DEVELOPING AN OXIDATIVELY STABLE OMEGA-3 RICH FOOD PRODUCT USING A COMBINATION OF ANTIOXIDANTS AND PROCESSING TECHNIQUES

by

JOSEPH ROBERT HYATT

(Under the Direction of Casimir C. Akoh)

ABSTRACT

This research focused on developing a lipid ingredient with ω-3 rich algal oil from *Schizochytrium sp.* which could be used to formulate shelf-stable food products. Gelation, microencapsulation, and antioxidants were used to develop yogurts with comparable physicochemical properties to in-store products. Initially, antioxidant efficacies were assessed with model systems using bulk soybean oil and oil-in-water (O/W) emulsion. Oxidative stability of bulk oil had the highest improvement using 1-*o*-galloylglycerol (GG) as antioxidant with a final TOTOX value of 206.82 compared to bulk oil at 232.09. A mixture of δ, β, γ, and δ tocopherols (TOC) used with O/W emulsion exhibited similar results with a TOTOX value of 196.72 compared to O/W emulsion at 352.88. Gelation was used to modify ω-3 fatty acid rich algal oil to develop desirable physical properties while also improving its oxidative stability to produce an oxidatively stable ω- 3 fatty acid (FA) source. FA analysis over an accelerated oxidation study showed 12% (w/w) monolaurin oleogel (12% M) outperformed other gels in oxidative stability, preventing oxidation of approximately 17.96% and 20.43% of EPA and DHA, respectively, compared to algal oil alone. Solid fat content (SFC) and thermal properties of 12% M indicate that it could replace saturated fat sources, with an average SFC of approximately 4.5% at 30 °C, similar to butterfat, and a

melting completion temperature of 59.38 °C. Antioxidants were combined with gelation and microencapsulation processing techniques to produce ingredients to formulate yogurt products. Combinations of gelation, microencapsulation, and antioxidants were produced, including a microencapsulated oleogel (MEOG) with GG as antioxidant (MEOG-GG) which exhibited significantly slower lipid oxidation than bulk oil alone as it prevented an average of 18.13 % of EPA and 12.55 % of DHA oxidation. Yogurt with MEOG-GG ingredient maintained a rheological profile and viscosity similar to store-bought yogurt and yogurt developed with butterfat. It also exhibited a significantly similar oxidative stability to both store-bought yogurt and yogurt made with butterfat. Yogurt made with MEOG-GG exhibited an average PV and *p*-AV of 7.17 ± 0.76 mmol O_2/kg of oil and 118.85 ± 0.98 abs/g, respectively. Yogurt with butterfat had values of 8.17 ± 0.29 mmol O_2/kg of oil and 149.71 ± 2.34 abs/g, for PV and *p*-AV, respectively.

INDEX WORDS: Antioxidant; Oxidative stability; ω-3 fatty acid; Gelation; Microencapsulation; Bulk oil; Emulsion

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DEDICATION

To my brilliant and beautiful wife, thank you for all of your help these last two years. This wouldn’t have been possible without you.

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CHAPTER 1

INTRODUCTION

Omega-3 fatty acids (ω-3 FA) are of increasing interest to the food industry due to their health benefits when consumed on a regular basis. The American Heart Association (AHA) suggests that increasing ω-3 FA content, such as EPA and DHA, of food products, can lead to a reduced risk for the development of cardiovascular diseases (Stone, 1996; Kris-Etherton, Harris, & Appel, 2002). Polyunsaturated fatty acids (PUFA) can act as energy storage material, and some PUFA support a wide range of biological functions, such as regulating inflammation and hormone production, and aiding neural development and function (Calder, 2017; Hall & Harwood, 2017). However, certain issues must be addressed before including ω-3 FA-rich lipid sources in food products. PUFA which include ω-3 FA and ω-6 FA are extremely susceptible to oxidation due to the presence of multiple double bonds (Galano et al., 2015). PUFA also face physical limitations as they are mostly liquid oil at ambient temperatures, whereas most saturated fat sources are solid or semi-solid at the same temperatures. These physical differences must be addressed before using PUFA-rich lipid sources in place of saturated lipid sources (Lucca & Tepper, 1994).

Adding antioxidants is an easy solution to help improve the oxidative stability of PUFAs and ω-3 FAs. However, this does not help to address the physical limitations of these lipid sources. Gelation and microencapsulation are two processing techniques which could be used to convert liquid oils to stable solid fats at ambient temperatures. Through a combination of adding antioxidants and processing techniques the oxidative stability and physical characteristics of liquid oils can be altered to a more

desirable state. Using these strategies, successful alteration of high PUFA oils can allow for their use as a replacement for saturated fat sources in food products.

Antioxidants are used as components of foods, cosmetics, and pharmaceuticals every day. These compounds delay or inhibit the onset of oxidation and deterioration of a product. The majority of antioxidants used in industry include synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ) are suspected of being carcinogenic (Lee et al., 2009). Due to adverse health effects of synthetic antioxidants, plant-derived phenolics have attracted interest due to their beneficial and functional properties as well as their natural status. Phenolics and phenolic acids are a large family of compounds that possess at least one aromatic ring substituted with one or more hydroxyl groups (Ho,1992). The phenolic acids are naturally occurring secondary metabolites of plants (Shahidi, 2015).

The phenolic compounds are potent antioxidants which function as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation (Ghasemzadeh & Ghasemzadeh, 2011). As these compounds exist abundantly in plants in nature, they may be extracted for use in products. However, solubility issues are of concern as phenolic acids are generally insoluble in lipid phase and only slightly soluble in aqueous phase with high temperature and time dependency (Daneshfar, Ghaziaskar, & Homayoun, 2008). Due to solubility problems, the efficiency of phenolic acids as antioxidants will differ based on what type of system they are dissolved in and the chemical structure of the selected phenolic acid.

For instance, tocopherols (TOC), the family of compounds known as vitamin E function as free radical scavengers which deliver a H atom to quench free radicals. At 323 kJ/mol, the O-H bond in tocopherols is approximately 10% weaker than in most phenols (Lide, 2006). Due to this weaker hydroxyl bond, TOC can be utilized while keeping a natural ingredient, vitamin E, on the food label.

However, since TOC only has a single hydroxyl group, they may have a lower antioxidant activity than other naturally occurring or synthetic phenolic compounds. Combining antioxidants or determining optimal concentrations with various antioxidants could help solve limitations in efficacy that the use of typical synthetic antioxidants presents, but doing so still won't provide a solution for the physical limitations of liquid oils such as algal oil.

Using bulk oil with a selected gelator under heat with time can yield oleogels, and using emulsion in place of oil can yield emulgels. Both gel types can be solid at refrigerated and ambient temperatures, which allows for their use in food products that would normally rely on saturated fat sources. This processing technique also allows for improvement of the oxidative stability of oil and emulsion as seen in previous research (Hwang, Fhaner, Winkler-Moser, & Liu, 2018; Willett & Akoh, 2019). The choice of a gelator can also enhance the oxidative stability of a chosen matrix material, such as monolaurin which has been shown to enhance the oxidative stability of lipid matrixes (Moradi, Tajik, Razavi Rohani, & Mahmoudian, 2016).

The development of microencapsulation products is yet another processing technique which can be employed to improve the physical characteristics of a PUFA-rich lipid source while also contributing to the oxidative stability of an oil. Encapsulation of oil reduces its exposure to light, moisture, and oxygen, all of which decrease the quality of the oil through oxidative deterioration, formation of undesirable flavor compounds, and production of free radicals (Sagiri et al., 2014). It is also important to note that with microencapsulation there is the potential for leaching of the internal phase. Using gelation with microencapsulation has been shown to significantly reduce the amount of internal phase lost through leaching (Willett & Akoh, 2019). Additionally, utilizing two processing techniques in tandem with each other could help prevent oxidation of the ω-3 FAs better than simply using them alone.

It is hypothesized that including antioxidants together with two different processing techniques could potentially allow for the use of PUFA-rich oils in place of saturated fat sources. Comparing a saturated fat source to an optimal combination of processing techniques and antioxidants is novel as most research only seeks to apply one of these variables. This research has been a piece wise project spanning multiple chapters which will be collected here. Ultimately the goal of this research is to develop an ω-3 FA-rich ingredient that can replace saturated fats in foods and nutraceuticals. To achieve this goal, the specific objectives and hypotheses are set as follows.

Objective 1: Investigate the effect that different antioxidants and combinations of antioxidants have on a bulk soybean oil and O/W emulsion model system. **Hypothesis 1:** Different antioxidants will exhibit different activities in different matrices, depending on factors such as the polarity of each antioxidant. Exploring and understanding how well these antioxidants perform in each matrix allows for the selection of an optimal antioxidant for a given matrix, such as bulk oil versus a more polar O/W emulsion system.

Objective 2: Characterization and comparison of oleogels and emulgels prepared from *Schizochytrium* algal oil using monolaurin and a mixture of monoacylglycerols and diacylglycerols (MAG/DAG) as gelators. **Hypothesis 2:** Stable oleogels and emulgels will have significant improvement on oxidative stability over non-gelled oil. Additionally, different gelators will affect the physical properties of developed gels with different results. Determining an optimal combination will lead to the development of an ω-3 FA-rich ingredient that could be used in place of saturated fat sources.

Objective 3: Combining antioxidants and processing techniques to improve oxidative stability of a Schizochytrium algal oil ingredient with application in yogurt. **Hypothesis 3:** A

combination of processing techniques and antioxidants can be used to develop an ω-3 FA-rich ingredient that could be used to replace saturated fat in a given food product, such as yogurt. This developed ingredient could then be used in the future as an alternative fat source for a healthier alternative in foods, cosmetics, nutraceuticals, and pharmaceuticals.

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CHAPTER 2

LITERATURE REVIEW

Lipids

Lipids are a major classification of biomolecule that are soluble in nonpolar solvents (IUPAC, 1997). The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes (Fahy et al., 2009). Lipids have applications in pharmaceutical, cosmetic, and food industries. Lipid substrates may include glycolipids, fatty acids (FA) and their derivatives, waxes, sphingolipids, sterols, and phospholipids, and some examples can be seen in Figure 2.1. In addition to their fundamental role in biochemistry, lipids play an important role in foods as they affect traits such as texture, structure, mouthfeel, flavor, and color. Typically, simple lipids are connected to a glycerol backbone, where they may contain varying numbers of different FA. For instance, lipids are found as either monoacylglycerols (MAG), diacylglycerols (DAG), or triacylglycerols (TAG) depending on the number of FA connected onto a glycerol backbone.

TAG are the major form of energy storage in most living systems, making up the majority of lipids in animal and plant tissues. In vegetable oils, TAG make up more than 95% of the total lipids (Scrimgeour, 2005). The differences in biochemical and physical characteristics of different TAG are based on the FA which differ from each other by such things as carbon chain length, number of molecules, conformation of the double bonds, and stereochemical position of the FA esterified to glycerol (Lichtenstein, 2013).

When FA are no longer attached to the glycerol backbone they are known as free fatty acids (FFA). Some FFA have no double bonds in the carbon chain and are known as saturated fatty acids (SFA), some FFA have one double bond on the carbon chain and are known as monounsaturated fatty acids (MUFA), while some have multiple double bonds and are known as polyunsaturated fatty acids (PUFA). All FA are aliphatic carboxylic acids with chain lengths of 4-22 carbon atoms and for most natural sources C18-chains are most common (Scrimgeour, 2005). Due to the biosynthesis pathway by which FA are synthesized they are almost entirely found in nature with even numbered carbon chains, and double bonds exist in a characteristic pattern of methylene interrupted *cis* double bonds.

SFA are the main form of energy storage fats in terrestrial animals, particularly stearic acid (C18:0). Plant life differs in that the majority of energy storage fats are not in the SFA form, but instead are made up of MUFA and PUFA such as oleic (C18:1ω-9), linoleic (C18:2ω-6), and linolenic (C18:3ω-3) acids being predominant (Scrimgeour, 2005). Marine oils, including algal, fish, and marine mammal oils are high in PUFA, and because of this they can function as an excellent source of omega-3 FA (ω-3 FA) such as eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) whose structures are included in Figure 2.2. These PUFA are desirable as healthier options over SFA, and the American Heart Association (AHA) suggests that increasing the ω-3 FA content of food products, particularly EPA and DHA, can lead to a reduced risk for development of cardiovascular diseases (Stone, 1996; Kris-Etherton, Harris, & Appel, 2002).

PUFA can also function as essential nutrients, known as essential fatty acids (EFA), including some ω-6 and ω-3 PUFA derived from linoleic and α-linolenic acids, respectively, by elongation and desaturation (Lichtenstein, 2013). PUFA are also required for a diverse range of

biological functions, including the regulation of inflammatory response and hormone production, and aiding neural development and function (Calder, 2017; Hall & Harwood, 2017). Dietary sources of ω-3 PUFA include those common PUFA shown in Figure 2.2 and stearidonic acid. The main source of α-linolenic and stearidonic acids are plant oils, but EPA and DHA are found mainly in fish and algal oils. Aside from the recommendations made by the AHA, ω-3 FA also likely contribute to lower incidence of cardiovascular diseases, renal disorders, autoimmune disorders, and cancers (Cicero, Reggi, Parini, & Borghi, 2012; Shahidi & Miraliakbari, 2005). Due to these benefits, use of PUFA in foods, cosmetics, and pharmaceuticals is desirable to both consumers and industries alike, however, there are both physical and chemical issues that arise when incorporating PUFA in place of traditional SFA.

Typically, SFA are used in most foods as the traditional fat source. However, SFA are now considered less desirable than MUFA and PUFA due to research that suggests that SFA may cause adverse health effects. For instance, except for stearic acid, SFA raise cholesterol levels while MUFA and PUFA may actually help lower or at least keep levels constant (Micha & Mozaffarian, 2010; Yu, Derr, Etherton, & Kris-Etherton, 1995). The majority of readily available FA are SFA, and in fact the majority of naturally occurring fats from dietary sources are SFA (Table 2.1). However, new techniques and technologies are making it easier to source the more desirable EPA and DHA content. Solving problems such as physical limitations and oxidative stability related to the use of PUFA-rich oils may allow us to replace SFA in more food products than what used to be possible.

Lipid Oxidation

Lipid oxidation is one of the leading causes of food waste and economic loss in the food industry. Lipids are the most important macromolecule when discussing the oxidative

degradation in food products. PUFA, particularly ω-3 FA are extremely susceptible to oxidation due to the presence of multiple double bonds (Galano et al., 2015). The general principal for lipid autoxidation contains three steps which includes initiation, propagation, and termination as shown in Figure 2.3. The first step, initiation, is prone to occur due to certain environmental factors such as the presence of light, heat, pigments, metal ions, or enzymes. Lipid oxidation will occur and propagate leading to the chain reaction seen in Figure 2.3 until finally, a terminating step occurs, preventing further oxidation.

