to 180℃ at a heating rate of 8℃/minute (Machado et al, 2023). Before sample injection, the injection syringe was rinsed thrice with hexane and thrice with the sample solution. A total volume of 2 µL of sample was drawn using the glass syringe and injected into the column through the injection port. After injecting the sample, the syringe was ejected and the start button was pressed to initiate the analysis. The used syringe was rinsed with hexane before being used for the next sample. A chromatogram indicating the fatty acid composition was generated for each sample for subsequent analysis and the percentage free fatty acid (% FFA) can be calculated as follows:

$$\% \text{ FFA} = \frac{\text{Area of Peak}}{\text{Total Area}} \times 100\%$$

3.3 Statistical Analysis

One way analysis of variance (ANOVA) was determined using version 26 of IBM Statistical Package for Social Sciences (SPSS) and the differences between the mean values for each type of analysis were compared at a confidence level of 95% using the Tukey's test.

CHAPTER 4

RESULTS

4.1 Thermal Behavior of RBO Oleogels

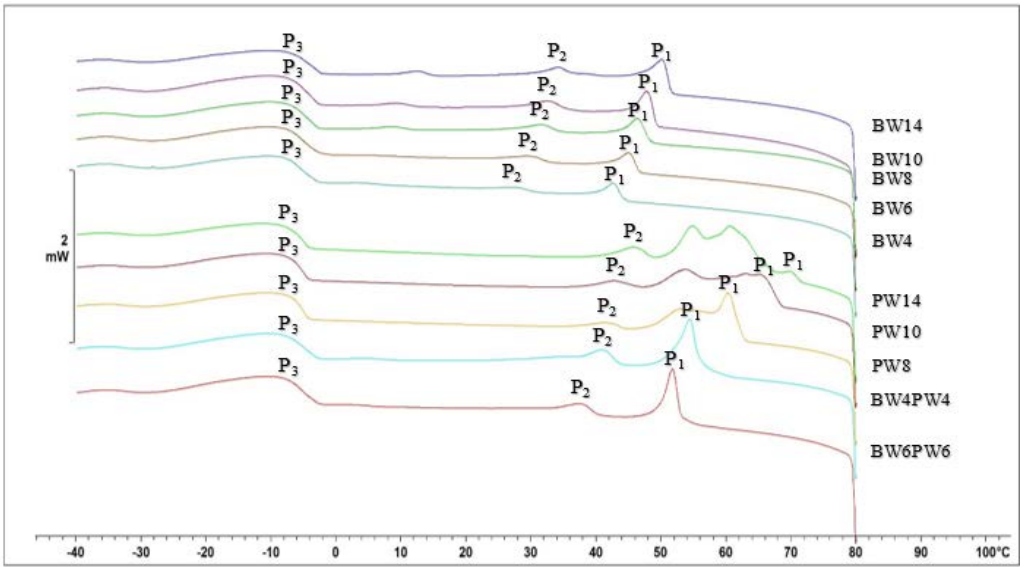


Figure 2: Crystallization profile of natural waxes-incorporated rice-bran oil based oleogels.

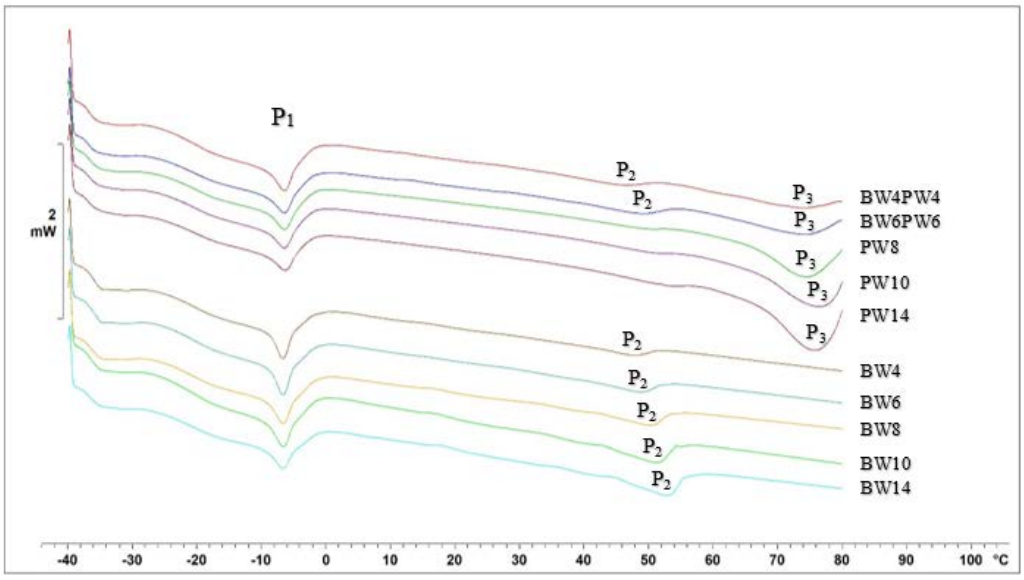


Figure 3: Melting profile of natural waxes-incorporated RBO-based oleogels.

Table 2: Crystallization onset temperature (T_{oc}), crystallization offset temperature (T_{fc}), enthalpy (ΔH_c) of RBO oleogel samples at 5°C/min interval.

Oleogel Samples	Transition Temperature (°C)			T_{oc} (°C)	T_{fc} (°C)	ΔH_c (J/g)
	P_1	P_2	P_3			
BW4PW4	51.79	37.95	-9.22	53.03	-26.52	12.95
BW6PW6	54.46	41.11	-9.22	55.99	-26.26	17.01
BW4	42.61	28.11	-8.81	44.25	-24.79	10.60
BW6	45.11	30.11	-9.22	46.52	-26.58	13.10
BW8	46.45	31.77	-8.56	48.27	-25.91	10.58
BW10	47.79	32.78	-8.88	49.25	-26.65	14.30
BW14	50.11	34.28	-8.56	51.50	-24.70	11.32
PW8	60.45	41.77	-8.89	62.43	-25.76	19.77
PW10	65.61	42.78	-8.89	68.36	-25.13	13.97
PW14	70.02	45.78	-9.89	71.38	-27.41	14.84

Table 3: Melting onset temperature (T_{om}), melting offset temperature (T_{fm}) and enthalpy (ΔH_m) of RBO oleogel samples at 5°C/min interval.

Oleogel Samples	Transition Temperature (°C)			T_{om} (°C)	T_{fm} (°C)	ΔH_m (J/g)
	P1	P2	P3			
BW4PW4	-6.46	46.37	73.86	-11.56	78.92	-14.57
BW6PW6	-6.53	49.21	74.53	-12.80	79.15	-17.19
BW4	-6.79	48.20	-	-10.15	50.68	-13.68
BW6	-6.71	48.53	-	-10.18	51.58	-15.16
BW8	-6.70	50.20	-	-11.30	52.96	-16.41
BW10	-6.71	51.19	-	-11.36	53.77	-16.62
BW14	-6.78	53.03	-	-11.66	55.03	-14.58
PW8	-6.45	-	66.44	-12.18	79.52	-7.78
PW10	-6.45	-	74.60	-12.43	79.51	-12.42
PW14	-6.37	-	76.42	-12.60	79.92	-11.79

*** -** indicates the absence of the peak(s) in the thermogram generated

4.2 Textural Analysis

Table 4: Effect of the type of wax, concentration of wax and combination of waxes on the mechanical textural properties (hardness, adhesiveness and cohesiveness) of RBO oleogels in comparison to a control sample (margarine).

Samples	Hardness	Adhesiveness	Cohesiveness
BW2	t.l	t.l	t.l
BW4	$127.87^{d} \pm 9.12$	$-106.46^{a} \pm 0.49$	$0.552^{a} \pm 0.003$
BW6	$159.69^{d} \pm 17.86$	$-149.13^{a} \pm 10.33$	$0.432^{abc} \pm 0.057$
BW8	$249.02^{cd} \pm 26.10$	$-243.80^{a} \pm 55.77$	$0.372^{bcde} \pm 0.075$
BW10	$369.58^{bc} \pm 60.14$	$-277.20^{ab} \pm 45.78$	$0.441^{abc} \pm 0.004$
BW14	$277.00^{cd} \pm 17.15$	$-222.86^{a} \pm 18.52$	$0.460^{ab} \pm 0.001$
BW1PW1	t.l	t.l	t.l
BW2PW2	t.l	t.l	t.l
BW4PW4	$558.89^{a} \pm 28.93$	$-333.37^{ab} \pm 1.35$	$0.277^{def} \pm 0.011$
BW6PW6	$537.55^{ab} \pm 35.66$	$-183.30^{a} \pm 87.51$	$0.182^{f} \pm 0.060$
PW8	$245.54^{cd} \pm 62.85$	$-91.26^{a} \pm 4.04$	$0.254^{ef} \pm 0.020$
PW10	$250.76^{cd} \pm 41.90$	$-145.44^{a} \pm 20.73$	$0.310^{cdef} \pm 0.001$
PW14	$560.33^{a} \pm 102.36$	$-244.36^{a} \pm 104.98$	$0.248^{ef} \pm 0.021$
Control	$692.45^{a} \pm 60.52$	$-701.38^{b} \pm 10.04$	$0.409^{bcd} \pm 0.011$

* t.l represents take liquid in which measurements for liquid samples are not taken

* mean values of the same superscripted alphabets do not have significant differences and vice versa

4.3 Colorimetry

Table 5: Effect of the type of wax, concentration of wax and wax combinations on the color of RBO oleogels in comparison to a control sample (margarine).

Sample	Color Parameters		
	L*	**a***	**b***
BW2	$51.16^{de} \pm 0.11$	$-0.40^{bc} \pm 0.03$	$7.35^{ef} \pm 0.63$
BW4	$34.42^{h} \pm 1.04$	$-0.71^{cd} \pm 0.22$	$6.64^{f} \pm 0.34$
BW6	$44.35^{fg} \pm 0.90$	$-1.53^{ef} \pm 0.03$	$10.54^{de} \pm 0.41$
BW8	$52.40^{d} \pm 0.50$	$-2.12^{fgh} \pm 0.15$	$13.10^{cd} \pm 0.18$
BW10	$59.70^{c} \pm 0.31$	$-2.75^{h} \pm 0.06$	$15.62^{bc} \pm 0.20$
BW14	$69.56^{b} \pm 0.37$	$-3.52^{i} \pm 0.07$	$14.87^{c} \pm 0.06$
BW1PW1	$48.11^{ef} \pm 0.77$	$0.04^{b} \pm 0.12$	$6.44^{f} \pm 0.36$
BW2PW2	$45.81^{fg} \pm 4.25$	$-0.86^{cd} \pm 0.41$	$7.53^{ef} \pm 2.55$
BW4PW4	$42.76^{g} \pm 0.47$	$-2.68^{h} \pm 0.26$	$9.00^{ef} \pm 0.48$
BW6PW6	$52.92^{d} \pm 0.39$	$-3.81^{i} \pm 0.14$	$13.40^{cd} \pm 0.32$
PW8	$52.69^{de} \pm 0.22$	$-1.07^{de} \pm 0.21$	$10.48^{de} \pm 0.19$
PW10	$52.32^{d} \pm 1.27$	$-1.87fg \pm 0.50$	$14.78^{c} \pm 2.62$
PW14	$57.19^{c} \pm 1.75$	$-2.47^{gh} \pm 0.16$	$18.36^{b} \pm 1.09$
Control	$86.67^{a} \pm 0.23$	$3.94^{a} \pm 0.04$	$39.74^{a} \pm 0.58$

* mean values of the same superscripted alphabets do not have significant differences and vice versa

4.4 Slip Melting Point Analysis

Table 6: Effect of the type of wax, concentration of wax and combination of waxes on the slip melting point of RBO oleogels.

Sample	Slip Melting Point (°C)
BW2	$25.0^{g} \pm 0.00$
BW4	$38.2^{e} \pm 0.76$
BW6	$45.0^{cd} \pm 0.50$
BW8	$47.0^{bc} \pm 0.00$
BW10	$48.2^{bc} \pm 0.76$
BW14	$49.8^{b} \pm 0.76$
BW1PW1	$25.8^{g} \pm 0.76$
BW2PW2	$32.7^{f} \pm 1.53$
BW4PW4	$43.5^{d} \pm 0.50$
BW6PW6	$46.2^{cd} \pm 1.26$
PW8	$30.0^{f} \pm 2.65$
PW10	$50.0^{b} \pm 1.73$
PW14	$54.0^{a} \pm 1.00$

* mean values of the same superscripted alphabets do not have significant differences and vice versa

4.5 Oxidative Stability Test

Table 7: The influence on the oxidative stability of the RBO oleogels stored under accelerated conditions over a span of 20 days determined using the peroxide value test.

Samples	Peroxide Value				
	Day				
	4^{d}	**8^{c}**	**12^{b}**	**16^{a}**	**20^{a}**
BW2	8.38^{a} ± 0.19	20.35^{ab} ± 4.21	43.45^{a} ± 6.64	57.68^{a} ± 4.02	59.79^{ab} ± 14.41
BW4	7.26^{ab} ± 0.47	22.00^{a} ± 1.76	27.57^{abcd} ± 8.59	49.65^{ab} ± 11.34	72.45^{a} ± 11.06
BW6	5.24^{cd} ± 0.30	15.29^{abc} ± 4.47	22.81^{bcd} ± 9.46	40.07^{bcd} ± 4.50	45.15^{bc} ± 10.23
BW8	8.08^{a} ± 0.28	20.58^{ab} ± 6.54	29.78^{abc} ± 0.58	36.34^{bcde} ± 4.85	42.25^{bcd} ± 8.67
BW10	5.02^{de} ± 0.41	12.91^{abc} ± 2.42	20.29^{bcd} ± 3.28	25.38^{def} ± 3.42	21.40^{def} ± 5.82
BW14	2.52^{f} ± 0.11	9.07^{c} ± 2.04	14.63^{cd} ± 3.54	25.43^{def} ± 2.73	15.89^{f} ± 4.13
BW1PW1	5.57^{cd} ± 0.38	18.44^{abc} ± 1.72	23.18^{bcd} ± 11.00	49.16^{ab} ± 6.56	39.56^{bcde} ± 9.52
BW2PW2	6.73^{abc} ± 0.50	16.14^{abc} ± 5.35	20.15^{bcd} ± 5.17	43.64^{abc} ± 8.00	30.19^{cdef} ± 7.04
BW4PW4	6.21^{bcd} ± 0.58	10.09^{bc} ± 2.97	33.07^{ab} ± 3.10	28.69^{def} ± 3.77	20.35^{def} ± 3.74
BW6PW6	3.51^{ef} ± 0.29	10.95^{bc} ± 2.96	12.02^{d} ± 2.61	22.09^{ef} ± 2.84	15.75^{f} ± 1.29
PW8	5.83^{bcd} ± 0.48	11.00^{bc} ± 3.45	11.70^{d} ± 5.13	29.25^{cdef} ± 1.79	26.90^{cdef} ± 9.72
PW10	6.03^{bcd} ± 0.40	14.68^{abc} ± 1.83	14.44^{cd} ± 3.68	27.63^{def} ± 1.20	26.42^{cdef} ± 2.08
PW14	5.17^{cde} ± 1.01	8.89^{c} ± 2.60	17.28^{bcd} ± 1.40	25.10^{ef} ± 1.80	17.21^{ef} ± 3.60
Control	5.47^{cd} ± 1.22	18.00^{abc} ± 2.91	14.15^{cd} ± 3.55	15.72^{f} ± 2.14	13.75^{f} ± 1.26

* mean values of the same superscripted alphabets do not have significant differences and vice versa

4.6 Oil Binding Capacity

Table 8: Effect of the type of wax, concentration of wax and combination of waxes on the OBC of RBO oleogels.

Sample	Oil Binding Capacity (%)
BW2	$97.41^{a} \pm 0.0167$
BW4	$99.44^{a} \pm 0.0049$
BW6	$99.81^{a} \pm 0.0033$
BW8	$100.00^{a} \pm 0.000$
BW10	$99.75^{a} \pm 0.0043$
BW14	$99.69^{a} \pm 0.0054$
BW1PW1	$73.55^{b} \pm 0.0566$
BW2PW2	$98.28^{a} \pm 0.0140$
BW4PW4	$99.54^{a} \pm 0.0043$
BW6PW6	$97.61^{a} \pm 0.0346$
PW8	$95.43^{a} \pm 0.0082$
PW10	$94.65^{a} \pm 0.0181$
PW14	$99.45^{a} \pm 0.0055$

* mean values of the same superscripted alphabets do not have significant differences and vice versa

4.7 Fatty Acid Composition

Table 9: Fatty acid compositions of RBO oleogels constructed with BW, PW and a combination of BW and PW.

Sample	% FFA								
	Myristic Acid	Palmitic Acid	Stearic Acid	Oleic Acid	Vaccenic Acid	Linoleic Acid	Linolenic Acid	Arachidic Acid	Lauric Acid
BW2	0.43	17.32	2.05	39.26	2.64	30.67	2.78	0.41	-
BW4	1.33	18.63	2.15	40.84	1.49	31.15	2.50	0.60	-
BW6	1.64	18.59	2.11	41.20	1.27	31.11	1.86	0.58	-
BW8	0.23	20.19	2.28	42.74	0.14	31.71	2.00	0.48	-
BW10	1.75	18.30	2.56	40.51	1.49	30.59	2.28	0.76	-
BW14	0.64	19.68	2.49	41.61	1.05	31.18	2.14	0.55	-
BW1 PW1	0.39	23.05	1.93	39.94	0.92	31.20	1.79	0.39	-
BW2 PW2	0.15	20.35	2.11	42.50	0.07	32.28	1.88	0.49	-
BW4 PW4	0.21	20.46	2.20	42.22	0.11	32.21	1.88	0.50	-
BW6 PW6	0.16	20.28	2.28	42.34	0.11	32.23	1.90	0.53	-
PW8	0.85	18.93	2.05	41.29	1.01	32.40	2.15	0.48	-
PW10	0.42	20.41	2.04	41.26	0.95	32.08	1.94	0.47	-
PW14	1.07	18.37	2.01	41.13	1.27	32.41	2.19	0.48	-
RBO	2.43	17.32	2.05	39.26	2.64	30.67	2.78	0.41	-
Control	3.31	35.18	5.54	34.20	2.66	13.12	1.05	0.00	4.94

* - denotes the absence of FFA in the sample tested

4.8 Rheological Analysis

Frequency Sweep Test

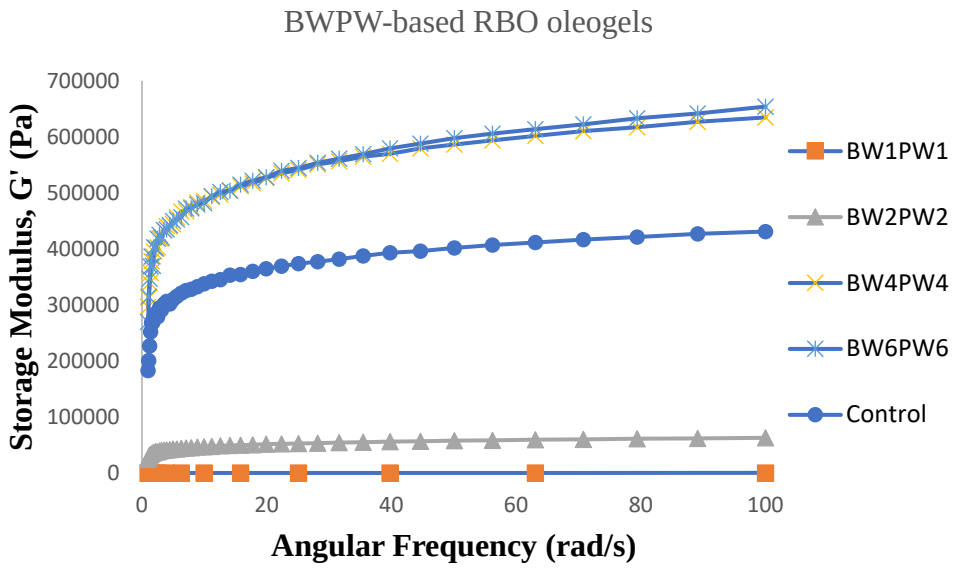


Figure 4: Changes in the storage modulus of the hybrid wax-based RBO oleogels over angular frequency.

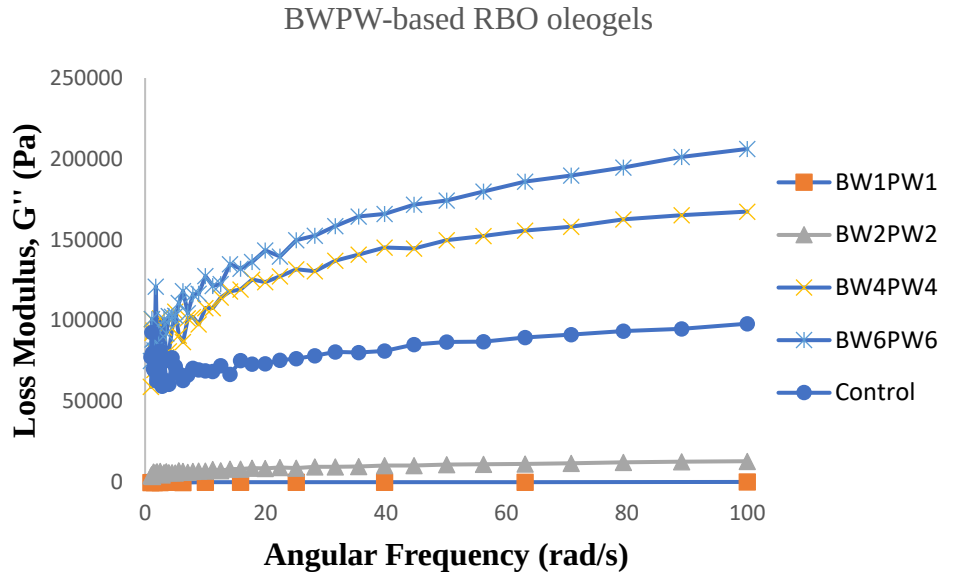


Figure 5: Changes in the loss modulus of the hybrid wax-based RBO oleogels over angular frequency.

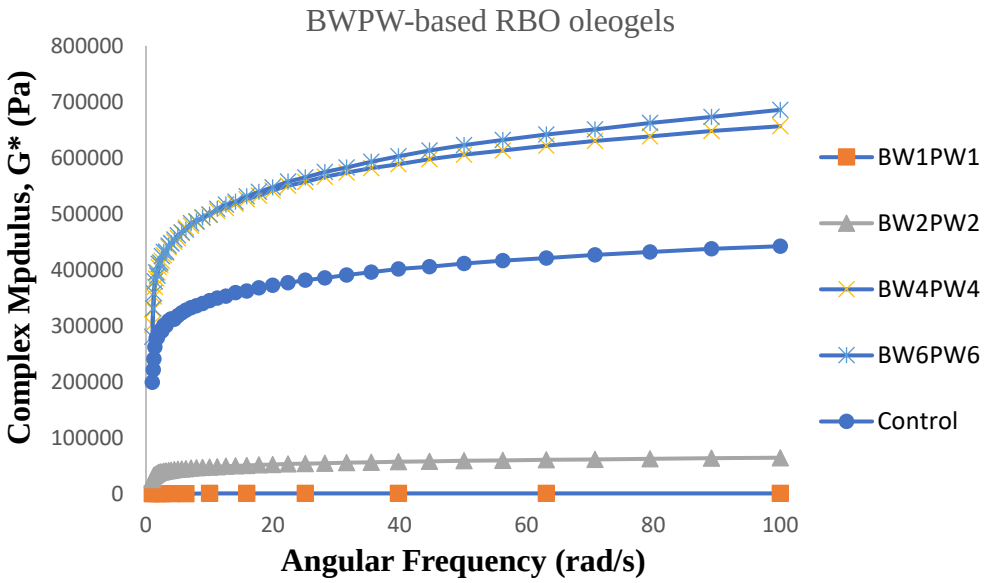


Figure 6: Changes in the complex modulus of the hybrid wax-based RBO oleogels over angular frequency.

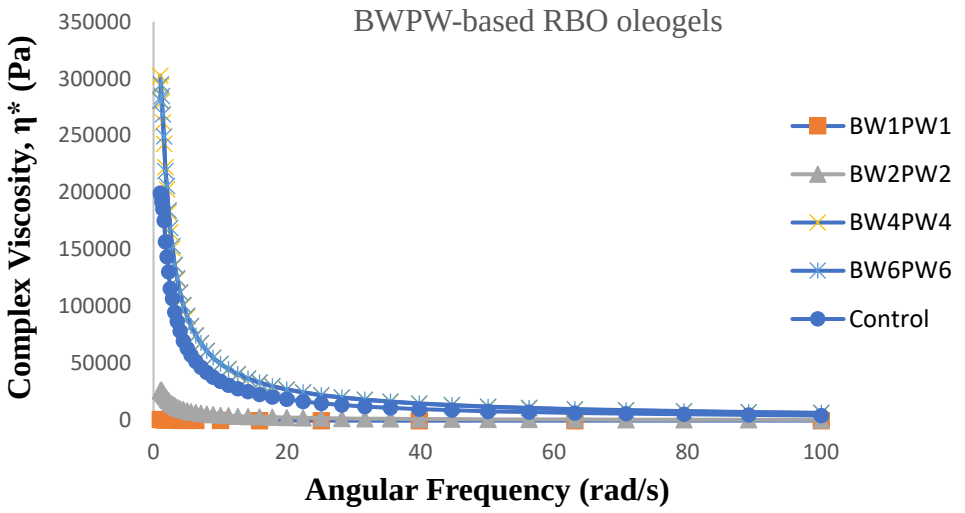


Figure 7: Changes in the complex viscosity of the hybrid wax-based RBO oleogels over angular frequency.

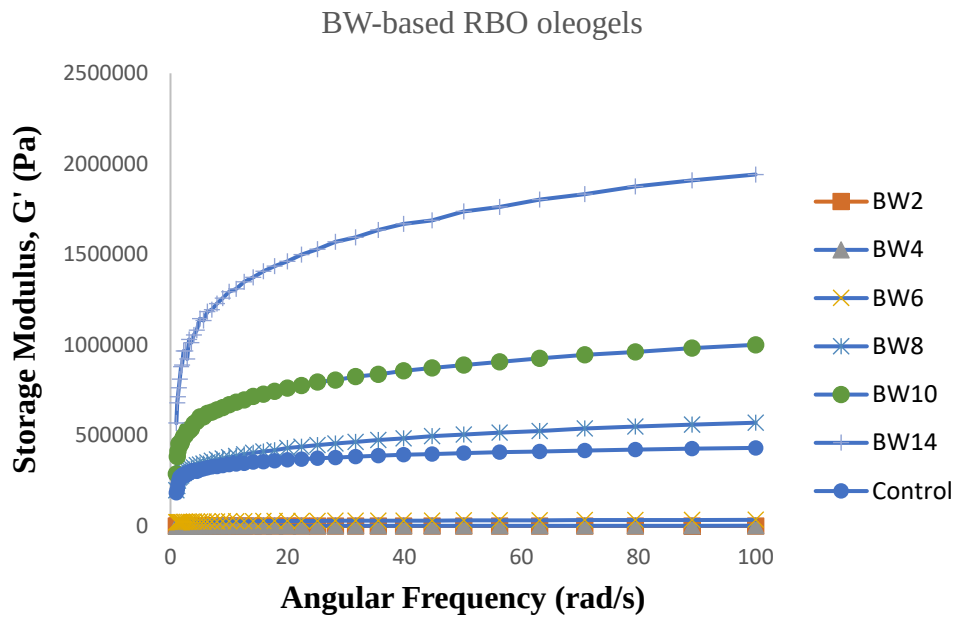


Figure 8: Changes in the storage modulus of the BW-based RBO oleogels over angular frequency.