As mentioned previously, lipid oxidation is a major cause for the deterioration of foods leading to economic loss for the food industry, but lipid oxidation can also lead to quality loss in foods which may have adverse health effects on consumers (Frankel, Huang, Kanner, & German, 1994; Vieira, McClements, & Decker, 2015). For these reasons PUFA are not currently utilized in most applications, but replacing SFA with PUFA is still a desirable objective and for this reason, overcoming lipid oxidation is of particular interest to the food industry. There are three main types of oxidation that lipids undergo that must be considered, autoxidation, photooxidation, and enzymatic oxidation (Frankel, 1980). The most common of which has already been mentioned, autoxidation, whereby a free radical chain reaction occurs via initiation, propagation, and finally termination. Preventing heat, light, and metal from interacting with FA is one of the easiest ways to prevent autoxidation as it can prevent the initial radical from forming.

Photooxidation refers to the development of free radicals produced by ultraviolet light irradiation (Frankel, 2012). Enzymatic oxidation occurs when certain enzymes, such as lipoxygenase, catalyze the oxidation of lipids. Prevention of enzymatic oxidation can be done by heat treatment to denature enzymes or by limiting oxygen exposure such as nitrogen environmental packaging of meat (Moon, Kwon, Lee, & Kim, 2020). Preventing photooxidation

is generally accomplished by protecting lipids from excess UV light. However, preventing autoxidation is more difficult due to the number of initiators which exist. The required components for autoxidation include unsaturated FA, an initiator, and reactive oxygen species. The key step of autoxidation is the initiation, when an unsaturated FA loses a proton and electron caused by an initiator, which then produces a lipid-derived free radical, $R_1^{\bullet}$ (Frankel, 1984). This can then lead to a chain reaction that occurs until no hydrogen is available or the chain is broken, which is how primary antioxidants help prevent the oxidation of lipids in food products (Figure 2.4).

Lipid oxidation, as seen in Figure 2.4, leads to primary and secondary products. Primary products include hydroperoxides, which are intermediates in the process of lipid autoxidation, and are the first stable products formed. However, heat, metals, light, and other prooxidants can cause the eventual decomposition of hydroperoxides through scission reactions (Frankel, 1984). This decomposition leads to secondary oxidation products which include alcohols, aldehydes, ketones, hydrocarbons, volatile organic acids, and epoxides (Mallia, Escher, Dubois, Schieberle, & Schlichtherle-Cerny, 2009).

Measuring lipid oxidation

Measuring oxidation in lipids can be based on chemical, physical, or sensory changes that occur with oils due to oxidation. The following is an extensive but not exhaustive list of various methods used to measure the effect of oxidation on lipids: chromatographic based analysis for changes in lipids, iodometric titration, ferric ion complexes, peroxide value (PV), 2-thiobarbituric acid reactive substances (TBARS) value, *p*-anisidine value (*p*-AV), carbonyl value, Rancimat, Oxidative Stability Instrument (OSI), and oxidation induction time (OIT) by differential scanning calorimetry (DSC) (Shahidi & Zhong, 2005). Every method for

measurement listed has a different measured factor for oxidation in oil. PV and *p*-AV measure primary and secondary oxidation products for oils, respectively, and because of this they are an excellent combination of tests to obtain a picture of overall oxidation in samples. OIT may be used as a predictor for the general oxidative stability of samples compared against each other, but without using multiple tests throughout the actual oxidation of an oil there is no way to know how an oil will oxidize. There are many methods for measuring the oxidation of oils, but in general, multiple tests will always be required to understand the complete picture of oxidation for a given oil.

Antioxidants

As mentioned previously the use of antioxidants is one of the easiest and most effective ways to improve the oxidative stability of oils in foods, drugs, and cosmetics. An antioxidant is defined as any substance that when present in low concentrations compared with an oxidizing substrate will work to significantly delay or prevent the oxidation of that substrate (Halliwell, 1995). Antioxidants are naturally occurring and prevent oxidative stress in biomolecular reactions for living organisms. These reactions allow antioxidants to reduce the risk of degeneration and disease in organisms by protecting lipids, proteins, carbohydrates, and DNA from oxidative stress (Halliwell, 1990; Parzonko, Czerwinska, Kiss, & Naruszewicz, 2013). Similar reactions also allow antioxidants to prevent lipid oxidation in food, cosmetics, and pharmaceutical products, increasing shelf-life and product quality. This increased shelf-life leads to less product waste, causing less financial loss, and improves the time which products can be profitable as well.

There are multiple mechanisms by which antioxidants prevent oxidation in products. These mechanisms are used to broadly categorize the different types of antioxidants. Primary

antioxidants are those that can stop the chain reaction of oxidation by scavenging the free radical intermediates. Secondary antioxidants, also known as peroxide scavengers, decompose hydroperoxides (ROOH) into nonreactive products before they decompose into alkoxy and hydroxy radicals. They are often used in combination with free radical scavengers (primary antioxidants) to achieve a synergistic effect. Still, some antioxidants can exhibit multiple effects and utilize different mechanisms and are called multiple function antioxidants (Elias & Decker, 2017).

The primary antioxidants, also called type-1 or chain-breaking antioxidants, inhibit oxidation by donating a hydrogen/electron to free radicals, which prevents free radical propagation. The formed antioxidant radicals are stabilized by delocalization of the unpaired electron around the resonance structures, such as the phenol ring, to form stable low energy radicals. The formed antioxidant radicals can further scavenge free radicals in the termination stage. Thus, one molecule of a primary antioxidant can stabilize two lipid radicals and receive an electron from another radical to form a stable non-radical (Young & Lowe, 2001). Secondary antioxidants, also called type-2 or preventive antioxidants, prevent oxidation by several different mechanisms which do not include converting free radicals. Their inhibitory effect comes from decomposing hydroperoxides to nonreactive species, replenishing hydrogen to primary antioxidants, and suppressing oxidation promotors such as metal ions. The use of secondary antioxidants as synergists is common.

Natural antioxidants

Antioxidants found in nature consist of phenolic acids, flavonoids, stilbenes, coumarins, lignans, and tannins which can be further broken down into more specific classifications as seen in Figure 2.5. These compounds exhibit a wide range of antioxidant activities *in vitro* and are

even thought to possibly exert protective effects against diseases such as cancer and cardiovascular diseases (Boudet, 2007). Among these natural antioxidants are tocopherols and tocotrienols, which are the compound family that make up what is commonly referred to as vitamin E. Tocopherols and tocotrienols are common in many different vegetable oils, which can be seen in Table 2.2. Natural vitamin E content in various oils protect the lipid content without the need for added antioxidants or complex solutions.

However, natural antioxidants (Table 2.2) cannot completely protect PUFA content in oils due to the unsaturated nature of the FA. PUFA breakdown much faster than the lipid content in vegetable oils, most of which would exist in either SFA or MUFA form of C18 FA (Frankel, Satuega-Gracie, Meyer, & German, 2002). To help protect against lipid oxidation in ω-3 FA other antioxidants that can be produced on demand may be incorporated into product. These antioxidants follow the same mechanisms previously discussed, but most literature suggest that they have higher antioxidant efficiencies than their natural counterparts when compared against each other in the presence of ω-3 FA (Rodrigues, et al., 2020).

Synthetic antioxidants

Synthetic antioxidants currently permitted for use in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary-butylhydroquinone (TBHQ). In addition, octyl gallate (OG) and dodecyl gallate (DG) are also used as synthetic antioxidants (Makahleh, Saad, & Bari, 2015). These antioxidants are added into foods to protect lipids within legally allowed maximum use concentrations as seen in Table 2.3. These synthetic antioxidants are regulated due to their potential toxicity at higher levels. The majority of currently utilized synthetic antioxidants are suspected of being carcinogenic (Stamatis, Sereti, & Kolisis, 1999). BHA and BHT are suspected of being responsible for liver damage and

carcinogenesis when used at high concentrations in laboratory animals (Powell, Connelly, Jones, Grasso & Bridges,1986; Grice, 1986).

Due to these adverse health effects of synthetic antioxidants, studies have been focused on the increasing potential use of natural antioxidants for food and lipid oxidation prevention but solubility issues limit their applications in foods. Phenolic acids, for example are basically insoluble in non-polar media such as fats and oils, and they're sparingly soluble in water-based media as they require either high temperatures or excessive time commitment to dissolve in such media (Daneshfar, Ghaziaskar, & Homayoun, 2008; Mota, Queimada, Pinho, & Macedo, 2008). In order to make natural antioxidants more viable in non-polar media hydrophilization and lyophilization can be employed to modify natural compounds to improve solubility in a given medium. For example, when gallic acid (GA) is esterified with glycerol, the aqueous solubility of the reaction product, 1-*o*-galloylglycerol (GG), is greatly enhanced by the hydroxyl groups of the glycerol moiety (Zhang & Akoh, 2020).

The development and implementation of underutilized natural compounds such as GG represent a possible solution for the oxidative stability issues of PUFA in food products. GG has a much greater solubility in polar media (Zhang & Akoh, 2020), and as of yet has no known adverse health effects (Nilson, Bender, & Darling, 1950). Additionally, the development of novel compounds with improved lipid solubility such as 1,2-dipalmitoylgalloylglycerol (DPGG) and 1,2-dioctanoylgalloylglycerol (DOGG) (Zhang, Hyatt, & Akoh, 2021; Zhang, Hyatt, & Akoh, 2022) could prove useful as an antioxidant in place of currently used synthetic antioxidants. However, without safer options for mass produced oil-soluble antioxidants, other methods for protecting PUFA-rich lipid sources may need to be explored alongside the use of commercially available antioxidants. Different processing techniques exist which could allow fats and oils to

not only be protected from oxidation but could also improve the physical characteristics of PUFA-rich lipid sources in such a way as to make them a viable replacement for traditional SFA-rich oils.

Processing Techniques

In addition to using antioxidants to improve oxidative stability of lipids, another potential method to prevent oxidation could include improving the physical characteristics of PUFA-rich oils in order to decrease exposure to sources of oxidation. Gelation and microencapsulation are two different forms of processing that could improve oxidative stability of oils. These techniques could prove to be helpful in preventing light and oxygen from permeating the desired product. Organogels, also known as oleogels, if the continuous phase is oil, are lipid gels that could potentially be used as an alternative to highly saturated fats (Co & Marangoni, 2012). Oleogels are a relatively recent discovery in the past decade and have a wide range of applications (Co & Marangoni, 2012; Patel et al., 2014 Patel & Dewettinck, 2015). Studies have shown that these oleogels may inhibit oil migration in chocolate, improve oxidative stability, be acceptable low saturated fat alternatives to shortening/margarines, and control the release of sensitive compounds such as antioxidants, bioactive compounds, and essential PUFA that are susceptible to oxidation (Co & Marangoni, 2012). Since this field of research is relatively new, the development of oleogels, gelators, gelling mechanisms, and functionality of these gels is of great interest to both academia and food industry. Microencapsulation is another possible method that may improve the oxidative stability of lipids. Microencapsulation is the process of coating tiny particles/droplets to form small (micro-sized) capsules. The core material, or internal phase, is typically an oil in food applications, however the internal phase can be solid, liquid, or gas (Bakry et al., 2016). Another added benefit regarding oils high in EPA and DHA is a potential

odor masking effect with microencapsulation which is of interest due to the common fishy off flavors exhibited by these oils. In fact, several studies have encapsulated fish oil using a variety of methods and encapsulating agents for this reason (Encina, Vergara, Gimenez, Oyarzun-Ampuero, & Robert, 2016, Willet, Martini, & Akoh, 2019). Typically, studies have been more interested in establishing microencapsulation methodology and textural property analysis than oxidative stability. Some studies have examined the effect of microencapsulation on the oxidative stability of oils and found that there is a protective effect as hypothesized (Willet, & Akoh, 2019). However, one potential problem with microencapsulation on its own the internal phase can leach out, causing loss of oil in the product. For this reason, several studies have successfully combined oleogelation with microencapsulation, improving texture, odor, and oxidative stability without sacrificing oil from the leaching effect (Sagiri, Sethy, Pal, Banerjee, Pramanik, & Maiti, 2012; Sagiri, Pal, Basak, Rana, Shakir, & Anis, 2014).

Gelation

Gels are colloids (aggregates of fine particles dispersed in a continuous medium) in which the liquid medium has become viscous enough to behave more or less as a solid. Currently, the most widely accepted definition of a gel is "a material that has a continuous structure with macroscopic dimensions that is permanent on the time scale of an analytical experiment and is solid-like in its rheological properties" (Flory, 1974). Gels can be classified into four general categories: well-ordered lamellar structures such as lyotropic phases, covalently linked polymer networks, entangled polymer networks held together by transient physical interactions, and disordered particulate structures (Flory, 1974). Gels are further classified based on the internal component that is being gelled. Gels with a gelled water-phase are called hydrogels, and gels with a gelled non-polar phase are called organogels or oleogels (if oil is

gelled). Organogels can also be further classified into two categories: polymeric organogels and low-molecular weight organogels. Most organogels that have been studied in literature are of the low-molecular weight type and are often crystalline dispersions of two phases: dispersed structurant (organogelator) and solvent being structured (oil).

As previously mentioned, another name for these organogels using oil as the continuous phase is an oleogel. Oleogels are low-molecular weight organogels with oil as solvent. These oleogels function through the observed phenomenon of immobilizing oil particles in a crystalline formation as discussed and explained in two extensive review papers (Marangoni & Edmond, 2012; Li, Liu, Bogojevic, Nedergaard Pedersen, & Guo, 2022). Other work using small-angle X-ray scattering with oleogels found that a crystalline nanotubular formation in a stacked helical structure entraps lipids to form a solidified gel (Bot, Adel, & Roijers, 2008). Fourier transform infrared spectroscopy (FTIR) was used to observe the hydrogen bonding in oleogels which supported the proposed mechanism of a composite polycrystalline network in oleogels (Zampouni et al., 2022).

Another form of gelation which could be used in future products might be emulsion gels. Emulsion gels or emulgels can be defined as a gel with a composite structure consisting of oil droplets within a gel matrix (Farjami & Madadlou, 2019). Emulgels are of particular interest due to their ability to substitute for the more unstable oil-in-water (O/W) emulsions used in pharmaceuticals, cosmetics, and foods. Emulsions are thermodynamically unstable systems and attempt to revert back into separate water and oil phases over time via various physicochemical mechanisms, including coalescence, gravitational separation, flocculation, Ostwald ripening and phase separation (McClements, 2014; McClements, Decker, & Weiss, 2007).

Using gelation to produce oleogels and emulgels in order to replace SFA-rich lipid sources and the less stable O/W emulsions could be a strategy to incorporate the desired ω-3 FA into foods. Utilizing this processing technique in addition to antioxidants could potentially allow for the development of ω-3 rich food products with shelf lives extended to match their SFA counterparts. Little research has been done to understand if the combination works well enough to ensure oxidative stability of a given food product. Simple products such as yogurt or a salad dressing could be developed in a trial test with a given saturated fat source, such as butterfat, and compared against a PUFA-rich lipid source to assess how well the combination of processing techniques and antioxidants protect lipids from oxidation.