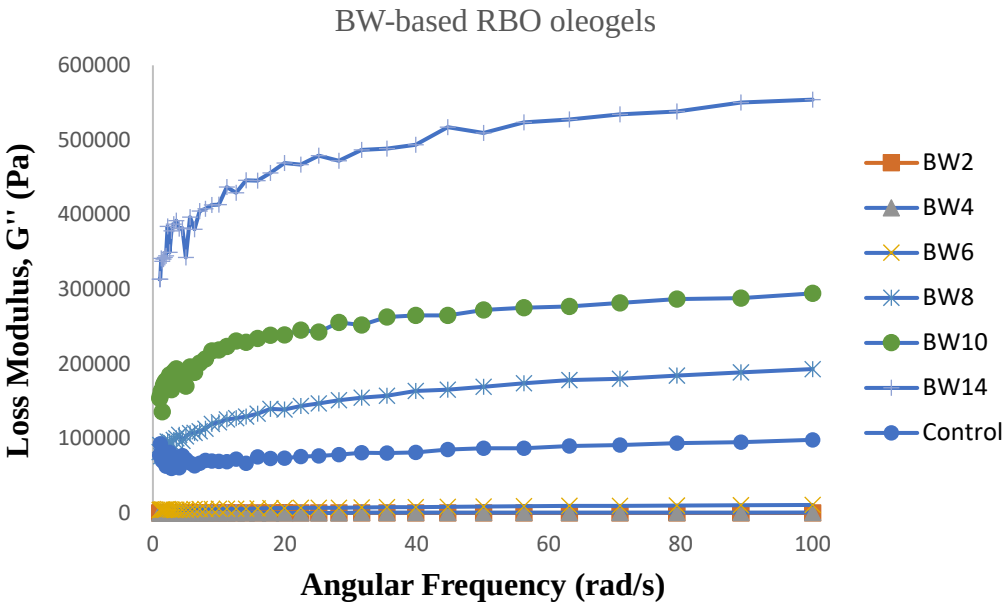


Figure 9: Changes in the loss modulus of the BW-based RBO oleogels over angular frequency.

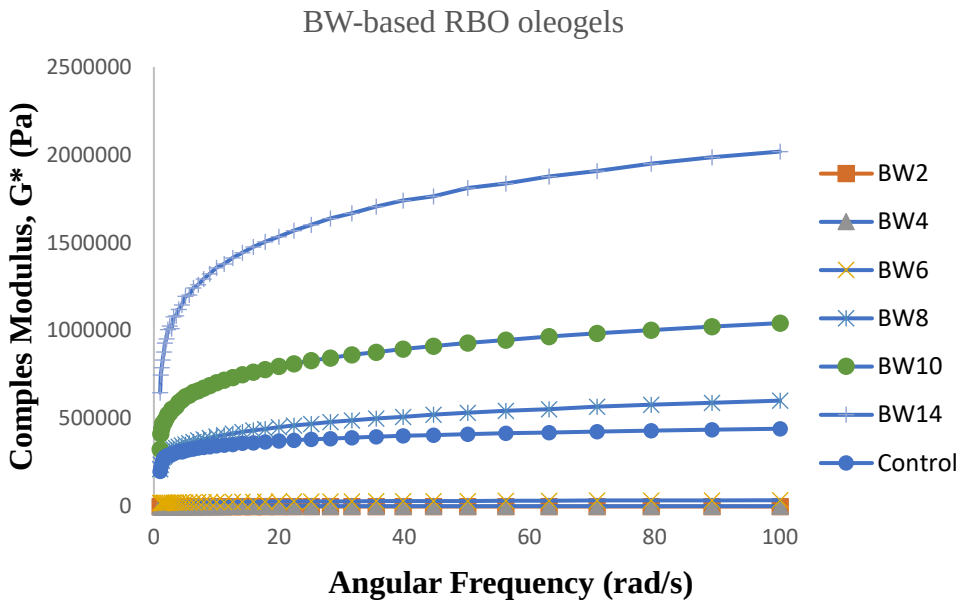


Figure 10: Changes in the complex modulus of the BW-based RBO oleogels over angular frequency.

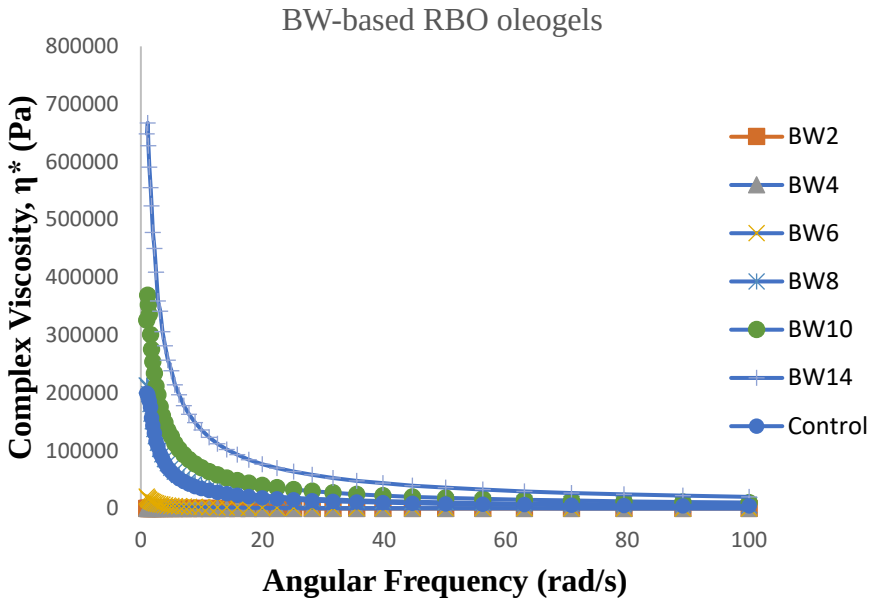


Figure 11: Changes in the complex viscosity of the BW-based RBO oleogels over angular frequency.

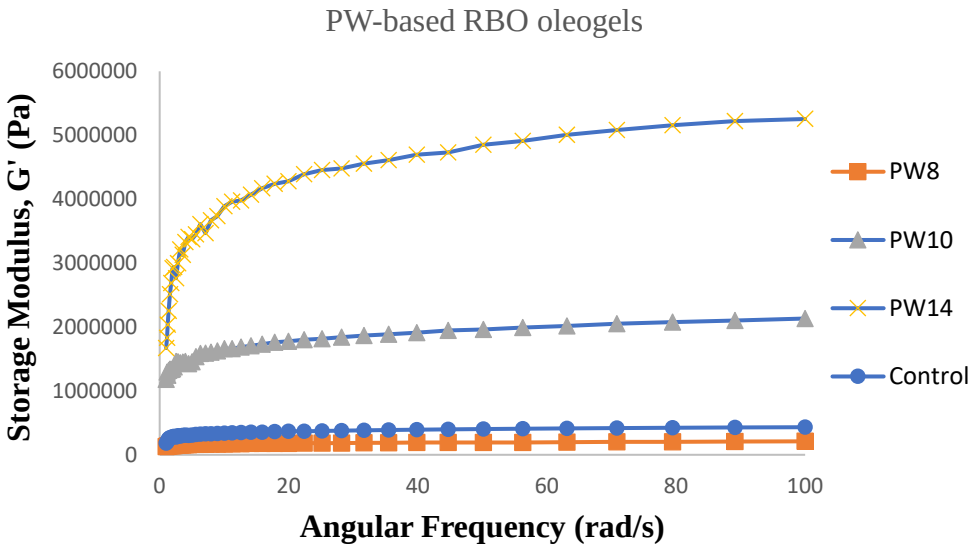


Figure 12: Changes in the storage modulus of the PW-based RBO oleogels over angular frequency.

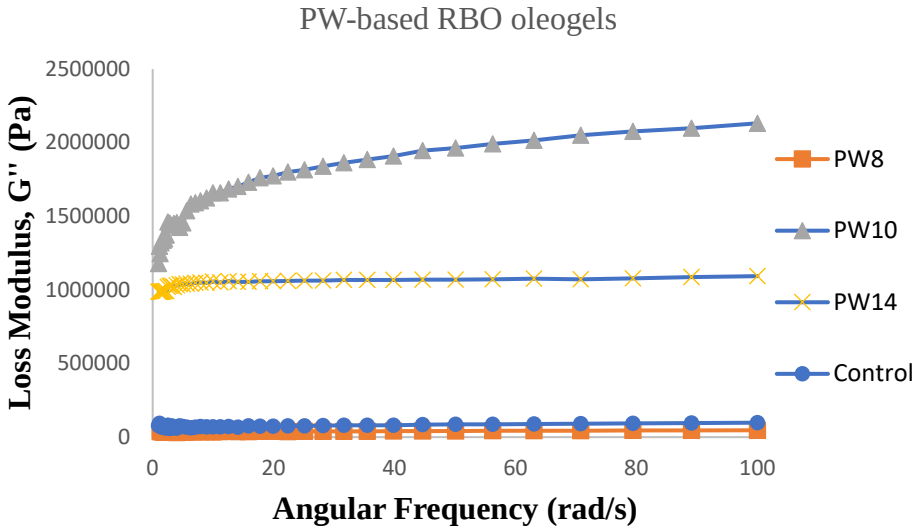


Figure 13: Changes in the loss modulus of the PW-based RBO oleogels over angular frequency.

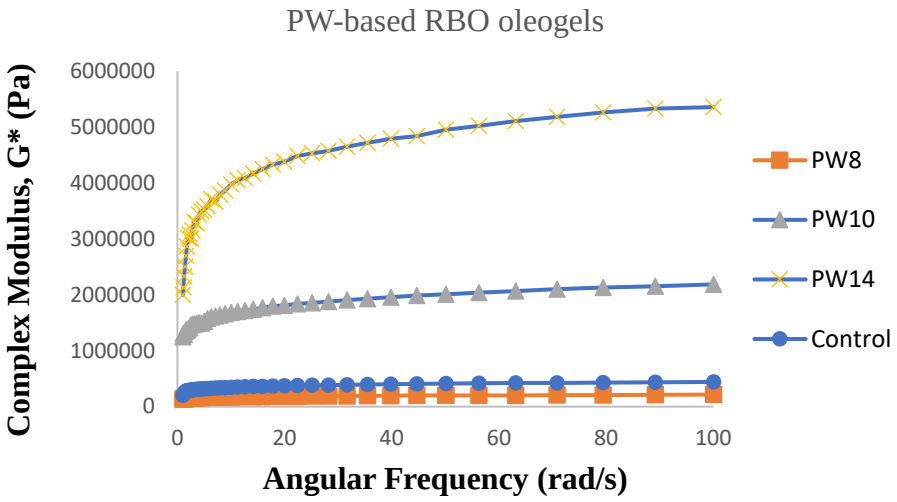


Figure 14: Changes in the complex modulus of the PW-based RBO oleogels over angular frequency.

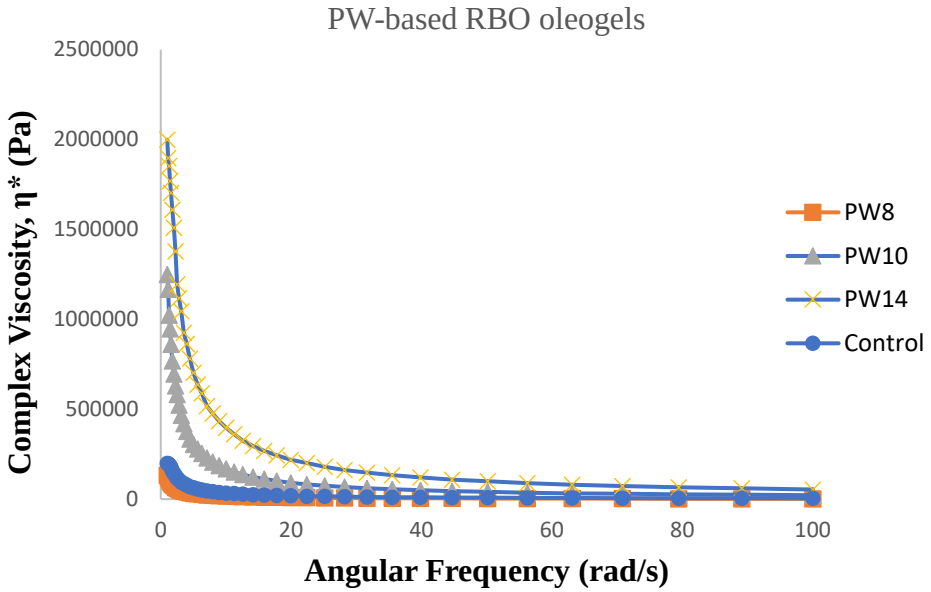


Figure 15: Changes in the complex viscosity of the PW-based RBO oleogels over angular frequency.

Dynamic Temperature Ramp

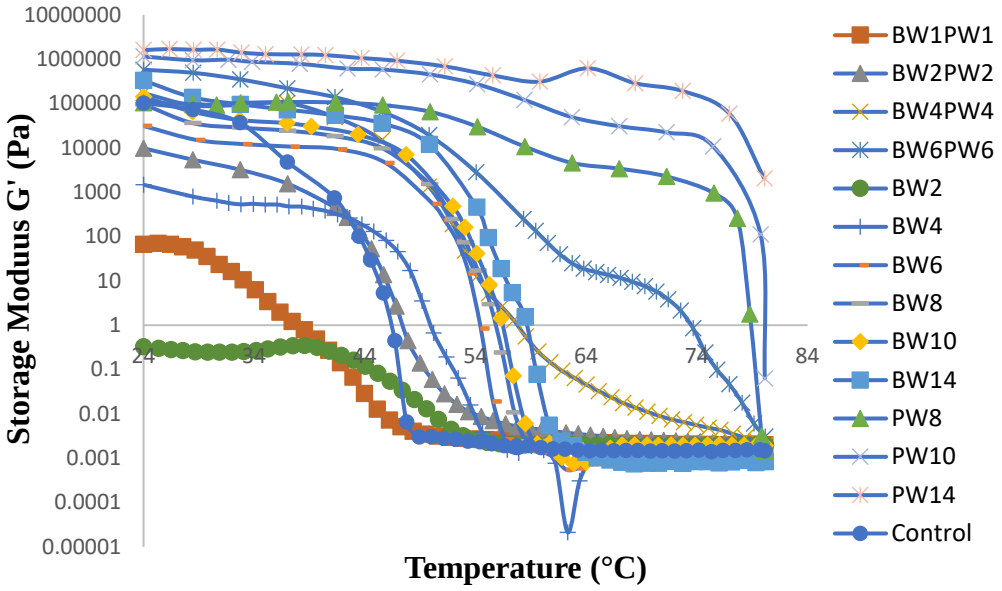


Figure 16: Changes in the storage modulus of the RBO-based oleogels structured with different concentrations and combinations of waxes over temperature.