Microencapsulation

Another method that may improve the oxidative stability of ω-3 PUFA is microencapsulation. While addition of antioxidants to the bulk oil is typically sufficient in improving oxidative stability, microencapsulation also masks some of the undesirable off flavors and odors associated with ω-3-rich oil (Encina, Vergara, Gimenez, Oyarzun-Ampuero, & Robert, 2016). Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to produce small capsules, with useful properties. In general, it is used to incorporate food ingredients, enzymes, cells, or other materials on a micro metric scale (Silva et al., 2014). The core material, or internal phase is typically an oil in food applications, however, the internal phase can be solid, liquid, or gas (Bakry et al., 2016). When these microcapsules are utilized, the internal phase gradually diffuses through the coating and releases outside the microcapsule. This gradual release allows for controlled release of the internal phase which is beneficial in many applications such as in drug delivery or controlling release of bioactive or health beneficial compounds (Bakry et al., 2016; Sagiri et al., 2014; Sagiri et al., 2012). The wall coating is

typically made up of carbohydrates (glucose syrup, maltodextrin, n-OSA starch, pectin, chitosan, lecithin, ethylcellulose, lactose, or corn syrup) or proteins (whey protein, caseins) in food applications (Encina et al., 2016; Bakry et al., 2016). These coatings offer a barrier that protects the internal phase from outside exposure. When lipids such as ω-3-rich oil are microencapsulated, their stability and shelf life increase due to the decrease in exposure to factors that cause lipid oxidation such as oxygen, light, heat, and moisture (Bakry et al., 2016).

This gradual release over time is acceptable in most applications where microencapsulation is applied, mostly in medicine, but it may not always be desirable for food applications (Sagiri et al., 2014). In this sense, it may be necessary to utilize gelation of the microencapsulated product in order to prevent excess leaching out of the internal phase (Willett & Akoh, 2019). Additionally, it is suspected that the combination of these processing techniques will lead to an improvement in oxidative stability, and while very little research has been done on the oxidative stability of the products using this combination (Sagiri et al., 2012, Sagiri et al., 2014; Willett & Akoh, 2019), there is virtually no data on the effect of these processing techniques when used in conjunction with selected antioxidants. Combining the two methods with antioxidants should allow for a physically stable product which can be used in place of current saturated fat sources. These products may potentially last as long on shelves as the currently used products utilizing saturated fat, and because of this the SFA-rich sources could be replaced with the healthier ω-3 FA-rich lipid sources.

Development of an ω-3 Rich Yogurt Food Product

There have been several studies which incorporate ω-3 rich oils in the development of a yogurt product (Bakry, Chen, & Liang, 2019; Gumus & Gharibzahedi, 2021; Ramadan, El-Said, El-Messery, & Mohamed, 2021; Matos, Afonso, Cardoso, Serralheiro, & Bandarra, 2021). The

main goal behind this research has been to replace saturated fat sources with an ω-3 PUFA-rich lipid source that mimics characteristics of highly saturated fats in terms of their physical properties (Bakry, Chen, & Liang, 2019; Gumus & Gharibzahedi, 2021). Most of this work has subsequently been focused on the health benefits from the developed yogurt and its use as an innovative functional food (Matos, Afonso, Cardoso, Serralheiro, & Bandarra, 2021; Ramadan, El-Said, El-Messery, & Mohamed, 2021).

While the health effects of a yogurt product rich in ω-3 FA makes for an interesting study, there is little research conducted on the actual oxidative stability of the developed yogurt. This is important not only from the cost analysis for implementing a product like this (Kumar, Balasubramanyam, Rao, Dixit, & Singh, 2017), it's also important from a health standpoint in that many products of oxidation are health hazards (Frankel, Huang, Kanner, & German, 1994; Vieira, McClements, & Decker, 2015). New research is required to develop an optimized ω-3 FA-rich ingredient for use in a yogurt food product. This work is necessary to understand the suitability of potential antioxidants, gelators, and formulations that could be used with a given ω-3 rich lipid source to develop a food product with similar physicochemical properties using ω-3 rich lipid as the saturated fat replacement.

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Tables

Table 2.1

Common fatty acids and dietary sources (Scrimgeour, 2005, with permission)

Fatty acid	Common name	Formula	Chain length	Significant Sources
4:0	Butyric	$CH_3(CH_2)_2CO_2H$	Short	Butter, dairy fats
6:0	Caproic	$CH_3(CH_2)_4CO_2H$	Short	Coconut, palm kernel
8:0	Caprylic	$CH_3(CH_2)_6CO_2H$	Short/medium	Coconut, palm kernel
10:0	Capric	$CH_3(CH_2)_8CO_2H$	Medium	Coconut, palm kernel
12:0	Lauric	$CH_3(CH_2)_{10}CO_2H$	Medium	Coconut, palm kernel
14:0	Myristic	$CH_3(CH_2)_{12}CO_2H$	Medium	Coconut, palm kernel
16:0	Palmitic	$CH_3(CH_2)_{14}CO_2H$	Long	Cottonseed, palm
18:0	Stearic	$CH_3(CH_2)_{16}CO_2H$	Long	Cocoa butter, tallow
18:1 9*c*	Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	Long	Cottonseed, olive, palm, rape
18:2 9*c*12*c*	Linoleic	$CH_3(CH_2)_4(CHCHCH_2)_2(CH_2)_6CO_2H$	Long	Corn, sesame, soybean, sunflower
18:3 9*c*12*c*15*c*	α-Linolenic	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6CO_2H$	Long	Linseed
22:1 13*c*	Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO_2H$	Long	High erucic rape
20:5 5c 8*c*11*c*14*c*17*c*	EPA*	$CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2CO_2H$	Long	Fish and animal fats
22:6 4*c*7*c*10*c*13*c*16*c*19*c*	DHA*	$CH_3CH_2(CH{=}CHCH_2)_6CH_2CO_2H$	Long	Fish and animal fats

*Abbreviations of the systematic names eicosapentaenoic acid and docosahexaenoic acid

Table 2.2

Tocopherol and tocotrienol contents (mg/kg) of select vegetable oils (Shahidi & Ambigaipalan, 2015, with permission)

Oil	Tocopherol				Tocotrienol			
	α	β	γ	δ	α	β	γ	δ
Borage[c]	-	-	150	1350	-	-	-	-
Camelina[a]	38	0.9	720	15	-		-	-
Coconut[b]	5-10	-	5	5	5	Trace	1-20	-
Corn[a]	180	11	440	22	9.4	-	13	2.6
Cottonseed[b]	40-560	-	270-410	0	-	-	-	-
Evening Primrose[c]	160	-	420	65	-	-	-	-
Linseed[a]	12	Trace	520	9.5	-	-	-	-
Olive[b]	1-240	0	0	0	-	-	-	-
Palm[b]	180-260	Trace	320	70	120-150	20-40	260-340	70
Peanut[b]	80-330	-	130-590	10-20	-	-	-	-

Sources: Adapted from [a]Schwartz, Ollilainen, Piironen, & Lampi (2008); [b]Shahidi & Naczk (2004); [c] Shahidi (2004)

Table 2.3 Maximum usage levels (Codex General Standards) permitted by Codex Almentarius Commission for synthetic antioxidants (Shahidi & Ambigaipalan, 2015, with permission)

Food Category	**Maximum Usage level (mg/kg)**			
	BHA	BHT	PG	TBHQ
Beverage whiteners	100	100	-	100
Milk powder and cream powder	100	200	200	-
Butter oil, anhydrous milkfat, ghee	175	75	100	-
Vegetable oils and fats	200	200	200	200
Lard, tallow, fish oil, and other animal fats	200	200	200	200
Fat spreads, dairy fat spreads and blended spreads	200	200	200	200
Fat emulsions mainly of type oil-in-water, including mixed and/or flavored products based on fat emulsions	200	200	200	200
Fat-based desserts excluding dairy-based dessert products	200	200	-	200
Edible ices, including sherbet and sorbet	200	100	50	200
Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	200	200	200	-
Cocoa and chocolate products	-	200	200	200
Imitation chocolate, chocolate substitute products	200	200	200	-
Confectionery including hard and soft candy, nougats, etc.	400	400	1000	200
Chewing gum	200	200	200	400
Decorations (e.g., for fine bakery wares), toppings (nonfruit) and sweet sauces	-	-	100	200
Whole, broken, or flaked grain, including rice	200	100	200	-
Breakfast cereals, including rolled oats	200	200	100	-

Figures

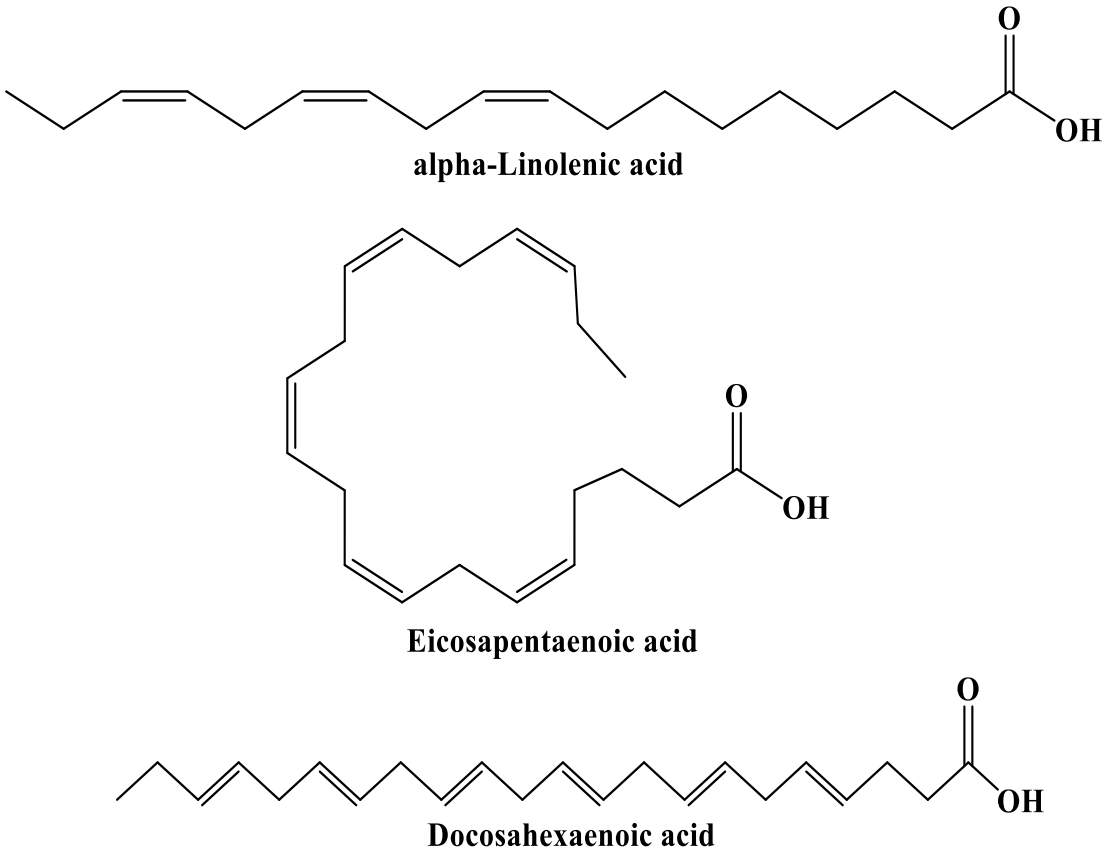
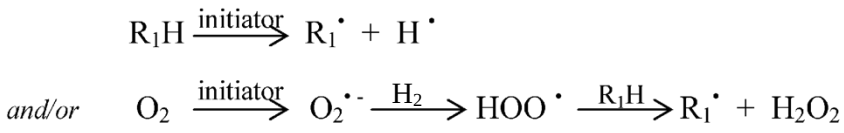


Figure 2.1 Structures of the most common ω-3 FA

Initiation:



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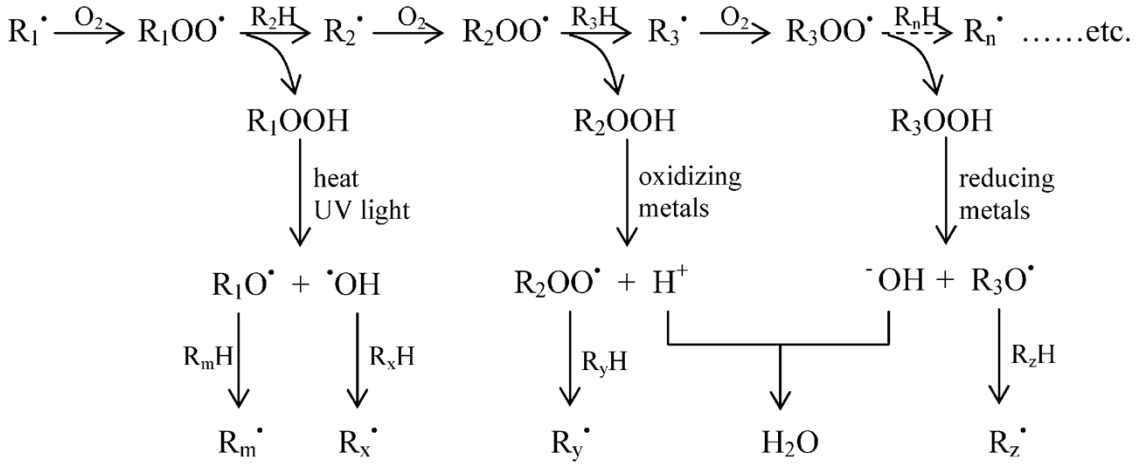

Termination:

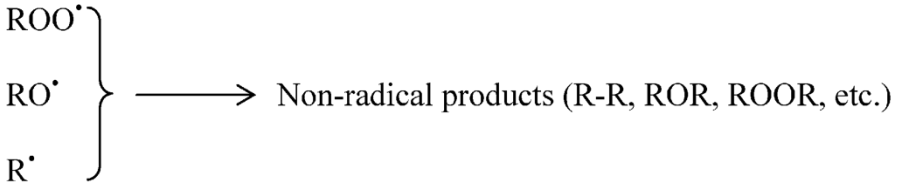

Figure 2.2 Simplified reaction mechanism of lipid autoxidation (Shahidi & Zhong, 2010, with permission)

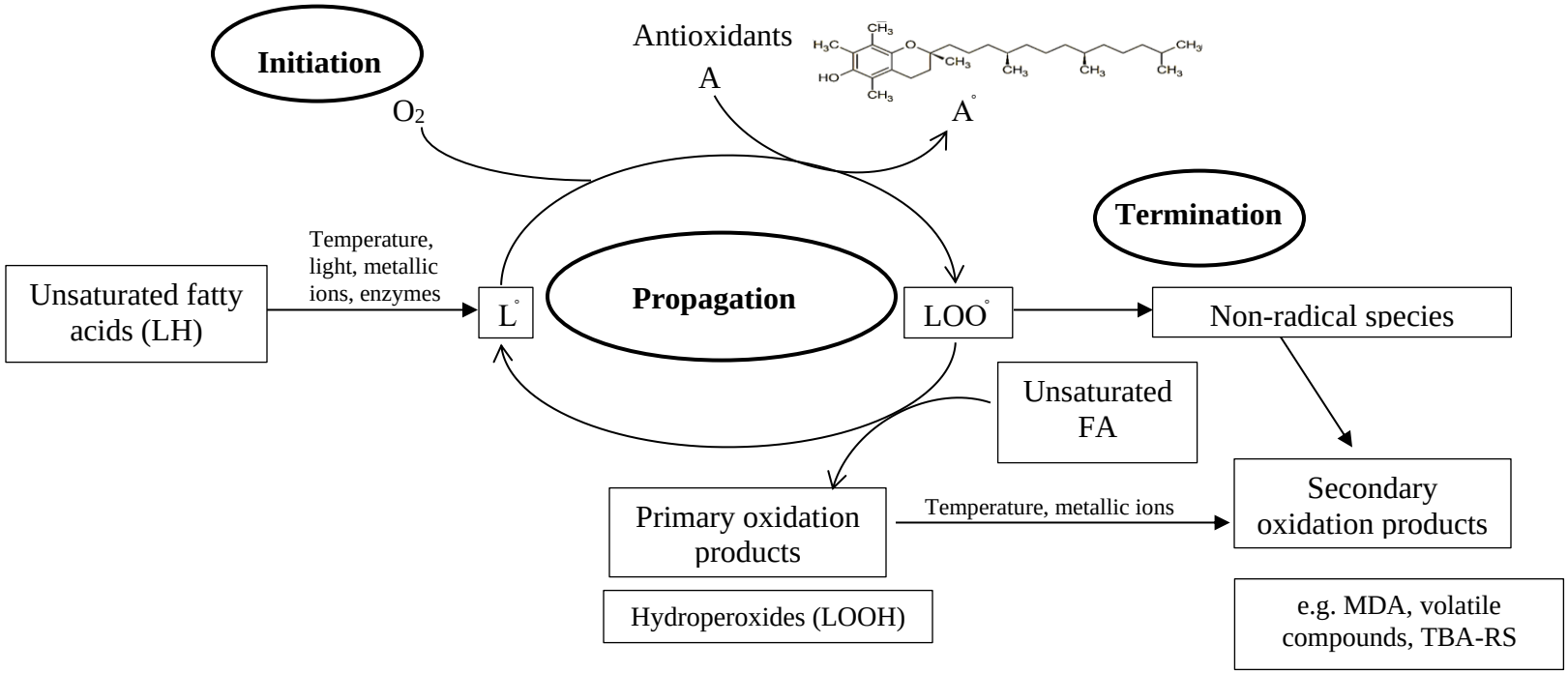

Figure 2.3 Simplified reaction scheme for lipid oxidation showing the role antioxidants take in chain-breaking lipid autoxidation (Guyon, Meynier, & Lamballerie, 2016, with permission)

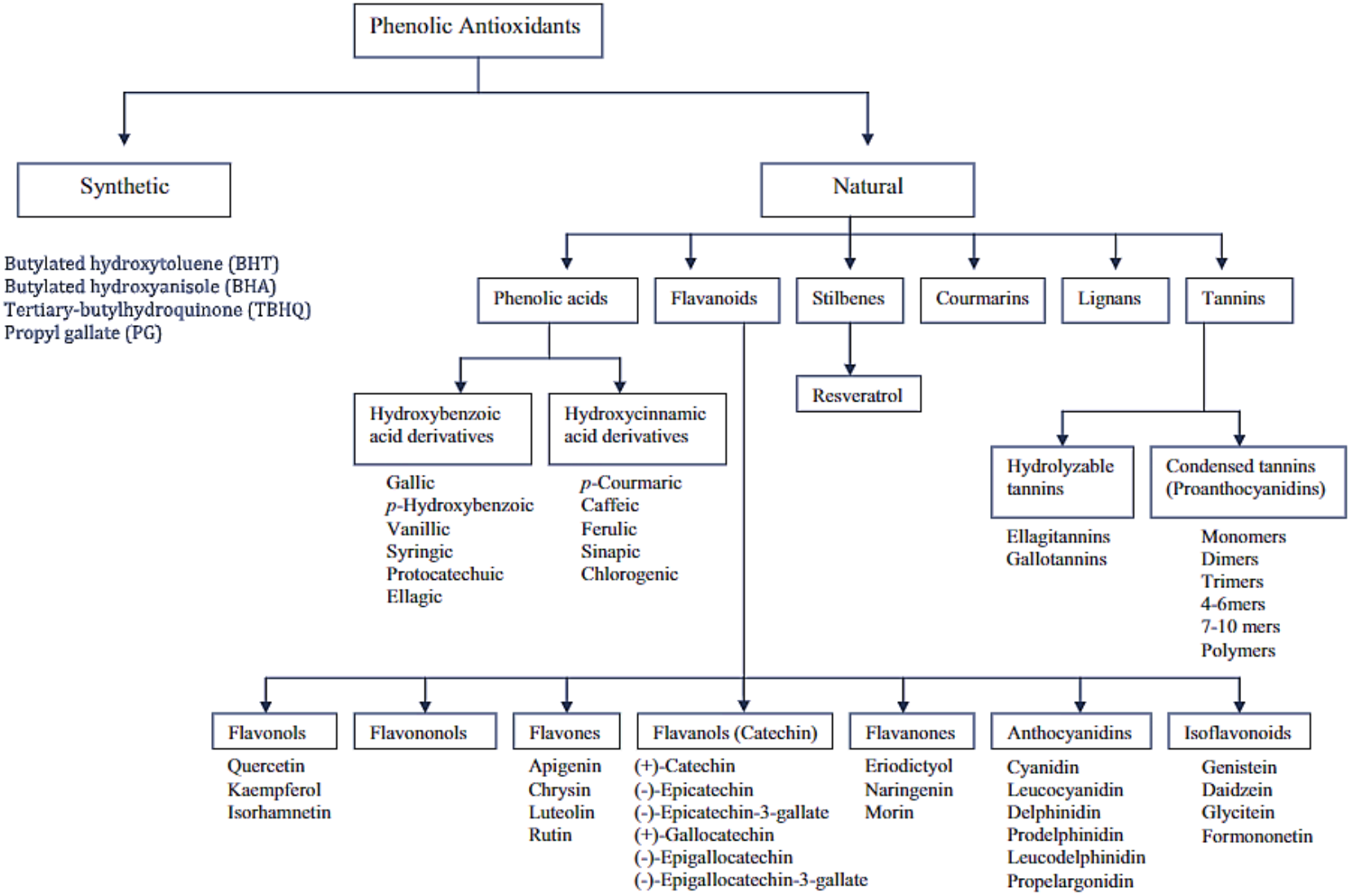

Figure 2.4 Classification of phenolic antioxidants (Shahidi & Ambigaipalan, 2015, with permission)

CHAPTER 3

COMPARISON OF ANTIOXIDANT ACTIVITIES OF SELECTED PHENOLIC COMPOUNDS IN O/W EMULSIONS AND BULK OIL[1]

[1] Hyatt, J. R., Zhang, S., & Akoh, C. C. (2021). *Food Chemistry*, 349, 129037. Reprinted here with permission of the publisher.

Abstract

Antioxidant activities of 1-o-galloylglycerol (GG), propyl gallate, rosmarinic acid (RA), tocopherols (TOC), and 1:1 combinations of GG/RA and GG/TOC were evaluated using in vitro assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+•), and ferric reducing antioxidant power (FRAP). Soybean oil stripped of TOC was utilized as bulk oil and as the oil phase in O/W emulsions for accelerated oxidation test with the selected phenolic compounds. Efficacies of antioxidants were evaluated by monitoring total oxidation (TOTOX) values and fatty acid profiles of oil and O/W samples during the accelerated oxidation. In bulk oil, GG outperformed other singular antioxidants, preventing 39.04% of oxidation for ω-3 fatty acids with a TOTOX value of 166.68. In emulsions, TOC outperformed other singular antioxidants, preventing 38.04% of oxidation with a TOTOX value of 196.72. Considering the polarities of the antioxidants and our testing systems, these results provide supporting evidence for the polar paradox theory.

Keywords: Antioxidant; Lipid oxidation; 1-o-Galloylglycerol; O/W emulsion; antioxidant in-vitro assays.

Introduction

Lipid oxidation is a major concern for the food industry as it causes food spoilage and rancidity, which then causes financial losses due to product waste. Furthermore, lipid oxidation products often have harmful effects adverse to human health. Polyunsaturated fatty acids (PUFAs) are some of the most susceptible to oxidation (Galano et al., 2015). This susceptibility is because the rate of reaction for lipid oxidation is directly proportional to the degree of unsaturation. In some cases, PUFA present in oils and emulsions not only function as energy storage material but are also involved in a diverse range of important biological functions. In cases such as this PUFA can act as an essential nutrient which can aid with the development and function of the human body (Briggs, Bowen, & Kris-Etherton, 2017). One important field of study is the use of antioxidants to prevent or slow lipid oxidation in food products.

One such field of study includes emulsions and emulsified food products. For the purpose of this present research, oil-in-water (O/W) emulsions of stripped soybean oil were studied to determine the effect selected antioxidants would have on oxidative stability. Soybean oil was selected for bulk oil tests as it is one of the largest supplies of oils used in the world at approximately 29% (Gerde & White, 2008), and it can be easily stripped to develop an unprotected oil to act as a baseline product free from antioxidant protection. For consistency, stripped soybean oil was used as the oil phase in O/W emulsions. O/W emulsions were developed to certain specifications to represent a selected food item, yogurt (Citta et al., 2017), which will be utilized for future studies.

Recently, a novel method for the enzymatic synthesis of 1-*o*-galloylglycerol, GG, was developed (Zhang & Akoh, 2019). Previous studies have shown that GG has considerable potential for use as an antioxidant, and it exhibits a higher antioxidant efficacy than other

common phenolic compounds (Zhang & Akoh, 2020). Additionally, when placed inside of a non-polar environment, such as a modified structured lipid, GG outperformed other antioxidants in terms of prohibiting oxidation (Zhang, Willett, Hyatt, Martini, & Akoh, 2021). However, to our knowledge, GG has not been tested in O/W emulsions before. O/W emulsions exist in a wide range of food products and are susceptible to spoilage/rancidity as a result of lipid oxidation. O/W emulsions need to be included in studies comparing the antioxidant ability of various compounds to fully understand the potential of such compounds as an antioxidant.

Typically, when antioxidants are compared in O/W emulsions, the results indicate that the more non-polar the antioxidant, the better it performs in polar media (Noon, Mills, & Norton, 2020). This phenomenon is known as the polar paradox theory (Porter, 1980). Since GG is relatively untested, it is still unknown how it may perform in this environment. By accessing GG against other antioxidants with a range of polarity and antioxidant combinations we seek to better understand the full antioxidant properties that other studies have previously sought to characterize (Zhang & Akoh, 2020). Additionally, this study will provide basis for follow-up studies which will utilize emulsion parameters optimized here.

The objectives of this study include providing a comparison on how selected phenolic compounds perform in both polar and non-polar systems. Additionally, we want to investigate the interactions between GG and other phenolic compounds in select 1:1 combinations, to assess whether such combinations are synergistic or antagonistic. Such combinations could prove useful in controlling lipid oxidation of food products currently on the market.

We report the antioxidant activities of GG, propyl gallate (PG), rosmarinic acid (RA), a mixture of α, β, γ, and δ tocopherols (TOC), as well as one-to-one mixtures for GG+RA, and GG+TOC accessed with *in-vitro* assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2'-

azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$), and ferric reducing antioxidant power (FRAP). Mixtures of GG with RA or TOC were included to assess potential synergistic effect with GG as a follow up on previous work (Zhang, Willett, Hyatt, Martini & Akoh, 2021). Furthermore, antioxidant efficacy was evaluated in bulk oil and O/W emulsions. Soybean oil was distilled to produce an oil without interference of pre-existing antioxidant to study the antioxidant activity of selected compounds more accurately. Additionally, the oxidation induction time (OIT) of bulk oil and antioxidants/combinations was measured using differential scanning calorimetry (DSC). Antioxidant efficacies of selected phenolic compounds in bulk oil and O/W emulsions were evaluated with accelerated oxidation tests, which included measuring peroxide value (PV), *p*-anisidine value (*p*AV), and calculating the total oxidation (TOTOX) value. The degradation of PUFA in oil and O/W emulsions were monitored by gas chromatography (GC).

Materials and Methods

Chemicals and reagents

PG, RA, and TOC were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The TOC mixture used contained 11.13%, 1.55%, 68.59%, and 18.73% of α, β, γ, and δ-tocopherols (w/w), respectively. Ryoto™ Sugar Esters, S970 and S1170, were obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). TWEEN® 80 was purchased from Sigma-Aldrich Chemical Co. Soybean oil without additives was purchased from H&B Oils Center Co. (Westchester, IL, USA). All other reagents and solvents were of analytical or HPLC grades and were purchased from Fisher Chemical (Fair Lawn, NJ, USA), Sigma-Aldrich Chemical Co. and J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). All materials mentioned, except soybean oil, were used without further purification steps.

Preparation of soybean oil and phenolic compounds

Naturally occurring TOC was stripped off of soybean oil through short-path distillation using the Chem Tech distillation model SP504-840 (Levelland, TX, USA). The short-path distillation used a temperature of 180°C and a vacuum maintained at 100 mTorr. The concentration of TOC was assessed before and after short-path distillation via normal phase HPLC at a wavelength of 292 nm according to American Oil Chemists' Society (AOCS) Official Method Ce 8-89 (American Oil Chemists' Society, 2011). For the analysis, an Agilent 1100 HPLC system connected to an Agilent 1100 series DAD as the detector equipped with an Agilent Zorbax Rx-SIL column of dimensions 5 µm x 4.6 mm x 250 mm (Agilent, Santa Clara, CA, USA) was used. The HPLC method utilized an isocratic flow with a mobile phase composed of an isopropanol: hexane, (v:v), 0.5:99.5 mixture. TOC standard mixture was analyzed and compared against soybean oil before distillation in order to quantify the presence of naturally occurring TOC. Standard curve for TOC using concentrations of 10, 5, 2.5, 1.25, and 0.625 µg/mL in hexane was created to quantify TOC presence in soybean oil. Soybean oil was stripped in order to ensure there was no antioxidant component prior to the addition of antioxidants. After distillation, stripped soybean oil was analyzed in triplicate to ensure no detectable amounts of naturally occurring TOC were present.

GG was synthesized in a previous study (Zhang & Akoh, 2019). Briefly, PG was dissolved in glycerol at a 1:25 mole ratio, and the enzyme, Lipozyme® 435, was added into the mixture at 23.8% (w/w) total substrates. The reaction took place in a double jacketed glass reactor with a circulating water bath (55°C) under constant stirring (200 rpm) for 120 h. Additional details dealing with purification are described elsewhere (Zhang & Akoh, 2020). Before testing, to alleviate any dispersion difference that might occur between antioxidants and

the matrix, GG and other phenolic compounds were dissolved in ethanol at a concentration of 1 mg/mL. Compounds were stored at -20°C until it was time to test.

Antioxidant assays

Ferric reducing antioxidant power (FRAP) assay

In order to assess the theoretical antioxidant efficiency of selected phenolic compounds outside of any protected matrix, several *in vitro* assays were conducted. FRAP assay was conducted according to previous studies (Benzie & Strain, 1996) with minor modifications (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). FRAP reagent was prepared in a 1:1:10 (v/v/v) ratio of 10 mM TPTZ (in 40 mM HCl), 20 mM $FeCl_3$, and 300 mM acetate buffer, pH 3.6. For comparison purposes, aqueous solutions of $FeSO_4 \bullet 7H_2O$ at concentrations of 100, 200, 500, and 1000 µM were used. The phenolic compounds GG, PG, RA, TOC, and mixtures GG+TOC and GG+RA were dissolved in water at various concentrations (25, 50, 100, 150, and 250 ppm) while using water as a blank. All reagents and samplers were prepared fresh on the day of analysis. The UV-Vis absorbance of samples were measured using a UV-1601 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at 593 nm. The results were expressed as the Fe^{2+} µM equivalents. All experiments were performed in triplicate.