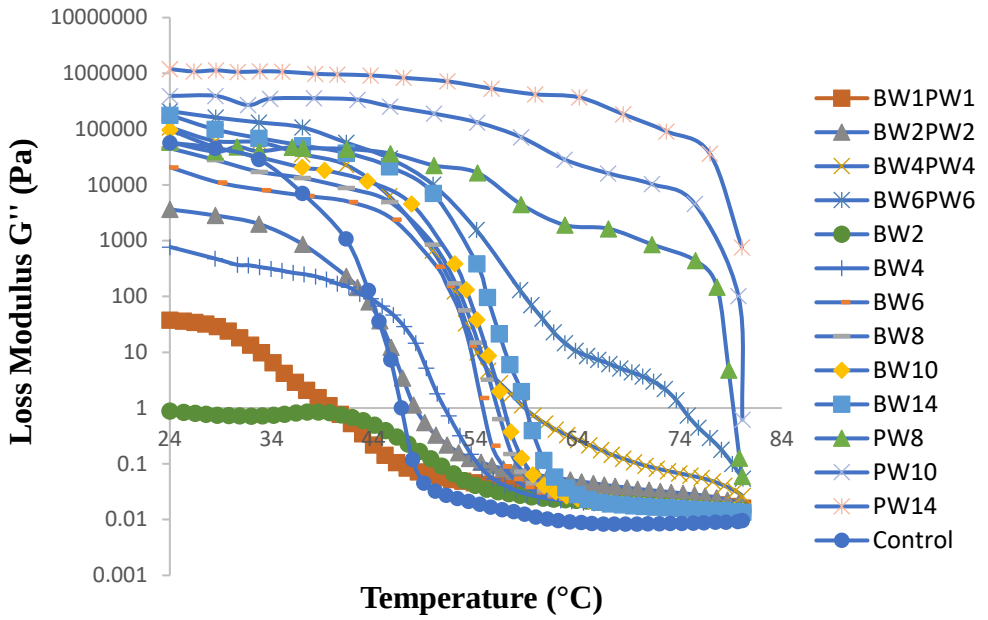


Figure 17: Changes in the loss modulus of the RBO-based oleogels structured with different concentrations and combinations of waxes over temperature.

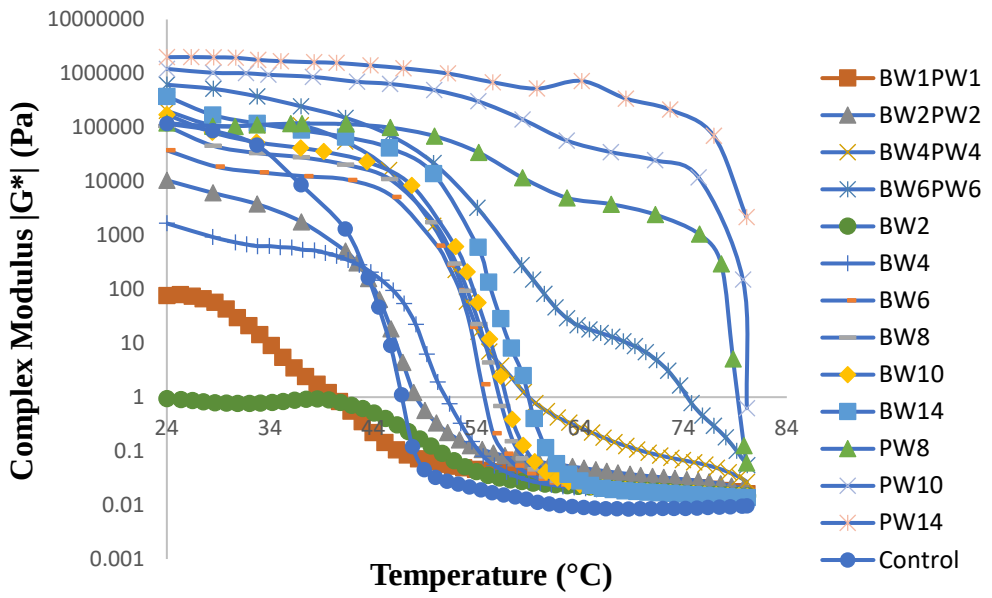


Figure 18: Changes in the complex modulus of the RBO-based oleogels structured with different concentrations and combinations of waxes over temperature.

Temperature Sweep Test

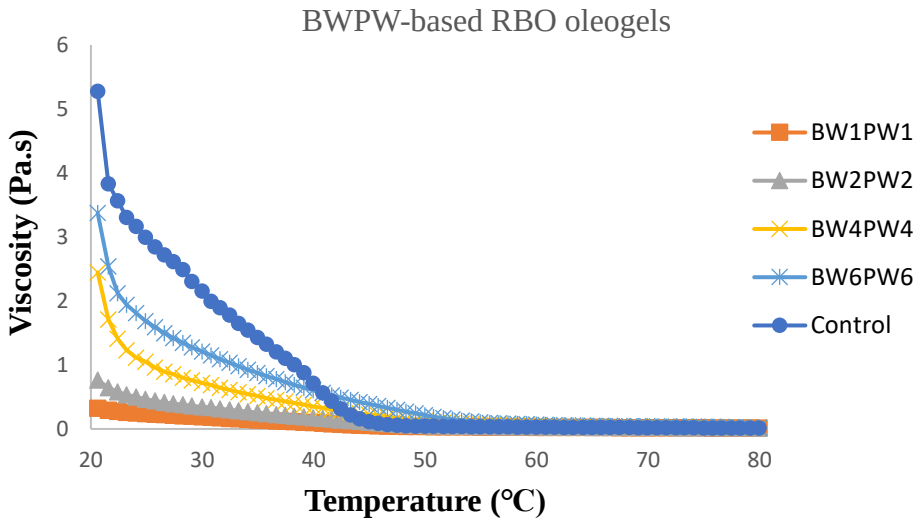


Figure 19: Changes in viscosity of the hybrid wax-based oleogels structured over temperature.

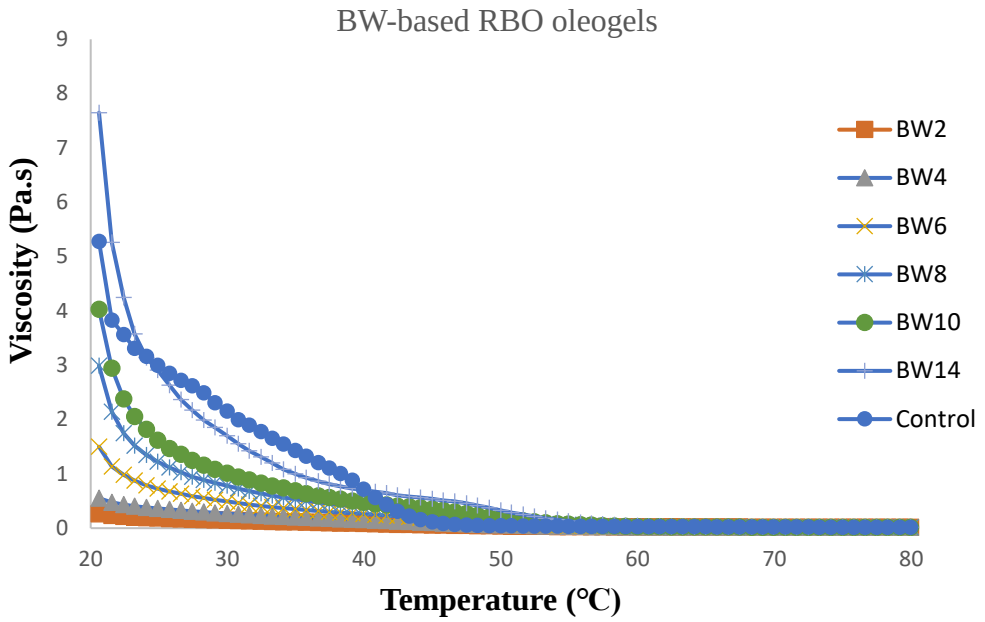


Figure 20: Changes in viscosity of the BW-based oleogels structured over temperature.

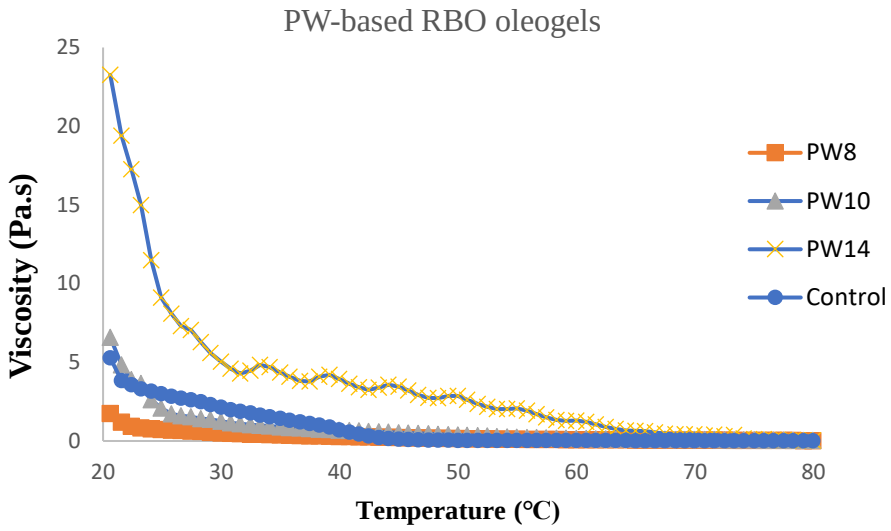


Figure 21: Changes in viscosity of the PW-based oleogels structured over temperature.

Shear Viscosity

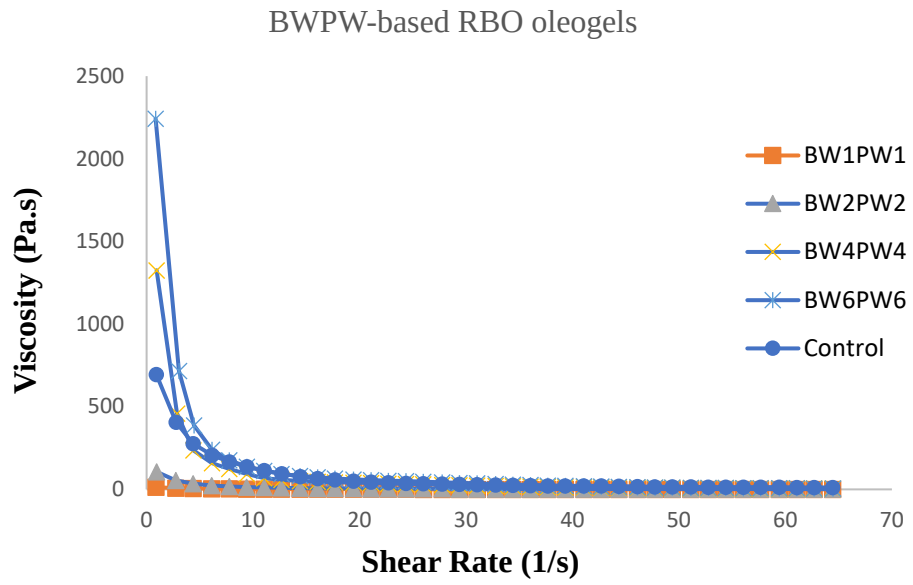


Figure 22: Changes in viscosity in hybrid wax-based RBO oleogels over shear rate.

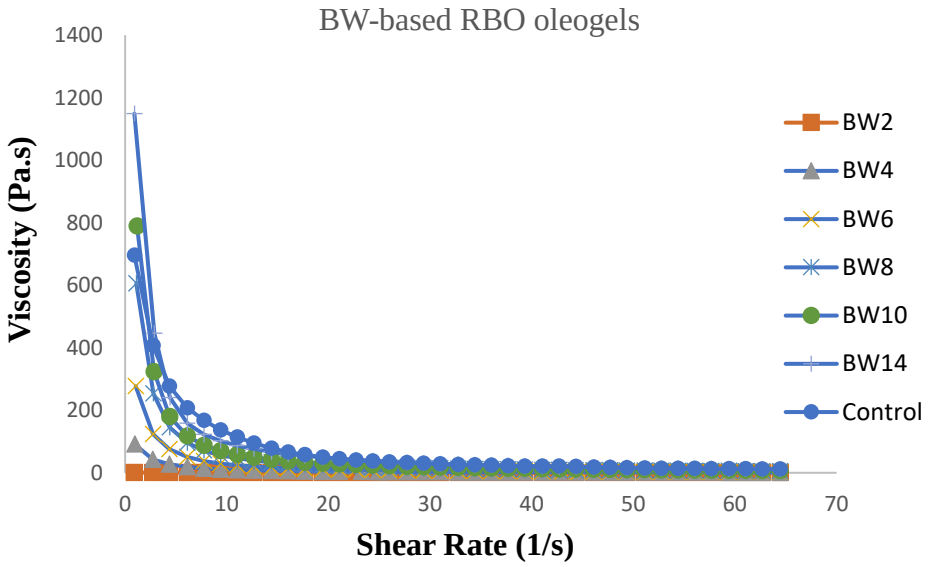


Figure 23: Changes in viscosity in BW-based RBO oleogels over shear rate.