2,2-Diphenyl-1-picrylhydrazyl scavenging (DPPH$^{\bullet}$) assay

DPPH$^{\bullet}$ scavenging assay was conducted according to a previous study (Compton, Laszlo, & Evans, 2012). Phenolic compounds and the mixtures were dissolved in methanol at varying concentrations (2.5, 5, 10, 15 ppm). DPPH was dissolved in methanol at a concentration of 200 µM. Equal amounts of DPPH$^{\bullet}$ and sample were then mixed and monitored at 517 nm for 30 min using the UV- 1601 spectrophotometer. Methanol mixed with DPPH$^{\bullet}$ instead of sample was used as the control. As a comparative method, an additional set of triplicate experiments per sample

were analyzed by mixing the same amounts of $DPPH^{\bullet}$ and sample as above, but then placed in low-light conditions before being analyzed at 517 nm after 30 min. All reagents and samples were prepared fresh on the day of analysis.

While the original method (Compton et al., 2012) allows for the determination of kinetics for antioxidants in the presence of $DPPH^{\bullet}$, the new comparative method does not, instead it offers faster analysis of samples without significant differences in results. As such, if the goal of analysis is to find the remaining percentage of $DPPH^{\bullet}$ without collecting data on the kinetics of the reaction, this modified method will allow for more efficient testing. The results were expressed as the remaining percentage of $DPPH^{\bullet}$ present after 30 min of reacting with sample. All experiments were performed in triplicate.

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate ($ABTS^{+\bullet}$) assay

$ABTS^{+\bullet}$ assay utilized method reported in previous studies (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999) with modifications (Phonsatta et al., 2017; Zhang & Akoh, 2019). $ABTS^{+\bullet}$ was generated by reacting 2.45 mM $K_2S_2O_8$ with 7 mM aqueous ABTS solution in a dark environment for 16 h. The generated $ABTS^{+\bullet}$ solution was then diluted with ethanol until an absorbance of 0.70 ± 0.01 was achieved at a wavelength of 734 nm. Selected phenolic compounds were dissolved in ethanol to obtain varying concentrations (2.5, 5, 10, 15, and 30 ppm). The standard for measuring against the antioxidant activity of samples with this assay was a Trolox™ standard curve using ethanol solutions at concentrations of 10, 20, 50, 100, and 200 µM. Ethanol was used in place of sample as a control. All reagents and samples were prepared fresh on the day of analysis.

A 100 µL aliquot of sample was mixed with 900 µL of the $ABTS^{+\bullet}$ solution, before the mixture was incubated in a dark environment for 6 min at 30°C. The absorbance of the sample

was measured at 734 nm, and the results were expressed as the decrease in absorbance after mixing the sample compared against control without antioxidant added. All experiments were performed in triplicate.

Interfacial and surface tension and particle size analysis

Interfacial and surface tension was measured following the method detailed in a previous study (Akoh,1992) in order to assess the potential emulsion stability of different emulsifiers at varying concentrations (Bourrel, Graciaa, Schechter, & Wade, 1979). Surface and interfacial tensions were determined with deionized water and tocopherol stripped soybean oil at room temperature (25°C) with a CSC-DuNouy Interfacial Tensiometer Model 70545 (CSC Scientific Company, Fairfax, VA). The mixture consisted of 5% stripped soybean oil and 0.1, 0.5, or 1.0% emulsifier with the remainder consisting of deionized water in a w/w/w mixture of constituents. The force (dynes/cm) was obtained for the surface tension and the interfacial tension. The tension was recorded as either apparent interfacial or surface tension, and correction factors were later applied to obtain the true interfacial and surface tensions. Each condition was tested in triplicate.

Preparation of O/W emulsions and particle size analysis

Ultrasonic homogenization was utilized in the preparation of emulsions. The benefits of ultrasonic homogenization as a way to quickly create nanoparticle-sized emulsions (nanoemulsions) with lower amounts of emulsifiers have been previously established (Khalid et al., 2015). Emulsions were prepared using a U.S. Solid 600W Ultrasonic homogenizer (U.S. Solid, Cleveland, OH, USA) with a 13 mm probe in a ten-minute time frame for 100 g emulsions with a 3 sec on/off pulse mode at 100% power. The temperature was controlled with a Cole-Parmer® Polystat® digital refrigerated circulating water bath (Cole-Parmer, Vernon Hills, IL, USA) which was set at a temperature of 1°C. The emulsions were made within a double-jacketed

reaction vessel to allow for a stable temperature throughout the homogenizing process, which was monitored and kept at 1°C (±0.1°C).

Emulsions were produced in triplicate using three emulsifiers, S970, S1170, and TWEEN® 80 at three concentrations 0.1, 0.5, 1.0% emulsifier. Emulsions included 5% by weight of stripped soybean oil and water at varying % by weight depending on the amount of emulsifier added. The emulsions were kept in low-light conditions at 25°C, and the particle size was analyzed every three days for a thirty day period. Particle size analyses were carried out using a Malvern Panalytical Mastersizer S with a small volume sample dispersion unit (Malvern Panalytical Ltd, Malvern, UK). Data analysis was completed using the Mastersizer software (Malvern Panalytical Ltd, Malvern, UK). Particle size analysis followed parameters set forth elsewhere (Kowalkska & Żbikowska, 2016). The particle size was measured in triplicate, and the increase in particle size over time was plotted to determine optimal emulsion parameters.

After the initial 30 days, it was determined from the preliminary results that the most stable emulsion was one with S1170 as the emulsifier at a concentration of 0.5% by weight. Large volume emulsions were made for the accelerated oxidation tests. For scaled-up preparation of emulsions, 1L of emulsion mixture was placed in a large beaker within an ice bath, and the same parameters for emulsion homogenization were used. The temperature of the mixture during processing was constantly monitored in order to keep the system at 1°C (±0.1°C). 1 mL aliquots of emulsion were removed at regular intervals to measure particle size; this was done in order to ensure large scale emulsions would have the same initial particle size as the initial test portions.

Accelerated oxidation test

The antioxidant ability of the selected phenolic compounds was assessed through several *in vitro* chemical assays in bulk oil and O/W emulsions. To minimize the differences in

distribution for the added phenolic compounds, the antioxidants were first dissolved in ethanol and then added into stripped soybean oil or emulsion. The control group consisted of stripped soybean oil or emulsion with ethanol in place of antioxidants and was tested in triplicate with samples. Both bulk oil samples and emulsion samples contained 100 ppm of antioxidants by oil weight, or ethanol in control group, which is within regulations on other standard antioxidants to be less than 0.02% of lipid content by weight (Tagnvaei & Jafari, 2015). Before the accelerated oxidation study, samples were placed under constant nitrogen flow using an Organomation 12-position N-EVAP (Organomation Associates, Inc., Berlin, MA, USA), to evaporate ethanol.

All samples for tests involving bulk oil were stored in identical 25 mL closed cap Corning vials (Corning, Corning, NY, USA) and placed in a Fisher Scientific Isotemp™ 500 series gravity convection oven (Fischer Scientific, Waltham, MA, USA). These samples were stored for thirty days at 90°C(±1°C) and were removed after 1, 3, 6, 10, 15, 20, and 30 days to test in triplicate. Initial tests were performed on day 0 to establish the initial baseline for oxidation. All samples for tests involving emulsions were stored in identical 200 mL closed cap bottles and placed inside the same gravity convection oven with the same testing regiment. However, the difference in size for sample containers was a necessity in order to have enough oil to complete the various tests needed during the accelerated oxidation study for the emulsions. Every day, samples were pulled from the oven and placed on the counter with caps off at 25°C for 5 min to further promote the oxidation of samples.

The PVs of oils and emulsions were assessed according to the AOCS Official Method Cd 8b-90 (American Oil Chemists' Society, 2011). *p*AVs were assessed on the same days as PV using the AOCS Official Method Cd 18-90 (American Oil Chemists' Society, 2011). The TOTOX value was calculated using PV and *p*AV. Samples were also analyzed for change in

fatty acid profile on days 0, 20, and 30. The change in fatty acid composition was determined by following AOAC Official Method 996.01 (Satchithanandam, Fritshce, & Rader, 2001) to prepare fatty acid methyl esters (FAME), and then analyzing FAMEs using an xs 6890 N GC system with an FID detector (Agilent, Santa Clara, CA, USA) and a Supelco SP-2560 capillary column (100 m x 0.25 mm ID, 0.20 µm film) (Sigma-Aldrich Co. St. Louis, MO, USA). GC analysis followed procedure set forth previously (Ifeduba & Akoh, 2013). Briefly, 1 µL of sample was injected at a split ratio of 50:1, the carrier gas (He) flow was 1.1 mL min^{-1} and the detector temperature was 250 °C. The oven was held at 140 °C for 5 min, then increased to 240 °C at a rate of 4 °C min^{-1}, and held for 15 min. Fatty acid composition analysis was conducted in triplicate for each sample, and results were expressed as average mol% and calculated based on relative peak area. In order to test emulsions for PV, *p*AV, and FAMES with the same methods as used for bulk oil, a 2.5 mL aliquot of emulsion which would contain the necessary amount of oil, 100 µg, was extracted and centrifuged at 2,000 rpm for 10 min before testing. This was done in order to obtain the required amount of oil from the resulting supernatant to test the oxidation of oil in the emulsion matrix.

Thermal oxidation using differential scanning calorimeter (DSC)

The antioxidant capability of the selected phenolic compounds and mixtures was also tested using a 204 F-1 Phoenix differential scanning calorimeter (Netzsch-Garätebau GmbH, Selb, Germany). Oil samples with 100 ppm of each antioxidant or mixture were placed in an aluminum crucible with a pierced cap. Samples were then placed inside the instrument and measured using an isothermal method wherein samples were heated from 0 to 135°C at a rate of 10°C min^{-1} under a constant nitrogen flow of 50 mL min^{-1}. Then, samples were stabilized for 3 min where gas flow was switched to pure oxygen at 50 mL min^{-1}. The oxidation induction time

(OIT) was determined from the onset of the exothermic peak by subtracting the stabilization and heating time (3 min) using Proteus thermal analysis software (Netzsch-Garätebau GmbH, Selb, Germany). All experiments were carried out in triplicate.

Statistical analysis

Statistical analysis of results was conducted using JMP® software (version 15, SAS Institute, Inc., Cary, NC, USA). Results were expressed as mean values ± standard deviation (SD). To create a predictive model for determination of initial particle size, the response variable was subjected to analysis of variance (ANOVA) with 2-way interactions. Tukey's honest significant difference (Tukey's HSD) test was used to determine differences between all experimental results for different sample types through all tests and the level of significance ($p < 0.05$) among them. All experiments were carried out in triplicate.

Results and discussion

Optimized emulsion parameters

The results for interfacial and surface tensions in Table 1 show the effect that emulsifier concentration and the hydrophilic-lipophilic balance (HLB) values have in emulsions. The emulsifiers selected were chosen due to their ideal HLB values. S970, S1170, and TWEEN® 80 have HLB values of 9, 11, and 15, respectively. These emulsifiers were selected as they fall between the desired HLB range (8-16) which is believed to be the optimal values for O/W emulsions (Griffin, 1949).

In Table 1, the emulsifier concentration at 1% seemed to be too high as the resulting interfacial and surface tension values were higher than that of the 0.5% emulsifier concentrations. However, the 0.1% concentrations also resulted in higher surface and interfacial tensions. The data gained from the interfacial and surface tensions analyses showed that S1170 at

0.5% concentration had the lowest interfacial (8.0 dynes/cm) and surface tensions (46.9 dynes/cm), however the results for S970 at 0.5% concentration were not significantly different. The interfacial tension decreased by approximately 40% with S1170 as emulsifier compared to the control without emulsifier. These results are further supported by the emulsion stability study.

S1170 at a 0.5% concentration had the lowest initial particle size and was significantly different than every other combination at 0.5% concentration as seen in Fig. 1. Overall, a regression model using emulsifier type, concentration, and surface and interfacial tensions as predictor variables to initial particle size could explain 94.1% of the variability in the response ($R2 = 0.941$) and this model can be found in the supplementary data (Table S1). Additionally, the JMP® output for this model which includes the predicted initial particle size graphed against the actual particle size can be seen in Fig. S1.

While the interfacial and surface tension results were determined to have a strong correlation to initial particle size in emulsions, this was a poor predictor of overall emulsion stability. This result could possibly save time and cost in the future when comparing the potential emulsion mixtures in future studies. However, for an accurate understanding of overall emulsion stability, it is recommended to use the particle size analysis parameters described above. It was determined with initial experiments that thirty days would not be sufficient for the experimental emulsions to undergo noticeable differences in droplet coalescence and flocculation. Thus, the particle size analysis was conducted in order to measure change in average droplet size (D3,2) in emulsions. An increase in average droplet size directly correlates with an increase in droplet aggregation and the slow coalescence of the two bulk phases, which means that the emulsion has separated as described previously (Mokhatab, Poe, & Mak, 2018). The change in interfacial area

between lipid and aqueous phases affects oxidative stability as this is the primary space where lipid oxidation will occur in emulsion (Noon, Mills, & Norton, 2020).

Fig. 1 shows the changes in particle size during the storage test. The largest particle sizes were those with the emulsifier concentration at 0.1% (w/w). At this concentration, S1170 and TWEEN® 80 showed no significant difference between each other, however, emulsions with S970 possessed the largest average particle size and exhibited a significant increase in droplet size over time. Emulsions with 1.0% (w/w) of emulsifiers performed in between the other two concentrations, with S1170 showing the smallest droplet size and S970 again having the highest average particle size of the three.

The emulsions containing 0.5% (w/w) of emulsifiers exhibited the smallest droplet sizes. There were significant differences between the particle sizes for all three emulsifiers with little change over the 30-day study. This lack of change indicates that over time the droplet coalescence was lower than samples containing other emulsifier concentrations, resulting in more stable emulsions. The emulsions made with TWEEN® 80 showed the highest particle size at this concentration, (0.5%, w/w), while S1170 had the smallest particle size with an average particle size of 0.472 μm. In addition to the lowest particle size, S1170 at 0.5% concentration exhibited the highest stability over the test period.

S1170 producing an emulsion with significantly lower particle size is likely, at least in part, due to the differences between HLB values compared with the other emulsifiers (Jafari & McClements, 2018). The differences in stability among different emulsifier concentrations however, are likely due to the droplet coalescence (Rao & McClements, 2012) and the differences in triggering Ostwald Ripening (Wooster, Golding, & Sanguansri, 2008), a mechanism whereby larger droplets begin to grow when smaller ones adhere to them (Ostwald,

1897; Donsi & Ferrari, 2016). These differences allowed the emulsions made using S1170 at 0.5% (w/w) concentration to maintain the smallest particle sizes for the entire 30-day storage test, while also showing the lowest interfacial and surface tension among all emulsion mixtures. Thus, this emulsion mixture was selected as the optimal system for the accelerated oxidation study.

Antioxidant activity

The phenolic compounds were tested for antioxidant activity by three common in vitro assays and by using thermal oxidation with DSC to assess OIT. The results of the in vitro assays and the thermal oxidation test can be found in Fig. 2. The antioxidant activities of the phenolic compounds tested in the DPPH• assay were expressed as IC50 (the concentration of tested compound required to inhibit 50% of DPPH•). The antioxidant activities of the phenolic compounds tested in the ABTS+• assay were expressed as EC50 (the concentration of tested compound required to induce 50% of ABTS+•). The results of the FRAP assay were expressed as EC1 (the concentration of test compound equivalent to 1 mM Fe2+ to be reduced from Fe3+). A summary of all in vitro assay results can be found in Table 2.

The results in Table 2 and Fig. 2 show that the 1:1 mixture of GG+RA exhibited the highest antioxidant activity (7.74 ppm) in the DPPH• assay with regards to IC50. The DPPH• assay measures the ability of tested antioxidants to stabilize free DPPH•, a stable nitrogen radical, over a period of time. The difference in results of antioxidants in the DPPH• assay can be explained by differences in the molecular structures of these phenolic compounds. Affected differences include the dissociation energy for hydrogen atoms from phenolic hydroxyl groups on the compounds as well as the differences in steric hindrance that would occur during reduction of the DPPH• radical (Shahidi & Naczk, 1995). The primary mechanisms of reaction

in the DPPH• and ABTS+• assay are believed to be combinations of HAT and SET mechanisms (Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo, & Hernández-Carlos, 2019). However, it is further supported by the kinetics of reaction, size, and shape of the compound that tocopherols and similar phenolic compounds primarily follow the HAT mechanism with DPPH• in polar media (Evans, Scaiano, & Ingold, 1992; Craft, Kerrihard, Amarowicz, & Pegg, 2012). So, the number and bond dissociation enthalpy of OH groups present on similar molecules could be a useful predictor of the antioxidant activity exhibited during DPPH• assay. The tested antioxidants and mixtures varied in the number of phenolic hydroxyl groups, so it can be partially explained that a combination of GG and RA, which include three phenolic hydroxyl groups from GG and four from RA, would exhibit the highest antioxidant activity during the DPPH• assay.

Additionally, the combination of GG and RA appeared to offer a plausible additive effect, as observed in previous work (Zhang, Willett, Hyatt, Martini & Akoh, 2021). GG+RA exhibited significantly better results than other antioxidants alone. Strong binary synergistic effects have been reported between RA and gallic acid (GA) in previous studies (Hajimehdipoor, Shahrestani, & Shekarchi, 2014). This may correlate with the additive effect between GG and RA as GG is a derivative of GA. As seen in Table 2, TOC had the second lowest average IC50 (8.48 ppm) indicating a strong antioxidant activity, however, it was not significantly different from RA, GG, or its mixture with GG. PG performed significantly worse than the other phenolic compounds tested. This could be explained by the steric hinderance caused by the close proximity of its three phenolic hydroxyl groups and its higher hydrophobicity, which negatively impacts its activity in a polar system (Zhang & Akoh, 2019; Asnaashari, Farhoosh, & Sharif, 2014; Lu, Nie, Belton, Tang, & Zhao, 2006). Overall, results for the DPPH• assay supports the

use of GG as an antioxidant within polar systems on its own or as a mixture with RA. An additive effect was observed with the mixture of GG and RA and it performed the best with the DPPH• assay. While RA, TOC, and GG all exhibited high DPPH• reducing ability as single antioxidants, it would require additional experimentation to establish if a true synergistic effect exists.

The next two assays, ABTS+• and FRAP, measured the reducing ability of tested phenolic compounds with two different ions. ABTS+• assay measures the reducing ability towards the ABTS+• radical ion in ethanol, while FRAP measures the reducing ability towards Fe3+-TPTZ in an acetate buffer with pH 3.6. Results for both assays provided supporting evidence that mixtures of GG+RA and GG+TOC possess some additive effects. The EC50 of TOC in ABTS+• (24.07 ppm) improved significantly when combined with GG (17.67 ppm). The EC1 of RA in FRAP (88.57 ppm) improved significantly when combined with GG (68.52 ppm). The large difference in the FRAP assay results between TOC (323.65 ppm) and other compounds could be explained by the effect that the acidic environment had on TOC (Kittipongpittaya, Panya, Phonsatta, & Decker, 2016). Previous studies provided evidence regarding GA, which also performs well in the ABTS+• and FRAP assay, that could be applied here for GG (Rice-Evans, Miller, & Paganga, 1996). The results of these two assays again support the use of GG as a potential antioxidant alone due to its own activity or in mixtures where additive effects were observed.

The OIT values determined with DSC shown in Fig. 2 resembled the results from the TOTOX values of bulk oil for the accelerated oxidation study seen in Fig. 3. The use of DSC for evaluating the efficiency of antioxidants in oils has been reported (Damasceno et al., 2013; Tsai, Hsu, Lin, Huang, & Lin, 2017). A previous study focused on establishing the correlation

between oil oxidation value and OIT (Zhang, Willett, Hyatt, Martini, & Akoh, 2021). Stripped soybean oil exhibited an OIT value of 27.70 ± 2.52 min whereas the highest OIT value determined came from the mixture of GG+TOC (85.97 ± 13.89 min). In the current study, every antioxidant in stripped soybean oil exhibited a significant difference with the Tukey's HSD test compared against oil without the addition of an antioxidant. The mixture of GG+RA (77.2 ± 1.42 min) was not significantly different from GG+TOC but showed a lower OIT. Overall, the OIT value of tested samples increased in the following order: oil < TOC < RA < GG < GG+RA < GG+TOC.

Accelerated oxidation study

The PVs and pAVs of the bulk oil and the O/W emulsions were tested as mentioned earlier. TOTOX values were calculated using the following equation: TOTOX value = 2(PV) + pAV and reported in Fig. 3. Additionally, changes in the unsaturated fatty acid profiles of stripped soybean oil over the course of the accelerated oxidation study are shown in Table 3. TOTOX values over time can be used as an indicator of the ability of antioxidants to prevent lipid oxidation. One important aspect of note is that the bulk oil and oil extracted from O/W emulsions examined on day 0 contained levels of oleic acid (OA) at 22.06 mol%, linoleic acid (LA) at 52.49 mol%, and α -linolenic acid (ALA) at 6.92 mol% which were in line with previous results on soybean oil (Daun, Eskin, & Hickling, 2011). As PUFAs degraded over time, the monounsaturated fatty acids (MUFA) content was increased due to the lower chance that MUFA will break down (Galano et al. 2015).

The TOTOX value of bulk oil showed a general agreement between the antioxidant activities of tested phenolic compounds and their ability to inhibit or slow down lipid oxidation. TOC was the only antioxidant that exhibited a TOTOX value that was not significantly different

from the oil without antioxidant in the bulk oil samples. This is supported by the changes in fatty acid composition shown in Table 3, as TOC was not significantly different in the mol % of two of the three fatty acids compared to oil on its own after 30 days of oxidation. The mixtures, GG+RA and GG+TOC, and GG alone have the lowest TOTOX values in bulk oil, and they also showed similar values of fatty acid composition after 30 days of oxidation. Additionally, after 30 days, ALA, the only ω-3 fatty acid in soybean oil, had average values between 4.49-5.05 mol % whereas in oil without antioxidant, ALA had an average value of 2.35 mol%. Compared with the starting value for ALA in bulk oil (6.92 mol%), these differences indicated that the three antioxidant groups were successful in preventing between 30.95-39.05 mol % of additional oxidation than stripped soybean oil without antioxidant for ALA. Similar results can be seen when comparing the values for LA, the only other major PUFA in soybean oil. TOC and PG were not significantly different in their ability to prevent oxidation in bulk oil.

The results for TOTOX values in Fig. 3 and the fatty acid composition over time in Table 3 for O/W emulsions showed some differences when compared to results for bulk soybean oil. TOC performed better than other antioxidants, and based on the fatty acid compositions, it was among the best antioxidants in terms of inhibiting oxidation of LA and ALA in O/W emulsions. GG+TOC and GG+RA were not significantly different from TOC, and were still among the lowest TOTOX values at the end of the 30 days in O/W emulsions. However, the final TOTOX value for GG (206.82) was significantly higher than TOC (196.72). When examining the ALA content for O/W emulsions after 30 days, TOC, GG+TOC, and GG+RA had average values between 3.08-3.78 mol%, while the emulsion without antioxidant exhibited an average ALA content of 1.49 mol%. When compared against the starting percentage of ALA in stripped soybean oil from O/W emulsions (6.02 mol%), these results showed that the three best groups of

antioxidants for the prevention of oxidation in O/W emulsions were successful in preventing between 26.41-38.04 mol% additional oxidation of ALA. One potential explanation for differences in antioxidant efficacies between different sample matrices is the interfacial phenomena which affects where each antioxidant is located in emulsion, as TOC and other lipophilic non-polar compounds will be located more in the lipid phase (Losada-Barreiro, Sánchez-Paz, & Bravo-Díaz, 2012).

The TOTOX values for bulk oil increase in the following order: GG < GG+RA < GG+TOC < RA < PG < TOC < Oil. While TOTOX values for O/W emulsions increase in the following order: GG+TOC < GG+RA < TOC < GG < RA < PG < Oil. For the most part, the polar paradox is supported for single antioxidants, while an additive effect seems to enable mixtures of antioxidants to perform better than expected. RA does not always follow expected results according to the polar paradox theory as seen in previous literature (Shahidi & Zhong, 2011). However, the results for TOC and PG are in line with results previously observed (Fukuzawa, Tokumura, Ouchi, & Tsukatani, 1982; Porter, Black, & Drolet, 1989). The results of TOC and GG support the polar paradox theory, which rationalizes the observation that polar antioxidants, GG in this instance, perform better in non-polar matrices, such as bulk oil, while non-polar antioxidants such as TOC, perform better in polar matrices, such as O/W emulsions (Porter, 1980; Shahidi & Zhong, 2011).

Conclusions

Optimized emulsion parameters were developed and the enzymatically synthesized GG was compared against five phenolic compounds or mixtures thereof for antioxidant ability in bulk oil and O/W emulsions. GG, GG+TOC and GG+RA showed the highest antioxidant ability in in vitro assays and in the accelerated oxidation study with bulk oil. In O/W emulsions, TOC

performed best, along with GG+TOC and GG+RA mixtures, supporting the polar paradox theory. Overall, GG alone and GG+RA and GG+TOC mixtures were shown to have potential for use as antioxidants in bulk oil, while TOC would be excellent for use in O/W emulsions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 3.1. Surface tension and interfacial tension reported in dynes/cm after applying appropriate correction factors. All three emulsifiers were tested at three percentage concentrations by weight, deionized water was used in place of emulsifiers as a control.

Emulsifier	HLB	Surface tension (dynes/cm) Concentration (%)			Interfacial tension (dynes/cm) Concentration (%)		
		0.1	**0.5**	**1.0**	**0.1**	**0.5**	**1.0**
S970	9	65.1 ± 0.29[b]	47.0 ± 0.38[a]	63.2 ± 0.52[c]	12.6 ± 0.09[b]	8.4 ± 0.30[a,b]	12.1 ± 0.06[b]
S1170	11	57.9 ± 0.19[a]	46.9 ± 0.23[a]	52.9 ± 0.15[a]	11.6 ± 0.06[a]	8.0 ± 0.18[a]	10.2 ± 0.23[a]
TWEEN 80®	15	57.8 ± 0.15[a]	51.2 ± 0.23[b]	58.1 ± 0.21[b]	11.3 ± 0.06[a]	9.9 ± 0.26[b]	11.4 ± 0.15[b]
Deionized Water (Control)		72.8 ± 0.12[c]	72.6 ± 0.34[c]	72.7 ± 0.12[d]	13.3 ± 0.15[c]	13.3 ± 0.41[c]	13.3 ± 0.23[c]

[a,b,c,d] Different letters within the same column indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Table 3.2 Antioxidant activities of 1-*o*-galloylglycerol (GG), propyl gallate (PG), rosmarinic acid (RA), tocopherols (TOC), and 1:1 ratio mixtures of GG with RA or TOC in $DPPH^{\bullet}$, $ABTS^{+\bullet}$, and FRAP assays.

Compounds	$DPPH^{\bullet}$ IC_{50} (ppm)	$ABTS^{+\bullet}$ EC_{50} (ppm)	FRAP EC1 (ppm)
GG	8.54 ± 0.24^{a}	13.15 ± 0.46^{a}	64.97 ± 0.45^{a}
PG	9.09 ± 0.32^{b}	14.02 ± 0.35^{a}	71.79 ± 1.43^{a}
RA	$8.70 \pm 0.13^{a,b}$	19.53 ± 0.66^{d}	88.57 ± 0.94^{b}
TOC	8.48 ± 0.12^{a}	24.07 ± 0.95^{e}	323.65 ± 6.30^{c}
GG+RA	7.74 ± 0.10^{c}	15.87 ± 0.44^{b}	66.50 ± 0.30^{a}
GG+TOC	8.55 ± 0.12^{a}	17.67 ± 0.55^{c}	68.52 ± 2.21^{a}

^{a,b,c} Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Table 3.3 Changes in unsaturated fatty acid composition of bulk oil phase and O/W emulsions with selected antioxidants

Time (day)	Sample	O/W emulsion sample			Bulk oil sample		
		Monounsaturated fatty acid (mol%)	Polyunsaturated fatty acids (mol%)		Monounsaturated fatty acid (mol%)	Polyunsaturated fatty acids (mol%)	
		OA[1]	LA[2]	ALA[3]	OA[1]	LA[2]	ALA[3]
0	**Oil**	20.91 ± 0.59	51.50 ± 0.45	6.02 ± 0.27	22.06 ± 0.02	52.49 ± 0.50	6.92 ± 0.09
20	**GG**	36.75 ± 0.96^{a,b}	38.19 ± 0.56^{a}	4.98 ± 1.08^{a}	25.32 ± 0.52^{c}	46.76 ± 0.30^{a}	5.27 ± 0.10^{a,b}
	PG	37.82 ± 2.70^{a}	38.73 ± 2.67^{a}	4.03 ± 0.56^{a}	27.41 ± 0.09^{b,c}	49.06 ± 0.63^{a}	4.56 ± 0.14^{b}
	RA	34.40 ± 1.29^{a,b}	41.78 ± 0.24^{a}	5.01 ± 0.22^{a}	28.34 ± 0.11^{b}	48.48 ± 0.50^{a}	6.10 ± 0.40^{a}
	TOC	27.59 ± 1.11^{b}	35.03 ± 1.39^{a}	5.44 ± 0.71^{a}	26.70 ± 0.75^{b,c}	40.41 ± 1.35^{b}	4.59 ± 0.60^{b}
	GG+RA	34.95 ± 0.05^{a,b}	39.18 ± 0.20^{a}	3.72 ± 0.32^{a}	26.91 ± 0.02^{b,c}	46.28 ± 0.18^{a}	5.95 ± 0.05^{a}
	GG+TOC	29.79 ± 0.33^{b}	39.19 ± 0.51^{a}	4.62 ± 0.10^{a}	27.56 ± 0.28^{b,c}	48.90 ± 0.14^{a}	5.88 ± 0.12^{a,b}
	Oil	39.38 ± 2.70^{a}	38.40 ± 2.32^{a}	3.22 ± 0.39^{a}	41.26 ± 0.84^{a}	34.09 ± 0.10^{c}	3.01 ± 0.02^{c}
30	**GG**	37.77 ± 0.12^{b}	32.06 ± 0.53^{a,b}	3.12 ± 0.11^{a,b}	34.15 ± 0.68^{c}	35.14 ± 0.70^{a}	5.05 ± 0.07^{a}
	PG	37.70 ± 0.30^{b}	33.73 ± 0.22^{a,b}	2.99 ± 0.06^{a,b}	39.21 ± 0.47^{b}	32.59 ± 0.45^{a,b}	3.01 ± 0.03^{b,c}
	RA	38.23 ± 0.15^{b}	32.27 ± 0.60^{a,b}	2.78 ± 0.18^{b}	38.04 ± 0.63^{b}	32.89 ± 1.55^{a,b}	5.01 ± 0.02^{a}
	TOC	34.53 ± 1.86^{c}	35.87 ± 1.13^{a}	3.78 ± 0.10^{a}	45.21 ± 0.71^{a}	32.75 ± 0.61^{a,b}	4.15 ± 0.01^{a,b}
	GG+RA	37.76 ± 0.14^{b}	34.59 ± 0.27^{a}	3.08 ± 0.05^{a,b}	34.04 ± 0.58^{c}	35.27 ± 1.50^{a}	5.01 ± 0.02^{a}
	GG+TOC	36.84 ± 0.45^{c}	34.32 ± 0.27^{a}	3.51 ± 0.05^{a,b}	39.22 ± 0.28^{b}	34.02 ± 0.59^{a,b}	4.49 ± 0.50^{a}
	Oil	49.06 ± 1.85^{a}	29.87 ± 0.28^{b}	1.49 ± 0.30^{c}	47.08 ± 0.91^{a}	29.78 ± 0.37^{b}	2.35 ± 0.24^{c}