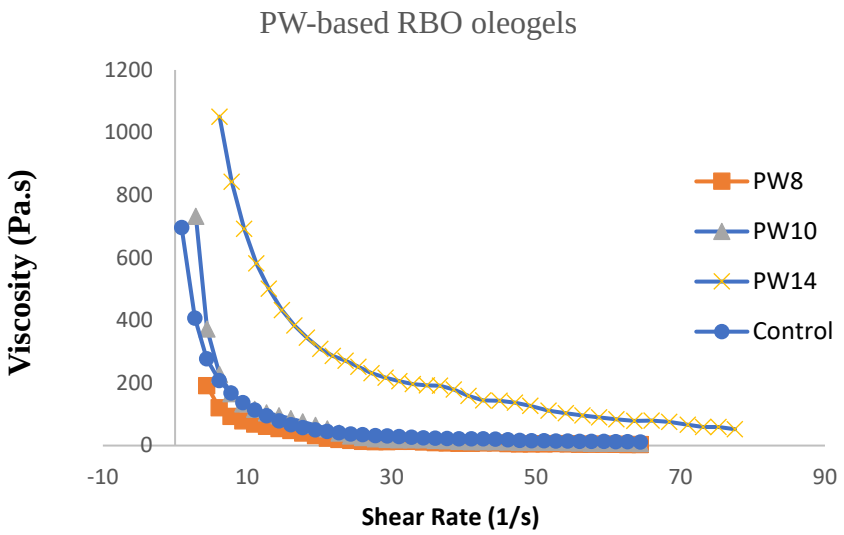
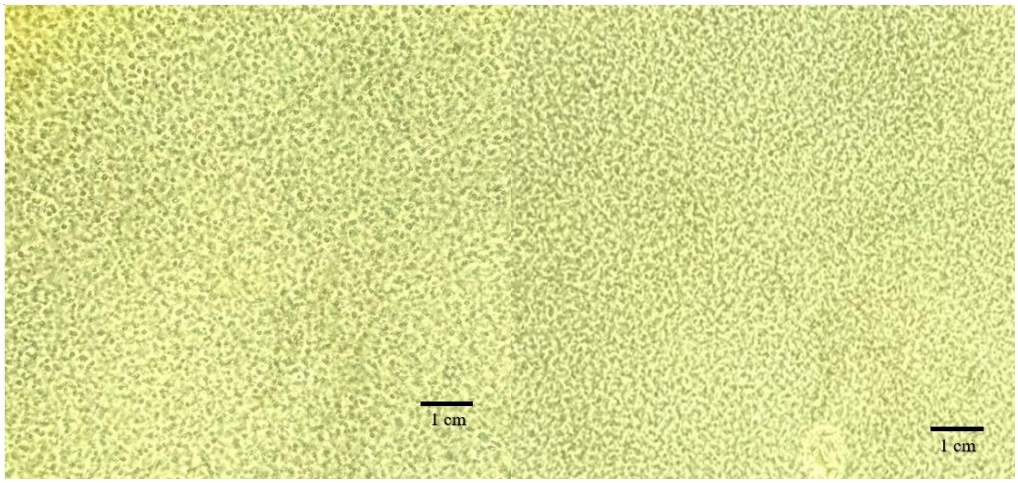


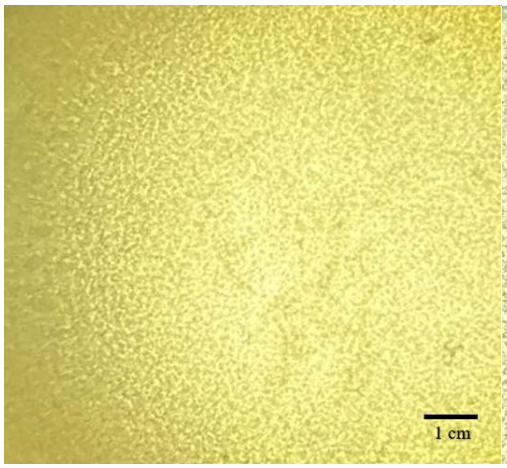
Figure 24: Changes in viscosity in PW-based RBO oleogels over shear rate.

4.9 Microscopic Analysis

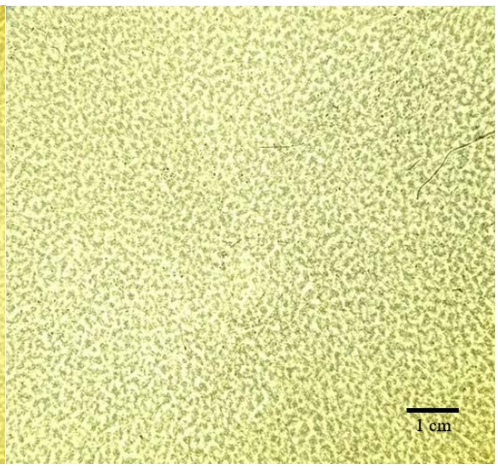


BW1PW1 BW2PW2

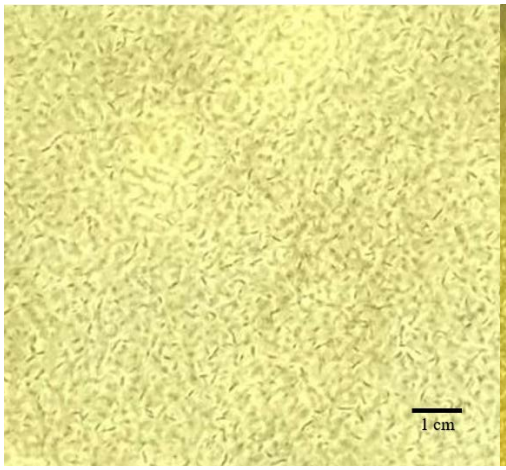
* 1cm: 25 µm



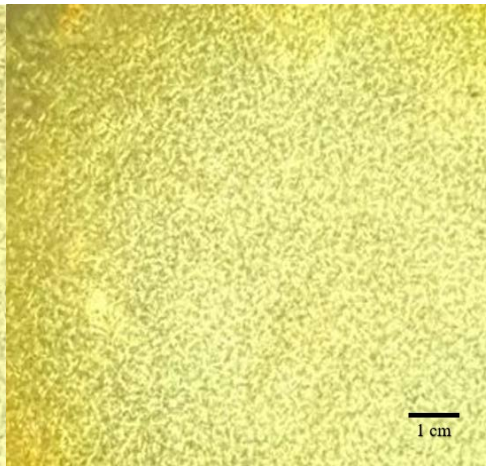
BW4PW4



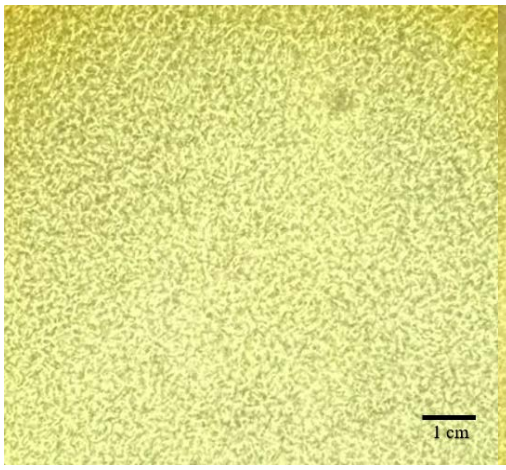
BW6PW6



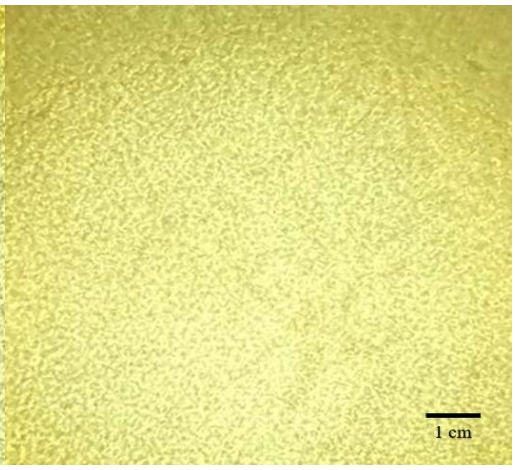
BW2



BW4

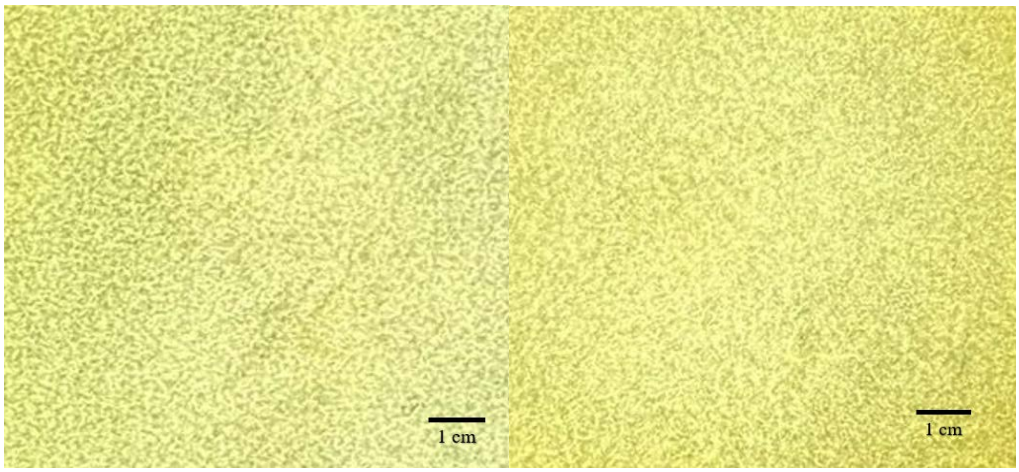


BW6

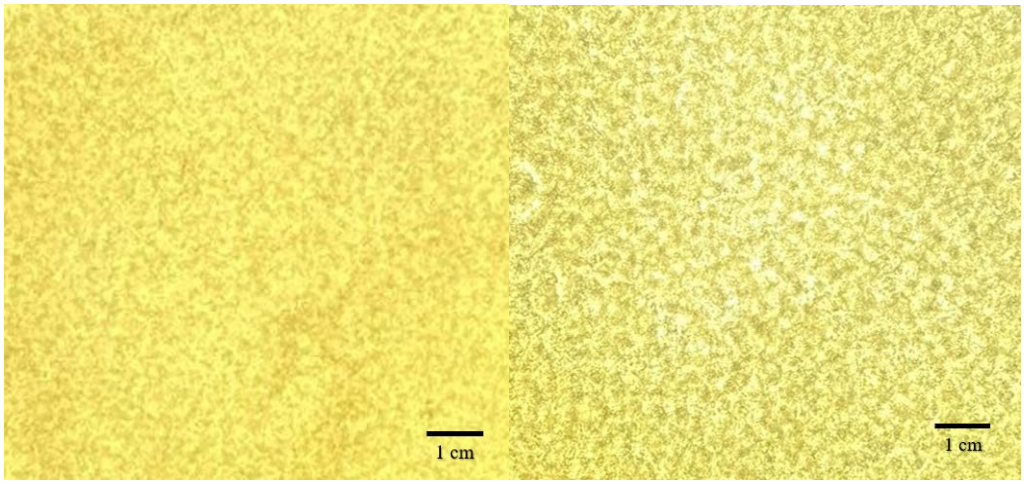


BW8

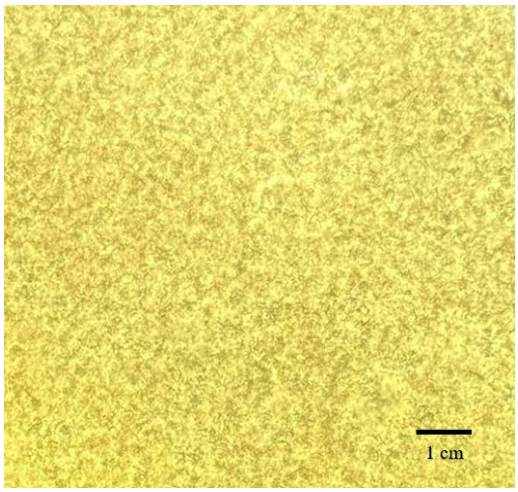
***** 1cm: 25 μm



BW10 BW14



PW8 PW10



PW14

* 1cm: 25 μm

Figure 25: Microstructure of the oleogel crystals in the prepared oleogel samples observed under 40× magnification.

CHAPTER 5

DISCUSSION

5.1 Differential Scanning Calorimetry

The thermal profile of the natural wax-structured oleogels was determined by DSC and the crystallization and melting curves for each oleogel sample were obtained as shown in Figure 2 and 3 respectively. A total of three exothermic peaks were obtained in the crystallization curve for all samples whereas three endothermic peaks were obtained for the mixed-wax oleogel samples and two peaks were obtained for the remaining samples in the melting curve. Referring to Figure 2 featuring the crystallization curve, out of the three exothermic peaks observed, the first two peaks for each sample were formed at different temperatures among the samples while the position of the third peak was almost constant among all samples. Furthermore, based on the trend of each crystallization curves, it can be observed that the oleogel samples which were structured with the same type of wax showed almost similar thermal behaviors. Besides the wax concentration, the main difference between the oleogel samples is the type of natural wax used for oil structuring. As a result, the wax concentration and type of wax used can account for the differences in the position of the peaks formed. On top of that, the enthalpy (ΔH_c) which is determined according to the area under the peak portrays an approximate amount of crystals present where a higher enthalpy will correspond to a higher rate of

crystallization and this can also correlate to other parameters including OBC as well as the microstructure of the oleogel samples (Zhang et al, 2023).

During the crystallization stage, the formation of each peak is dominated and determined by the individual composition of both the BW and PW. In a study done by Pang et al (2020), the wax ester content of the natural wax is mainly responsible for the position of the first peak which is when the oleogel samples first form crystals. In addition, since both the BW and PW are mainly composed of wax ester of about 71% and 80% to 85% respectively, this then explains the position of the first peak for PW relative to the position of the first peak for BW (Lan, 2019; Helmenstine, 2018). According to Table 2, the onset temperature, which is the temperature at the first peak is generally higher for oleogel samples containing PW as compared to those containing only BW. Furthermore, as observed in Figure 2, the intensity of peak 1 shifted to lower temperature as the concentration of wax decreases which is applicable to all samples in this case. This shows that a higher composition of wax esters can possibly allow crystallization to occur faster and as such, when the concentration of wax decreased, there will be comparatively lesser wax esters and hence the crystallization can only occur at lower temperatures as the sample cools further. The opposite is true when the concentration of wax increased. As shown in Table 2, when the concentration of PW increased from 8% w/w to 10% w/w and then to 14% w/w, the onset temperature increased from 62.43℃ to 68.36℃ and then 71.38℃ respectively. Similar trend was also observed for BW-structured and mixed wax-structured oleogel samples. The appearance of peak three which showed an almost constant thermal behavior among all the samples can be

explained by the presence of highly unsaturated triacylglycerols (TAG) which are found in RBO used as the continuous phase for the construction of oleogels.

During the melting phase, the melting point of waxes in turn becomes the differentiating factor in terms of the position of the peak particularly near the end of the melting curve. A total of three peaks were obtained for BW4PW4 and BW6PW6 whereas a total of two peaks were obtained for the remaining oleogel samples. Similar to peak 3 observed in Figure 2, peak 1 of all the oleogel samples in Figure 3 also displayed similar thermal behaviors which is also attributed to the presence of highly unsaturated TAGs from RBO, resulting in a low melting temperature (Devi and Khatkar, 2016). Comparing the values of peak 3 from the crystallization curve in Figure 2 and peak 1 from the melting curve in Figure 3, the melting point of the unsaturated TAGs for all samples ranges between -6.79℃ to -6.37℃, higher than the crystallization temperature ranging between -8.56℃ to -9.89℃, which is a reasonable and logical result since the crystallization temperature would always be below the melting temperature. When the temperature further increased, the melting of the oleogel samples were evident based on the subsequent formation of peaks 2 and 3. The formation of three peaks from the melting curves of BW4PW4 and BW6PW6 as shown in Figure 3 suggests that the mixed-wax oleogels are displaying the properties of both the BW and PW in which the second peak correspond to the melting point of BW and the third peak to that of PW. This can be inferred again from Figure 3 which shows that BW-structured oleogels only formed peaks 1 and 2 whereas PW structured oleogels on the other hand only formed peaks 1 and 3. Therefore, BW4PW4 and BW6PW6 which are the intermediates will form all three peaks

since they contain both BW and PW. Besides that, the peaks were also observed to shift towards a higher temperature as the concentration of the wax increased. This instance is similar to a preceding study conducted by Suriaini et al (2023) which also showed similar observations whereby increasing the concentration of the wax will in turn increase the melting point of the oleogel. This was evident in all samples in this study. Referring to Table 3, when the concentration of PW increased from 8% w/w to 10% w/w and then to 14% w/w, the melting temperature increased from 66.44℃ to 74.60℃ and then to 76.42℃ respectively. Similar trends were observed for BW and mixed-wax oleogels. Besides the concentration of the wax in affecting the melting temperatures, the type of wax used also greatly affects the melting temperature of the oleogels. Generally, BW has low melting point which ranges from 62℃ to 65℃ and PW in contrast is considered as the natural wax with the highest melting point of about 82℃ to 86℃ (Patel, 2020; Marie, 2014). This then explains the relatively higher melting temperatures of PW incorporated oleogels relative to oleogels that are structured with BW alone. Based on the tabulated values from Table 3, PW oleogels have melting temperature ranging from 66.44℃ to 76.42℃ whereas BW oleogels have melting temperature ranging from 48.20℃ to only 53.03℃. Oleogels consisting a mixture of BW and PW showed the melting temperature of BW at peak 2 (46.37℃ and 49.21℃) and melting temperature of PW at peak 3 (73.86℃ and 74.53℃) from BW4PW4 and BW6PW6 respectively.

Overall, the thermograms generated indicated that the type of wax and concentration used played a significant role in influencing the thermal behaviors of the oleogel samples.

5.2 Textural Properties

Out of the 13 oleogel samples prepared, 10 of them managed to form gel successfully. The remaining three were identified as ‘take liquid’ due to their runny consistency which could not be analyzed for their textural properties. Preliminary studies on PW have also shown that to form PW-based RBO oleogels, a higher concentration of PW was required due to the scarce formation of crystals at low PW concentration and the nature of the PW crystals morphology. On the other hand, for BW, preliminary studies have shown that BW can structure RBO at much low concentration as compared to PW, however, the BW threshold concentration is 3% w/w based on literature which was why BW2 did not manage to form gel. Several primary mechanical properties including hardness, adhesiveness and cohesiveness were measured for the 10 successfully gelled samples using texture profile analysis which compresses the sample twice to stimulate the chewing action of teeth.

Hardness is typically used to measure the sample’s resistance to deformation by a compressive force. Based on the results obtained in Table 4, a general trend was observed whereby increasing wax concentration produced RBO oleogels with more rigid texture which was somewhat comparable to a study done by Zhao (2019). However, based on the statistical analysis, it can be observed that the synergistic effect of BW and PW did produce RBO oleogels with a harder texture that had no significant difference in comparison to the hardness of 14% PW oleogel as well as the control sample. All BW-based oleogels were also found to have no significant difference in hardness from lower concentrations of

PW oleogels (8% and 10%) which indicated that PW contributed to much of the stiffness and rigidity in the RBO oleogels which increased the resistance of PW-based RBO oleogels to deformation by compressive forces. Among the BW-based oleogels, 10% BW was found to be significantly harder than the two lowest concentrations of BW-based oleogels (4% BW and 6% BW). Hardness value obtained for 14% BW was lower than anticipated but this phenomenon could possibly be attributed by a high level of deformation during the first compression with poor deformation recovery or known otherwise as resilience after the first compression.

Adhesiveness which is the area of negative force measured under the curve is used to determine the force needed to retract the probe away from the sample after the first compression (Kim et al, 2022). According to Table 4, there was significant difference in adhesiveness for 10% BW, 4% BW 4% PW and the control sample from the rest of the RBO oleogels but no significant difference in adhesiveness among the three aforementioned samples, indicating that the adhesiveness of 10% BW, 4% BW 4% PW showed the highest resemblance to that of the control sample.

Cohesiveness is a measure of the ability of the sample to withstand deformation until the point where the sample dissociate, which is highly dependent on the strength of the internal bonds holding the sample together and this can be an alternative analysis to the efficiency of the wax and wax concentration during

gelation (Smewing, 2014). Cohesiveness is typically associated with the resilience of a sample where there is an indirect relationship between the two and since hardness is somewhat a measurement of resilience, the two parameters (hardness and cohesiveness) will therefore display contrasting values as observed in Table 4 (Tanislav et al, 2022). Based on the results obtained in Table 4, significant difference in cohesiveness from the control samples were observed in 4% BW, 6% BW 6% PW, 8% PW and 14% PW. The control sample was also found to exhibit unusually high cohesiveness value even though it already has a high hardness value which could be attributed to the strong hydrogen bonds introduced in the process of partial hydrogenation. Among the samples with no significant difference for cohesiveness from the control sample, 10% BW RBO oleogel was found to exhibit cohesiveness property that resembles to that of the control sample which had both high hardness and cohesiveness values.

5.3 Colorimetric Analysis

Based on the results obtained in Table 5, all the RBO oleogel samples in general are lighter in color in comparison with the control sample. 14% BW in particular had the darkest color of 69.56 ± 0.37 among all the RBO oleogel samples while 4% BW had the lightest color of 34.42 ± 1.04 among all the RBO oleogel samples. The lightness of the control sample was also found to be significantly different from all of the oleogel samples, indicating that it is significantly darker than all the oleogel samples prepared. The darkest (14% BW) and lightest (4% BW) also had significant differences in their respective lightness compared to

the rest of the oleogel samples. The lowest concentration from each wax(es) combination was also darker than its corresponding counterparts at higher wax concentration. For instance, among all the BW-based RBO oleogels, 2% BW was the darkest among them and this was similarly observed in PW-based RBO oleogels as well as the hybrid wax RBO oleogels. It can be inferred that the color of oleogels with low wax concentration could be attributed to the color of the RBO which in this case masked the color of the corresponding wax(es) that was added in low concentration. In both the single wax-structured RBO oleogels, there was a general increasing trend in darkness with some exception for a few samples as the concentration of the wax increases but no synergistic effect was observed for the hybrid wax oleogels on the lightness value. As the wax concentration increases, the wax crystal network is more vast and this will proportion and disperse out the oil molecules, allowing the respective wax(es) to dominate the color of the resulting oleogel produced.

For the a* values, a positive a* value will indicate a higher redness while a negative a* value will indicate a higher greenness. From the results obtained in Table 5, all of the RBO oleogel samples were observed to have negative a* values, indicating a greater proportion of green hue among the oleogel samples with the exception of 1% BW 1% PW having positive a* value (0.04 ± 0.12) which show that this sample appears redder than the other RBO oleogel samples. Based on the trend for a*, it can be observed that the greenness of the oleogels increases with increasing wax concentration. The control sample however portray a higher degree of redness as opposed to the RBO oleogel samples. 6%

BW 6% PW appeared to be the greenest among the RBO oleogel samples whereas 1% BW 1% PW appeared to be the reddest among all the RBO oleogel samples as shown in Table 5. The degree in greenness observed in the BW-based oleogels is comparable to the BW-based oleogels from a study done by Moghtadaei and coworkers (2018) whereas the degree in greenness observed in the PW-based oleogels is in agreement with the PW-based oleogels from another study done by Ogutcu and Yilmaz (2014).

In terms of b*, a positive b* value will indicate a higher degree of yellowness whereas a negative b* value will indicate a higher degree of blueness. Based on the results obtained in Table 5, all the RBO oleogel samples contain a higher degree of yellow hue than blue but were significantly less yellow in comparison to the control sample. A general increasing trend could be observed whereby increasing the wax concentration increases the yellowness of the RBO oleogel samples but this was not very evident in the BW-based oleogels as 2% BW was observed to be more yellow than 4% BW while 10% BW was also observed to be more yellow than 14% BW but the b* values between the two pairs of samples were not significantly different. The slight difference could arise from environmental factors or slight variations in the color of the replicated oleogel samples prepared from different batches which also ensure that potential errors during the preparation of the RBO oleogel samples have been taken into account. Among the RBO oleogel samples, 14% PW was the yellowest (18.36 ± 1.09) whereas 1% BW 1% PW was the bluest (6.44 ± 0.36).

Overall, at low wax concentration, the color of the resulting oleogels is more commonly controlled by the color of the continuous phase (RBO) whereas at high wax concentration, the color of the resulting oleogel samples will be dependent on the concentration of wax(es) incorporated.

5.4 Slip Melting Point Analysis

Slip melting point is a versatile, cheap, fast and reproducible method of measuring the melting and crystallizing properties of fats and oils and is commonly used to classify oils and fats based on the composition and types of fatty acids present in them (Azmil Haizam, Lin and Kuntom, 2008). The melting point of the RBO oleogels was measured at the temperature when the column of RBO oleogel inserted into the capillary tube rises as a result of hydrostatic pressure. Based on the trend observed from Table 6**,** the slip melting point was found to increase with increasing concentration of wax. The results in within expectations as higher wax concentration will produce RBO oleogels which will have a more compact crystal structure with stronger hydrogen bonds and van der waals forces of attraction. Moreover, higher amount of oleogelator present will also provide a vast and extensive three-dimensional network to entrap more oil molecules, which then produces RBO oleogels with higher rigidity at higher wax concentration. As a result, to overcome the strong bonds and forces of attraction, a higher temperature would be required. Comparing with the melting points of RBO oleogels obtained from DSC analysis as shown in Table 3, the slip melting point analysis may be lacking in terms of precision since it does not scrutinize

the melting and solidifying behavior of the RBO oleogels as much of that of DSC analysis. On top of that, the slip melting analysis can be subjective as it requires visual inspection of the rise in the column of sample physically and it is also bound to be influenced by environment factor like temperature since it is carried out in an open system while DSC is done in a closed system. To further elaborate on the results obtained in Table 6, the slip melting point of 14% BW oleogel was found to be significantly different from the rest of the samples. The slip melting point of 4% BW oleogel was also significantly different from the rest of the samples. Slip melting points for BW-based RBO oleogels above 4% BW were not significantly different and slip melting points for BWPW-based RBO oleogels above 2% BW 2% PW were also not significantly different. However, the slip melting points for the three different concentrations of PW (8%, 10% and 14%) were found to be significantly different. Another observation derived from Table 6 is that the hybrid wax RBO oleogels were observed to exhibit slip melting points higher than when BW was used alone at the same concentration. This displayed the synergistic effects between BW and PW whereby the presence of BW allows gelation to occur at low concentration of PW which cannot happen if PW was used alone at low concentration above the threshold gelation concentration for BW while the presence of PW allows the innately low melting point BW to attain a higher melting temperature.

5.5 Peroxide Value as a Function of Oxidative Stability

The RBO oleogel samples were incubated at 60℃ in a dim environment to accelerate the rate of oxidation and peroxide value measurement was done every

four days for a total of 20 days to monitor the changes in peroxide value in analyzing and predicting the oxidative stability of the samples.

Peroxides are primary products of lipid oxidation which are typically broken down in the latter stages of lipid oxidation and the degradation process can be accelerated at high temperatures (Pakseresht et al, 2023). Therefore, the measurement of peroxide value near to the end of the incubation period may be inefficient as most of the primary products have been converted into their corresponding secondary products which cannot be detected through the peroxide value test. With reference to Table 7, there is no significant difference in the peroxide value measurement on days 16 and 20. However, there is significant difference in peroxide value measurement among days 4, 8 and 12. This means that the primary products are likely to have broken down after day 12 of incubation which led to a subsequent decline in peroxide value measured from day 12 to day 16.

From Table 7, it can be observed that increasing concentration of BW does increase the oxidative stability of the oleogels as the peroxide values on days 4, 8 and 12 can be seen to decrease as the concentration of wax increases. However, BW8 in particular showed otherwise and had the least oxidative stability among the BW-based oleogels. Similar trend was observed for the hybrid wax oleogels with BW4PW4 having the least oxidative stability. In the case of PW-based oleogels, there was no clear relationship between the effect of PW concentration on the oxidative stability of the PW-based RBO oleogels. Nevertheless, among

the three variations of RBO oleogels, PW-based RBO oleogels have the highest oxidative stability as the peroxide value remained below 20 even up till day 12. Depending on the origin and sources of PW, the chemical composition may differ. However, PW in general are derived from a type of palm native to Brazil, known as Copernica prunifera and is mostly composed of saturated fatty acid esters (Backe, 2017; Afzaal et al, 2022). The high composition of saturated fatty acid esters from PW could then be the possible factor rendering PW-based RBO oleogels a higher oxidative stability. Synergistic effect was also observed in the hybrid wax RBO oleogels whereby the more oxidation susceptible BW-based RBO oleogels shown higher tolerance to oxidation when PW was incorporated. This is evident from Table **7** which showed BW1PW1 having a lower peroxide value than BW2 on days 4, 8 and 12. However, the synergistic effect was not shown in BW4PW4 as the peroxide value escalated to above 30 on day 12 which was the highest among all the samples. Despite that, the sharp increase in peroxide value was not significantly different from all the samples. Therefore, it could be due to possible errors which may have arisen during the iodometric titration.