[1] Denotes oleic acid

[2] Denotes linoleic acid

[3] Denotes α-linolenic acid

[a,b,c,d] Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Figures

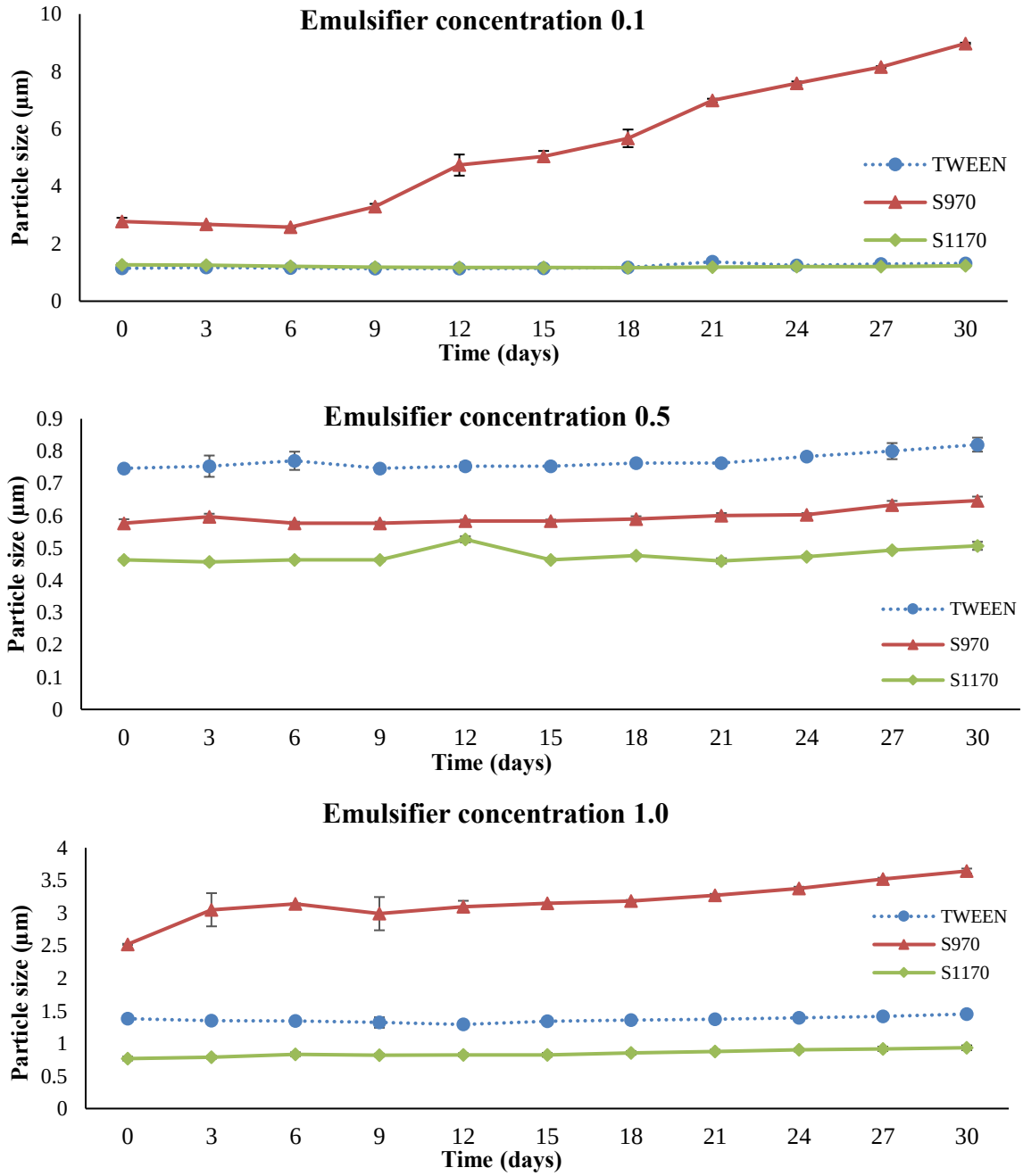

Figure 3.1 Change in particle size analysis surface mean, D[3,2], in µm, over time for the 30-day particle size analysis study. From left to right, top to bottom, emulsifier concentrations of 0.1% by weight, emulsifier concentration of 0.5% by weight, and emulsifier concentrations of 1.0% by weight.

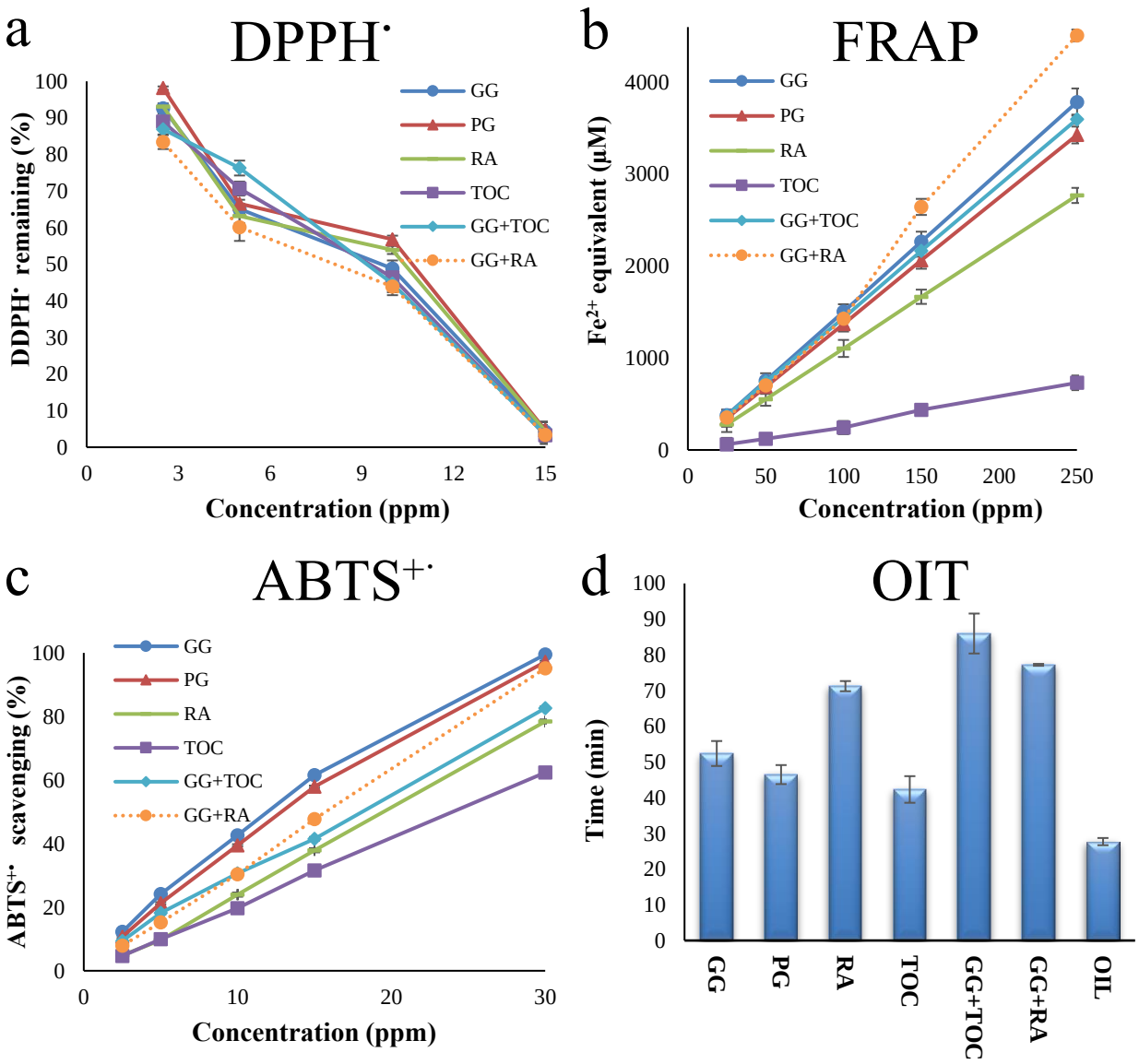

Figure 3.2 Graphs depict (a) results from the DPPH· assay for the tested antioxidants at various concentrations, (b) results from the FRAP assay for the tested antioxidants at various concentrations, (c) results from the $ABTS^{+\bullet}$ assay for the tested antioxidants at various concentrations, and (d) oxidation induction time (OIT) of stripped soybean oil with the tested antioxidants determined by differential scanning calorimeter (DSC).

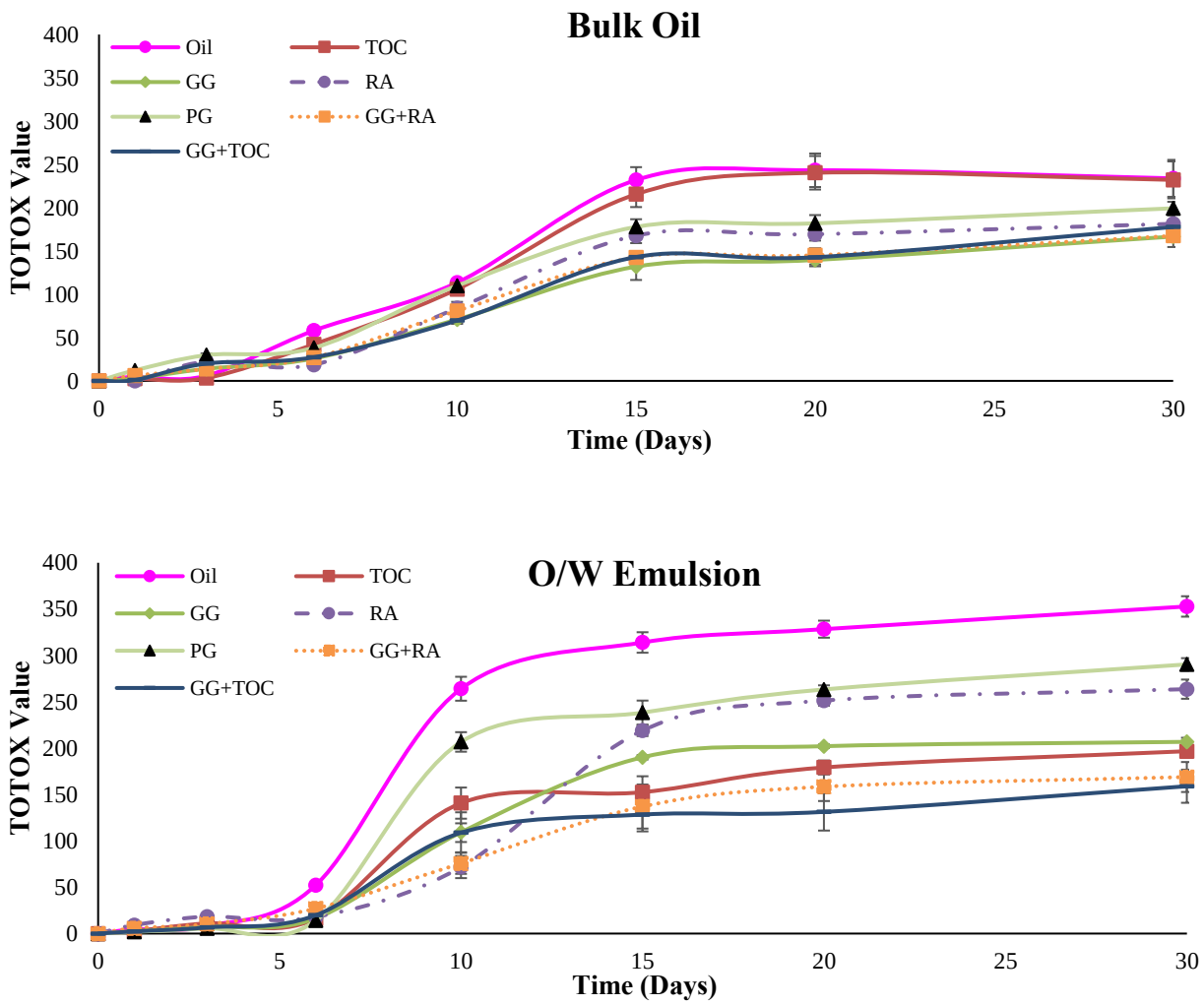

Figure 3.3 Top graph depicts change in TOTOX* values of bulk soybean oil samples with selected antioxidants during the 30-day accelerated oxidation study. Bottom graph depicts change in TOTOX* values of O/W emulsion samples, with selected antioxidants during the 30-day accelerated oxidation study. The concentration of antioxidants in all samples, except for oil or O/W emulsion without antioxidant, was at 100 ppm.

*TOTOX value = 2(PV) + *p*AV, PV = peroxide value, *p*AV = *p*-Anisidine value.

CHAPTER 4

CHARACTERIZATION AND COMPARISON OF OLEOGELS AND EMULGELS PREPARED FROM *SCHIZOCHYTRIUM* ALGAL OIL USING MONOLAURIN AND MAG/DAG AS GELATORS [1]

[1] Hyatt, J. R., Zhang, S., & Akoh, C. C. Submitted to Journal of American Oil Chemists' Society, February 22, 2022.