5.6 Oil Binding Capacity

The gelation capabilities of natural wax is an imperative intrinsic characteristic of natural wax-structured oleogels as it reflects on the efficiency of gelation which will subsequently affect the use of oleogels in many food applications. The structure of the oleogels is mainly provided by the formation of crystal

networks from the added wax and the extent of immobilization of the oil molecules within the crystal network will largely depend on the strength of the hydrogen bonding and intermolecular forces of attraction between the oil and oleogelator molecules. A preceding study done by Barroso et al (2022) reported that rate of cooling of the oil-gelator mixture upon complete dissolving of the gelator could potentially affect the OBC of the resulting oleogel as rapid cooling would more likely result in the formation of smaller, even wax crystals which will form a more compact and rigid oleogel structure whereas slow cooling may form large, uneven sized wax crystals.

According to Table 8, the OBC results showed that no significant difference in the OBC values was observed from all the RBO oleogel samples with the exception of 1% BW 1% PW being significantly different from the rest. This shows that the synergistic effects of BW and PW could possibly only work above the 1% critical wax concentration for both waxes in order to achieve a higher OBC. Even though the OBC results for 1% BW 1% PW was significantly different to 2% BW and 2% BW 2% PW, there was little difference in the visual appearances of the three variations of RBO oleogels which was also similarly observed in the study of Airoldi et al (2022). As such, it shows that the OBC of the RBO oleogels could also be related to the temperature of the environment in which the analysis of carried out since all the samples were left in the freezer overnight for the same amount of time before they were centrifuged. Prior to that, 1% BW 1% PW, 2% BW 2% PW and 2% BW were all observed to be in liquid phase during the storage period at ambient room temperature. However, upon removing the samples from the freezer, the 1% BW 1% PW RBO oleogel was

observed to have signs of melting at ambient room temperature and this may perhaps highlight the insufficiency of waxes to entrap the oil molecule which led to a higher amount of unbound oil molecules in the sample. Nevertheless, the oleogelators were shown to have formed good interactions with the RBO given the high OBC values that was calculated. Comparatively, BW was probably more efficient in entrapping the RBO molecules than PW due to the almost perfect OBC values from all the BW-based RBO oleogel samples.

5.7 Fatty Acid Composition

Depending on the sources of raw materials, refining and other processing conditions, the fatty acid composition of RBO available in the markets may differ accordingly. Nevertheless, RBO is generally a healthier choice of oil compared to other variations like coconut and palm oil due to their distinctively higher polyunsaturated and monounsaturated fatty acid profiles as compared to the latter two types of oils mentioned. The prominent type of saturated fatty acid present in RBO is palmitic acid which takes up about 21% of the fatty acid composition of RBO, with minute amounts of stearic acid at around 2.9% (Wright, 2021). Apart from palmitic and stearic acids, myristic acid was also present in the analysis of the RBO in this study. In addition, the two most abundance unsaturated fatty acids in all the RBO oleogel samples as well as that of RBO were oleic and linoleic acid and the results were found to align with a study carried out by Sobolev et al (2022) on BW-based sunflower oil oleogels. Saturated fatty acids are generally more resistant to oxidation as compared to unsaturated fatty acids and this is crucial during the storage of oils. Since RBO

is largely composed of unsaturated fatty acids, the RBO-based oleogels may be susceptible to oxidation which will in turn depend on parameters such as OBC and the crystal morphology of the wax used to structure RBO. According to Table 9, the detection sequence of the fatty acids in the samples are based on the fatty acid chain length, with lauric acid (C12:0) having the shortest chain length, followed by myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1-cis (n9)), vaccenic acid (C18: 1-trans-11), linoleic acid (C18:2-cis (n6)), α-linolenic acid (C18:3 (n3)) and arachidonic acid (C20:4 (n6)) (Siram et al, 2019).

Referring to the results obtained in Table 9, in general, the fatty acid composition of the oleogel samples did not deviate much from that of RBO since RBO was used as the continuous phase during oil structuring. Among all the oleogel samples, 2% BW in particular had the highest resemblance of fatty acid composition to that of RBO which could be due to the fact that BW was added in very minute concentration hence making it difficult for detection of the wax's fatty acid composition. Other than myristic acid for 2% BW at 0.43%, the other components of fatty acids were exactly similar to that of RBO. Nevertheless, in comparison to the control sample (margarine), the saturated fatty acids (myristic, palmitic and stearic acids) content was comparatively higher than the rest of the analyzed samples, with palmitic and stearic acids almost doubling the amount detected in majority of the remaining samples. Furthermore, with reference to the data in Table 9, the control sample was found to contain much lesser monounsaturated and polyunsaturated fatty acids which include oleic acid,

linoleic acid, linolenic acid as well as arachidonic acid. An additional type of saturated fatty acid, lauric acid was also detected only in the control sample at around 4.94%. The fatty acid composition of RBO was also in large agreement of a similar finding done by Latha and Nasirullah (2014). On top of that, the variations in unsaturated fatty acid composition between the samples can possibly be explained due to changes in the fatty acid contents during storage as well as the type of wax used to structure RBO (Issana, 2022).

Overall, the findings encompassing the fatty acid composition of RBO-based oleogels structured with BW and PW in this study suggest that the RBO oleogels indeed had a healthier lipid profile in comparison to commercial margarine and therefore, they have great potential to become healthier alternatives to commercial margarine. Nevertheless, the fluctuations observed in some of the fatty acids content need to be monitored closely to gather more reliable data on the influence of either the incorporated wax or storage on the actual fatty acid composition of the oleogels.

5.8 Rheological Measurements

5.8.1 Frequency Sweep Test

Frequency sweep test was done to evaluate the time-dependent characteristics of oleogels within the linear viscoelastic region (LVR) to avoid deformation of the samples (Ramli et al, 2022). Referring and comparing Figures 4, 8, 12 (storage

modulus) and Figure 5, 9, 13 (loss modulus) for hybrid wax, BW and PW respectively, all the samples have shown higher G' than G'' throughout the frequency sweep test which indicated that the oleogel samples portray a more distinctive elastic behavior than viscous behavior. From Figures 8 and 12, samples PW14, PW10 and BW14 were found to be more frequency dependent as evident from the steeper increase in G' with increasing angular frequency and this may possibly mean that these three samples only portray little of gel characteristics and seemed to appear more "solid-like" in comparison to the remaining samples (Wang et al, 2022). This finding is also similar to another finding by Chai et al (2022) who reported in their findings that increasing BW concentration produced oleogels which are more frequency dependent as they tend to be more brittle to external forces and have poor malleability and ductility. Other than the aforementioned three samples, the other samples were largely in agreement with preceding study by Martin-Alfonso et al (2022) where samples exhibiting gel-like behavior will display gradual increment of G' with increasing angular frequency whereas samples with G' that is constant with increasing angular frequency or known otherwise as frequency independent are classified as strong gels. Referring to the results in Figures 7, 11 and 15 on the complex viscosity, it can be observed that all samples in general portray a decreasing trend in complex viscosity with increasing angular frequency. The two lowest BW-based oleogels, namely BW2 and BW4 were the least resistant to flow with BW2 having complex viscosity lesser than 10^1 Pa (obscured from the figure) which explains their low viscosity while BW1PW1 was the least resistant to deformation for the hybrid wax oleogel. Synergistic effect was observed as BW2PW2 as well as BW6PW6 were found to be more resistant to flow than

when BW was used alone to structure the RBO at the same concentration. Nevertheless, in general, BW-based oleogels with concentration above 6% BW were observed to be more resistant to flow perhaps due to a more extensive crystal network available to form stronger bonds and forces of attraction while PW-based RBO oleogels in general were largely resistant to flow due to the presence of possibly stronger bonds and forces of attraction between the oil molecules and PW crystals.

5.8.2 Dynamic Temperature Ramp

A dynamic temperature ramp test was done for the determination of the viscoelastic properties in the oleogel samples with increasing temperature and this analysis is highly associated with the thermal behavior of the oleogels shown in DSC as well as their corresponding slip melting points. The G', G'' and G* from Figures 16, 17 and 18 respectively were found to decrease with increasing temperature. Out of all the samples, only BW2 showed prominent viscous properties than elastic properties even at low temperatures and this could be due to the gelation occurring below the critical concentration for BW which resulted in BW2 having a more viscous property. Referring to Figure 16, at higher temperatures, majority of the curves showed a sharp decline in G', indicating the melting of the samples which is characterized by reducing elastic properties and increasing viscous properties (Lupi et al, 2018). As the oleogel transitioned from possessing elastic properties to losing its elastic properties, a crossover point occurs where the curve for G' will intersect with that of G'' and from that point of intersection, the value for G'' will dominate over the value for G' (Lim, Hwang and Lee, 2016). By analyzing the temperature at which the sharp decline

occurred in Figure 16, the temperature at the start of the decline was observed to be consistent with the melting temperature of the oleogels at peaks 2 and 3 derived from the thermogram accordingly based on the samples as shown in Table 3 and this is finding is supported by a research carried out by Martins et al (2016) who also found corresponding results from the melting point derived from thermogram which tallies with the phase angle where the samples transitioned into a molten state. For instance, referring to the tabulated results for thermogram in Table 3, the melting temperature for BW14 was determined to be 53.03℃ and by plotting this value into the G' curve for BW14 in Figure 16, this particular melting temperature can be observed to be situated very closely to the sharp turning point which resembles almost like a 90° angle. With that, it can be concluded that the semi-solid and solid samples in general are able to retain their elastic properties up to the point where they start to melt. G* on the other hand measures the overall resistance of the samples to deformation by temperature and based on the general trends observed in Figure 16, oleogels with higher wax composition of its type are shown to be more resistance to deformation and it can be observed that oleogels containing PW are generally more resistant to deformation due to the intrinsically higher melting point of PW and also attributed to its stiffness and rigidity (Kulkarni and Shaw, 2016).

5.8.3 Temperature Sweep Test

With reference to Figures 19, 20 and 21, the viscosity of all oleogel samples regardless of the type and concentration of wax used decreased as the temperature increases. The main reasoning behind this observation is due to a change in state particularly for semi-solid and solid oleogel samples or known

as the gel-sol transition as the temperature increases which can be explained based on the kinetic particle theory (Floter et al, 2021). The researchers also suggested that the kinetics of the oleogels may be vital in various applications in foods because the phase changes may be desired in certain food to impart the organoleptic properties but not necessarily in all food applications. To explain from a kinetics point of view, when the temperature increased from 20℃ to 80℃, the oil and oleogelator molecules which were initially held in a fixed position start to accumulate heat energy that will be convert to kinetic energy. As the kinetic energy increases, which is contributed by both the heat energy and mechanical energy from shearing, the molecules will vibrate at a faster rate, which then ultimately overcomes the energy required to hold the secondary bonds and weak intermolecular forces of attractions between the oil and oleogelator molecules (Contreras-Raminez et al, 2022). The semi-solid and solid oleogel samples will first form an intermediate known as liquid crystals and as the heating temperature continues to increase, the intermediate state will be dominated by complete liquid state when the heating temperature surpasses the melting points of both the oil and oleogelator (Ract, da Cruz and Pereira, 2019). This increases the flow behavior of the RBO oleogels at increasing temperature which is indicated by the decrease in viscosity. On top of that, it is worth noting that the type and concentration of wax do influence the viscosity of oleogel at different temperatures as portraited from their thermal behavior in the DSC analysis (Park, Campanella and Maleky, 2022). Among all the samples, PW14 showed the most prominent viscosity which was higher than that of the control sample and it required a higher temperature to achieve the same consistency as the other oleogel samples. This can be attributed to the high melting point of PW

too since a similar concentration of oleogel made with BW showed a comparatively lower viscosity when measured at the same temperature.

5.8.4 Shear Viscosity

Based on the results obtained in Figures 22, 23 and 24, all the oleogel samples regardless of the type and concentration of wax used for structuring displayed shear-thinning properties which can be observed from the decreasing viscosities at an increasing shear rate. The results obtained are also comparable to a preceding study done by Espert et al (2022) which also reported that all the oleogel samples showed shear-thinning behavior which is evident with decreased viscosity at increasing shear rate. This can be explained due to the disruption of the hydrogen bonding and van der waals forces holding the oil molecules and oleogelator molecules together which breaks up the structure of the oleogel samples progressively as the shearing forces increases (Li et al, 2022). The concentration of the wax incorporated was also shown to influence the viscosity of the oleogels at an increasing shear rate. Based on the trends observed in for each combination of wax(es) from Figures 22, 23 and 24, the lower the concentration of wax used, the smaller the shear rate required to reduce the viscosity to a certain point and the opposite is true for oleogel samples with higher concentration of wax. This trend is consistent with a study done by Kwon and Chang (2022) which reported that higher concentration of the emulsifier or oleogelator in this case will result in a higher viscosity and in turn lesser flow behavior. The resistance of the oleogel samples to shear can also be explained by their textural properties, in particular their hardness. With reference to Table

4, 2% BW, 1% BW 1% PW and 2% BW 2% PW are three of the oleogel samples in liquid form and this corresponds to the results shown in Figures 22 and 23 whereby an increasing shear rate has almost no effect on reducing the viscosities of these three oleogels which are taken as liquid. In general, for BW-structured oleogels, increasing the concentration of BW was found to increase the hardness of the oleogel samples which also corresponds to the higher shear rate required to reduce the viscosity to a common point. For instance, to reduce the viscosity to 400 Pa.s, a comparatively higher shear rate was needed for BW14 as compared to BW10.

5.9 Microscopic Analysis

Based on the microscopic images from Figure 25, needle-like crystals were predominantly observed in BW structured RBO oleogels whereas spherulitic crystals were observed in PW structured RBO oleogels which seems to be overlapping one another. The needle-like crystals from BW structured RBO oleogels was similarly reported in the BW structured peanut oil from a study by Zbikowska et al (2022). The overlapping of the spherulitic crystals observed in PW structured RBO oleogels from this study can be supported by the preceding findings from Silva et al (2021) on their carnauba wax based-olive oil oleogels which was further explained that the overlapping of the spherulitic crystals was the main reason for the rigid structure of PW-based oleogels but could only gel liquid oil at high wax concentrations due to the usually small size of the spherulitic crystals. On top of that, it was also observed that as the wax concentration increased, the BW-based and PW-based RBO oleogels formed a

more compact crystal structure which can be seen from the closely packed needle-like crystals in BW-based RBO oleogels and multiple overlapping of the spherulitic crystals in PW-based RBO oleogels. This observation can be further supported by Kamali et al (2019) whom reported that the increment in wax concentration will result in the repositioning of the wax crystals thus generating different variations of microscopic images at various concentrations of wax.

In the case of the hybrid wax RBO oleogels, the microscopic images showed that PW crystals were more dominant in the microstructure of the hybrid wax oleogels. However, the PW crystals appeared smaller than when it was compared with the microscopic images of 8%, 10% and 14% PW oleogel samples. This could be attributed to the low concentration of PW in the hybrid wax oleogels which dispersed the crystals further from each other in the organic solvent, preventing the overlap of spherulitic crystals. Nonetheless, gelling at low concentration of PW was possible owing to the presence of BW with provide the required synergistic help in structuring of RBO. This could have probably happened when the needle-like crystals from BW bridged the gap between the neighbouring spherulitic crystals of PW which allowed the oil structuring to occur. This was however more evident in 4% BW 4% PW as well as 6% BW 6% PW RBO oleogels based on Figure 25.

5.10 Future Study

Oleogels have huge potential for the market in the future especially with the increasing health consciousness among consumers in their choices of diet. However, there are still plenty of room for improvement in the physicochemical properties of oleogels which requires extensive and conclusive optimization of the plethora of different combinations and concentrations of oleogels that could be produced. Firstly, the organoleptic of oleogels could be explored for their feasibility and applications in food products and consumers general acceptance which are still lacking in present literature studies as well as in this study. This would also allow the evaluation of the potential of oleogels for the market in the future. Moving on, the microstructure of oleogels can also be evaluated using a polarized light microscope instead of an inverted microscope used in this study principally to attain a clearer and better understanding of the microstructure of the hybrid wax oleogels so that the synergistic effects of the two waxes can be studied more thoroughly as explained by Silva, Barrera-Arellano and Ribeiro (2021) that the incorporation of two or more oleogelators is gaining interest in most oleogels studies as the synergistic effects from each contributing oleogelators can possibly outweigh the beneficial properties of an oleogelator used alone in structuring the liquid oil.

5.11 Limitations of Study

With oleogels being a newly developed novel creation in hopes of replacing the harmful and detrimental effects of trans and saturated fatty acids in conventional

fats, there are bound to be limitations. Since researches on oleogels are still in a very premature stage, it is necessary to bridge the knowledge gap between the effect of oleogelators and how they interact with various food ingredients, how the properties of oleogelators will change under different food processing parameters such as freezing, boiling, baking et cetera (Park and Maleky, 2020). To further elaborate, present studies are still putting hefty focus on the intrinsic properties of oleogels with very little emphasis on the extrinsic properties in influencing the oleogels. The sensory aspects of oleogels are also still inconclusive on how potential consumers will perceive them in food based on the present studies available. Moreover, it is also crucial to ensure that the oleogelators used from time to time are food-grade and that they are in sufficient supply as well as available from inexpensive yet accessible sources.

CHAPTER 6

CONCLUSION

In conclusion, the present study focuses on physicochemical properties of 13 oleogel samples with some comparisons made with margarine used as a control sample. The results obtained from most analysis were within expectation and were mostly explainable. The combination of BW and PW has also shown to portray desirable synergistic effects in some of the physicochemical properties of the RBO oleogels, particularly in the two thermal analysis (DSC and slip melting point analysis) carried whereby the addition of PW was found to increase the melting point of hybrid wax oleogels which was found to be lower when BW was used alone to gel RBO. The synergistic effect contributed by BW was more prominently allowing the gelation to occur at low concentrations of BW and PW used which could not be achieved in this case if PW was used alone to gel RBO at the same concentration. As a result, two particular oleogels, namely BW4PW4 and BW6PW6 showed potential for further study to scrutinize the synergistic potentials of BW and PW in other physicochemical properties not done in the present study. In terms of the oxidative stability of oleogels in comparison to the control sample (margarine), the RBO oleogels still had comparatively lower oxidative stability due to the presence of higher amounts of unsaturated fatty acids in them as highlighted in the fatty acid composition analysis by GC-FID. Conversely, the control sample also contained high amounts of saturated and trans fatty acids produced via the process of partial hydrogenation which rendered the control sample a higher oxidative stability.

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APPENDICES

APPENDIX A

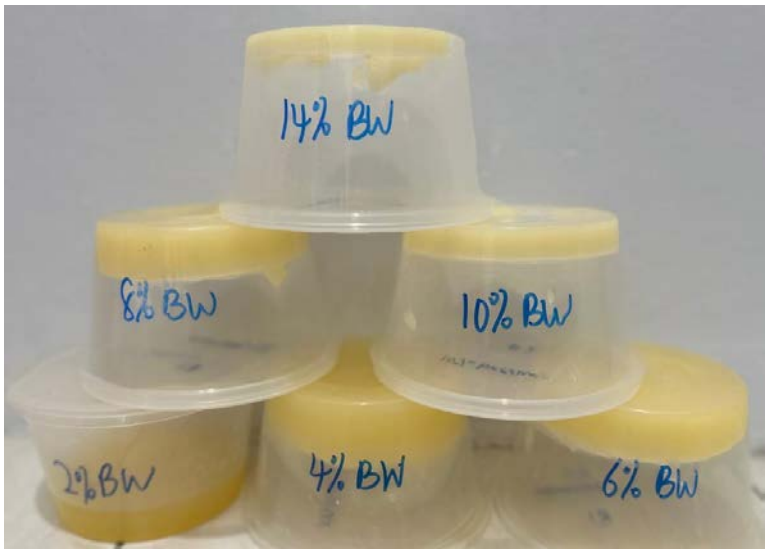

Figure A: Visual aspect of RBO-based oleogels made from various concentrations of beeswax.

APPENDIX B

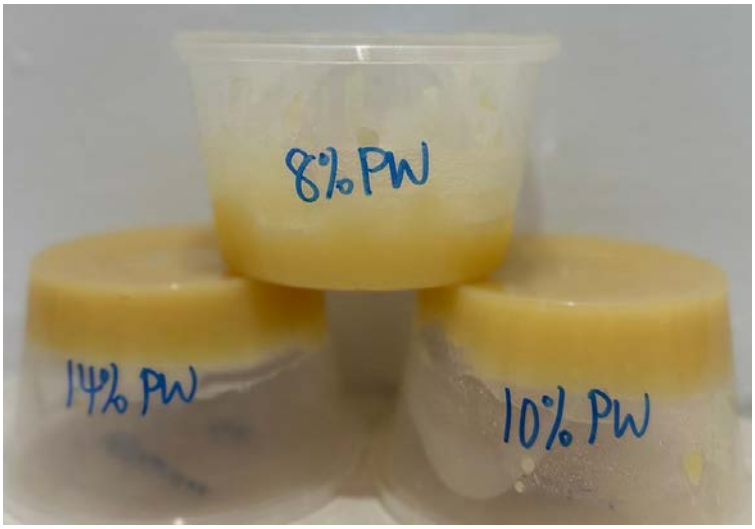

Figure B: Visual aspect of RBO-based oleogel made from various concentrations of PW.

APPENDIX C

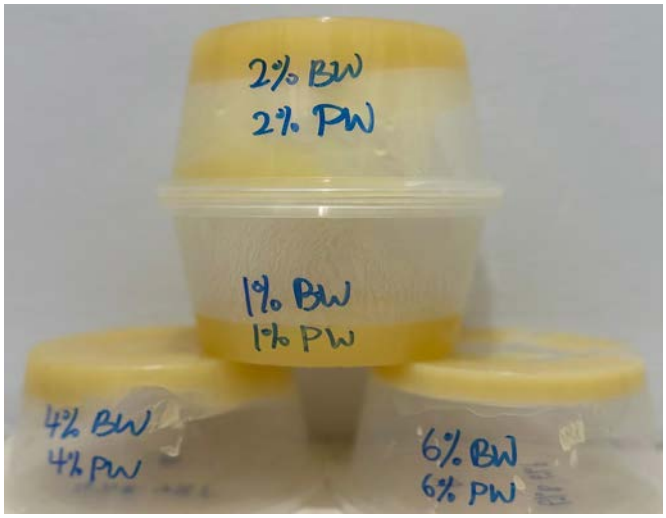

Figure C: Visual aspect of RBO-based oleogel made from various concentrations of BW and PW.

APPENDIX D

Figure D: DSC furnace with the hermetically sealed aluminum pans loaded in the respective chambers, with "S" representing the sample chamber and "R" representing the reference chamber.

APPENDIX E

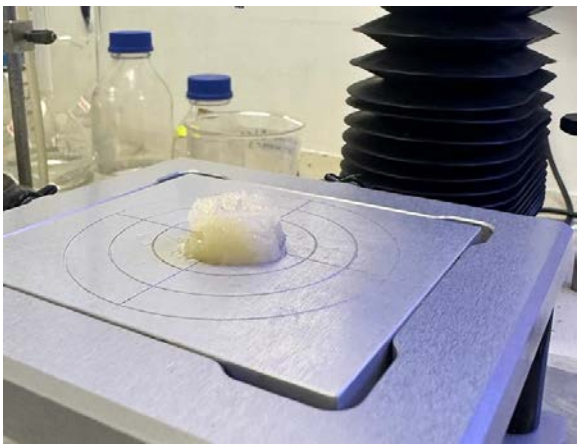

Figure E: Shaped oleogel sample (2 cm × 2 cm × 2 cm) loaded onto the sample loading platform.

APPENDIX F

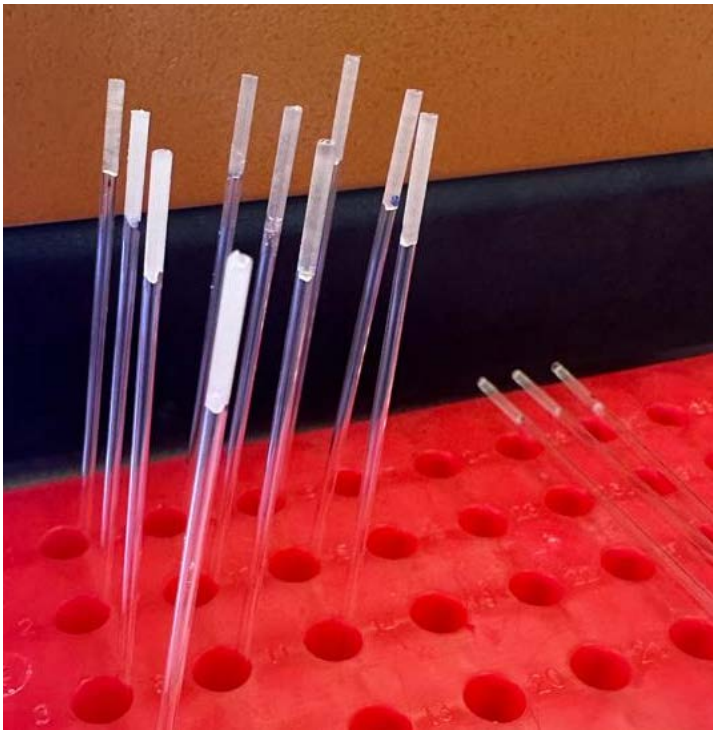

Figure F: Oleogel samples filled to a 1 cm mark in capillary tubes.

APPENDIX G

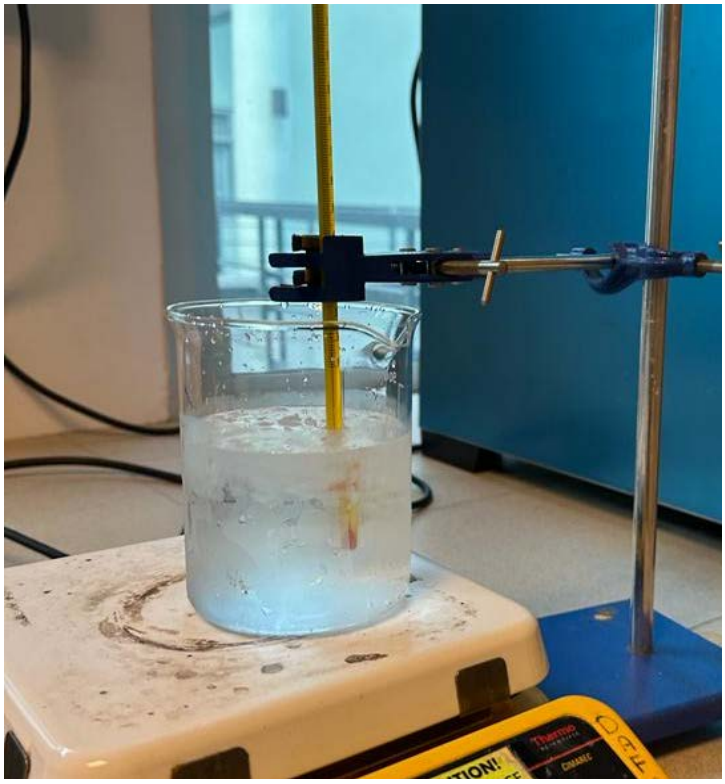

Figure G: Set-up for slip melting point analysis.

APPENDIX H

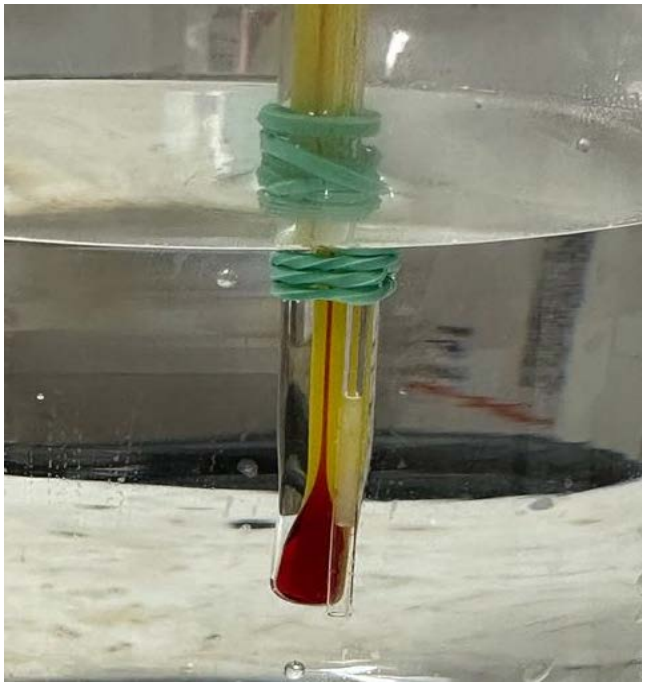

Figure H: Rising column of the oleogel sample measured as its slip melting point.

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