Abstract

Oleogels and emulgels were developed with winterized algal oil from *Schizochytrium spp*. rich in ω-3 fatty acids (FAs) to overcome physical limitations of using a highly unsaturated lipid source in food applications. Both gel types were developed using monolaurin or a combination of mono- and diacylglycerols (MAG/DAG) as the gelator at concentrations of 8, 10, or 12%, w/w, in oil or emulsion. A 14-day accelerated oxidation study was conducted using peroxide value, *p*-Anisidine value, and change in FA composition to measure the level of oxidation. Oleogel and emulgel samples exhibited a higher oxidative stability than bulk algal oil and oil-in-water emulsion as control groups, respectively. The 12% monolaurin oleogel outperformed others in oxidative stability, preventing oxidation of approximately 17.96% and 20.43% of EPA and DHA, respectively, compared to algal oil. Physical characteristics including thermal behavior, solid fat content (SFC), rheology, morphology, and polymorphism were studied. Results indicated that MAG/DAG oleogels and monolaurin emulgels were the most physically stable. The SFC of 12% MAG/DAG oleogel at 30 °C was 10.27% whereas 12% monolaurin oleogel was only 4.51%. Both gel types developed with monolaurin and MAG/DAG could be used for different applications as they exhibited desirable qualities such as oxidative stability and improved physical characteristics.

Keywords: Gelation; ω-3 Formulation; Oxidative stability; Algal oil

1. Introduction

Polyunsaturated fatty acids (PUFA), particularly those of omega-3 fatty acids (ω-3 FA) are extremely susceptible to oxidation due to the presence of multiple double bonds (Frankel, Satue-Gracia, Meyer, & German, 2002). However, increasing PUFA content in food products has been of particular interest due in part to the reduced risk of heart disease and stroke associated with an increased intake of PUFA content (Stone, 1996). The American Heart Association (AHA) suggests that increasing the ω-3 FA content of food products, particularly EPA and DHA, can lead to a reduced risk for development of cardiovascular diseases (Stone, 1996). The increased interest to include higher levels of ω-3 FA signifies that improving the oxidative stability of these susceptible groups is of importance not only to the food industry but to other industries employing these various lipids such as pharmaceuticals and cosmetics. It is also important to note that PUFA-rich lipid sources are not easily used in the same fashion as saturated fat sources (Willett & Akoh, 2019). Thus, research is needed to help develop PUFA-rich lipid sources with physicochemical properties similar to saturated fat sources. If the oxidative stability of those PUFA-rich lipid sources can be improved it will enhance and increase their use in many products in the future.

One possibility for improving the physical characteristics of a particular PUFA source while also improving the oxidative stability is through the formulation of oleogels. Due to their chemical structure, PUFA usually have melting points below 0 °C, but by physically converting those PUFA into oleogels with the use of gelators, they can form semi-solid gels at ambient temperatures. The gelators chosen for the purpose of this research were monolaurin and a mixture of monoacylglycerol and diacylglycerol (MAG/DAG). The selected gelators performed best against a battery of other potential gelators in a screening test prior to the onset of this study. To the best of our knowledge these gelators have been

included in limited research for the purpose of food grade gels. MAG/DAG has been studied while monolaurin has been used in niche studies as a medicinal gel (Mancuso et al., 2020).

Monolaurin has a known HLB value which falls in the lipophilic range (~7) (Park et al., 2018), while the HLB value for the combination of MAG/DAG will depend on the actual composition of different MAG and DAG included as well as the ratio of the two in the gelator used. However, MAG and DAG are commonly used emulsifiers. Monolaurin has also been reported to possibly enhance the oxidative stability of lipid matrixes (Moradi, Tajik, Razavi Rohani, & Mahmoudian, 2016). The selected ω-3 FA-rich lipid source, algal oil from *Schizochytrium spp.*, also has not been well researched in terms of its use in the formulation of food-grade gels. Oleogels present a unique approach to solve oxidative stability issues while also developing desired physical characteristics for lipid sources rich in PUFA (Willett & Akoh, 2019) . Another underutilized processing technique explored in this study is the formulation of emulsion gels (emulgels). Emulgels present unique challenges with experimentation due to the presence of water in sample matrix, but they also possess the same benefits oleogels confer but in an emulsion form. Such gels open more possibilities for use as food ingredients or for use in cosmetic and pharmaceuticals.

The objective of this study was to formulate oleogels and emulgels with *Schizochytrium* algal oil and analyze the physicochemical characteristics of these gels. To our knowledge no oleogels or emulgels have been developed using this lipid source. If the physical properties of the developed gels are suitable for use as a replacement of saturated fat sources, then these gels may well prove useful in food, pharmaceutical, and the cosmetic industry. The oxidative stability of gels was studied to determine if any protective effect was achieved. It is hypothesized that the development of these solid gels will not only allow PUFA-rich oils to be used in place of SFA, but also help slow the oxidation of a susceptible oil without the use of antioxidants. Thus *Schizochytrium spp.* algal oil can act as an excellent benchmark, as

it is highly inundated with ω-3 PUFA, with the purchased oil used herein having approximately 6.5% and 35% EPA and DHA, respectively. Once gels are developed and the physicochemical characteristics are better understood additional research could potentially allow for these gels to be used in a wide range of applications.

2. Materials and Methods

2.1. Chemicals and reagents

Algal oil was purchased from Baoding Faithful Industry Co. (Baoding, China). Monolaurin was purchased from Inspired Nutrition (Salem, OR, USA), and the MAG/DAG mixture used was Grindsted® Mono-Di HV52 K-A, purchased from Danisco USA Inc.® (New Century, KS, USA). Ryoto™ Sugar Ester: S-1570, S-1170, and S-970 (with HLB values 15,11, and 9, respectively) were obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Tween® 80 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents and solvents were of analytical or HPLC grades and were purchased from Fisher Chemical (Fair Lawn, NJ, USA), Sigma-Aldrich Chemical Co. and J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). All materials mentioned were used without further purification steps.

2.2. Selection of gelators and emulsifier

Monolaurin and MAG/DAG mixture were selected as gelators through initial screening tests against other plausible gelators. Typically, a combination of gelators with different HLB values is ideal due to the improved physical properties seen in developed gels (Co & Marangoni, 2012). However, combining different gelators such as sucrose stearates, DAG and TAG combinations, and select phytosterols all exhibited a lowered ability to adequately form gels with algal oil and oil-in-water (O/W) emulsions. Other gelators including monolaurin and the MAG/DAG mixture were tested at a higher concentration than those used for the purpose of this study, at 15% w/w.

This cursory test acted as a screening to find the best gelator for algal oil and O/W emulsion. Monolaurin and the MAG/DAG mixture not only developed a stable gel at 15%, w/w, concentration but also formed promising oleogels and emulgels at the lowered concentrations that would be utilized in testing for the purpose of this study, 8, 10, and 12% (w/w).

The selection of emulsifier and emulsifier concentration for O/W emulsions followed parameters set forth elsewhere (Hyatt, Zhang, & Akoh, 2021). First, the interfacial and surface tension of four selected emulsifiers at three concentrations, 0.1, 0.5, and 1.0% were determined via a CSC-DuNouy Interfacial Tensiometer Model 70545 (CSC Scientific Company, Fairfax, VA) following parameters discussed in a previous study (Akoh, 1992). Interfacial and surface tensions were measured in order to determine potential stability of different emulsifiers at varying concentrations for developed emulsions (Bourrel, Graciaa, Schechter, & Wade, 1979). Additionally, particle size analysis was performed on sample emulsions following parameters mentioned elsewhere (Kowalska & Żbikowska, 2016). Particle size analyses were carried out using a Malvern Panalytical Mastersizer S (Malvern Panalytical Ltd, Malvern, UK) with a small volume sample dispersion unit. Data analysis was completed using the Mastersizer software (Malvern Panalytical Ltd, Malvern, UK).

Overall, the best performing emulsifier and concentration was S1170 at a concentration of 0.5% (w/w/w) with 5% algal oil and 94.5% deionized water. Emulsions developed using S1170 at 0.5% concentration had the smallest initial particle size with an average of 0.481 μm, and the lowest interfacial and surface tensions with averages of 7.9 and 46.6 mN/m, respectively. These values were significantly different from the results of other combinations of emulsifier and concentration.

2.3. Preparation of oleogels and emulgels

Oleogel preparation followed parameters set forth elsewhere with modifications (Willett & Akoh, 2019). Oleogels were developed in triplicate by dissolving either monolaurin or MAG/DAG in 10 g of algal oil at 90 °C. Oleogels were prepared with concentrations of 8, 10, or 12% gelator (w/w). Gel mixtures were stirred constantly for 10 min until fully dissolved and then transferred to Ace Glass vials purchased from VWR™ (Radnor, PA, USA). Samples were flushed with nitrogen using an Organomation 12-position N-EVAP (Organomation Associates, Inc., Berlin, MA, USA) and placed at 4 °C to develop the gel network and to store for further analysis. All oleogel treatments were prepared in triplicate.

Emulgels were prepared by first developing emulsions following steps from previous studies (Hyatt, Zhang, & Akoh, 2021). Emulsions were made in bulk using a U.S. Solid 600W Ultrasonic homogenizer (U.S. Solid, Cleveland, OH, USA) with a 13 mm probe for 10 min with a 3 sec on/off pulse method at 80% power. Temperature was controlled using a Cole-Parmer® Polystat® digital refrigerated circulating water bath (Cole-Parmer, Vernon Hills, IL, USA) set at 1 °C. Emulsions were made within a double-jacketed reaction vessel to allow for a stable temperature throughout the homogenizing process, which was monitored and kept at 1 °C (± 0.1 °C).

Once bulk O/W emulsions were developed the preparation of emulgels followed parameters from another study with slight modifications (Chang, Hu, Huang, Hseih, & Ting, 2020). Emulgels were developed by dissolving either monolaurin or MAG/DAG mixture at 8, 10, and 12 % gelator (w/w) with 10 g of developed emulsion at 50 °C while stirring for 30 min until fully dissolved. This temperature and time was selected as it allowed gelators to dissolve while not affecting the stability of developed emulsions. Once dissolved, emulgel mixtures were then placed in the same type of glass vials as oleogels and flushed with nitrogen in the same

manner as oleogels. Mixtures were then stored at 4 °C to set the gel and to store for further analysis. All emulgel treatments were prepared in triplicate.

2.4. Accelerated oxidation test

For the purpose of the accelerated oxidation test, 10 g of oleogel or emulgel were placed into Reacti-vials™ within a Reacti-Therm™ heating and stirring module (Thermo Fisher Scientific, Waltham, MA, USA) fitted with aluminum heating blocks. The Reacti-Therm™ module was set at 60 °C, and samples were removed for testing on days 4, 7, 10, and 14 with an initial test on day 0. The peroxide values (PV) of oils and emulsions were assessed according to the AOCS Official Method Cd 8b-90 (American Oil Chemists' Society, 2011). *p*-Anisidine values (*p*-AV) were assessed on the same days as PV using the AOCS Official Method Cd 18–90 (American Oil Chemists' Society, 2011). PV and *p*-AV tests were conducted in triplicate, and all reagents were prepared fresh on the day of analysis. Values were reported as mean ± standard deviation (SD).

Fatty acid composition was also measured for algal oil in oleogel and emulgel samples on days 4, 7, 10, and 14 with an initial test on day 0. This was done in order to track the change in fatty acid profile over the accelerated oxidation study. The change in fatty acid composition was determined by following AOAC Official Method 996.01 (Satchithanandam, Fritshce, & Rader, 2001) to prepare fatty acid methyl esters (FAMEs) and analyzed using an Agilent 6890 N GC system with an FID detector (Agilent, Santa Clara, CA, USA) and a Supelco SP-2560 capillary column (100 m x 0.25 mm ID, 0.20 µm film) (Sigma-Aldrich Co., St. Louis, MO, USA). GC analysis followed procedure set forth previously (Hyatt, Zhang, & Akoh, 2021). Briefly, 1 µL of sample was injected at a split ratio of 5:1, the carrier gas (He) flow was 1.1 mL min^{-1} and the detector temperature was 250 °C. The oven was held at 140 °C for 5 min, then increased to 240

°C at a rate of 4 °C min^{-1}, and held for 15 min. In order to analyze PV, *p*-AV, and FAMEs of oleogels and emulgels with the same methods, a 1.0 mL aliquot which would contain the necessary amount of oil, 50 mg, was pulled and centrifuged at 2,000 rpm for 10 min, in order to extract required oil. The required amount of oil was extracted from the resulting supernatant to test the oxidation of oil in the gel matrix. FAMEs analysis was conducted in triplicate for each sample, and results were expressed as average mg/g concentrations and normalized percentages for notable fatty acids and calculated using an internal standard, heptadecanoic acid (C17:0). Normalization was carried with data from FAMEs analysis to help correct for the difference in saturated fatty acid content among different gelator concentrations.

2.5. Thermal oxidation measured with differential scanning calorimeter (DSC)

The oxidative stability of oleogel and emulgels were measured using a 204F-1 Phoenix differential scanning calorimeter (Netzsch-Garätebau GmbH, Selb, Germany) to determine the oxidation induction time (OIT) of samples. The test followed parameters in previous research (Hyatt, Zhang, & Akoh, 2021; Zhang, Willett, Hyatt, Martini, & Akoh, 2021) with slight modifications for both sample types. Oleogels were analyzed using 10 ± 0.5 mg aliquots placed in aluminum crucibles with pierced caps against a pierced blank empty crucible. Emulgel samples were analyzed against a pierced blank crucible which held an equivalent amount of water to offset interference caused by water present in the emulgel (Pollastri, Porter, McIntosh, & Simon, 2000). Samples were heated from 40 to 105 °C at a rate of 20 °C min^{-1} under constant nitrogen flow at 50 mL min^{-1}. At 105 °C, after a 3 min stabilization, gas flow was switched to oxygen at 50 mL min^{-1}. The OIT of the sample was calculated as the onset time of the exothermic peak subtracted from stabilization time (3 min) and heating time (2.5 min). All experiments were carried out in triplicate and results were reported as mean ± SD.

2.6 Characterization of oleogels and emulgels

2.6.1 Thermal behavior and solid fat content

The DSC mentioned previously was also used to analyze the thermal behavior for both oleogel and emulgel following AOCS Official Method Cj 1-94 (American Oil Chemists' Society, 2011). The crystallization onset and melting completion temperatures were measured using Proteus thermal analysis software (Netzsch-Gerätebau GmbH, Selb, Germany). Solid fat content (SFC) was determined using an MQC benchtop NMR analyzer (Oxford Instruments, Abingdon, UK) following AOCS Official Method Cd 16b-93 for non-stabilizing fats (American Oil Chemists' Society, 2011).

Prior to experimentation, calibration standards (Oxford Instruments, Oxfordshire, UK) had SFC values of 0, 32.6, and 70.5%. SFC was measured for oleogels and emulgels between 0 °C and 60 °C at intervals of 5 °C. All experiments were conducted in triplicate and results were reported as mean ± SD.

2.6.2 Rheological properties

The rheological properties of emulgels and oleogels were analyzed using an HR-2 Discovery Hybrid Rheometer (TA Instruments, New Castle, DE, USA). A parallel plate (diameter 40 mm, gap of 1 nm) was used during measurements. Results were obtained and analyzed using parameters described in a previous study (Willett & Akoh, 2019). Temperature was controlled with a Peltier Plate Temperature System (TA Instruments, New Castle, DE, USA). All experiments were conducted in triplicate. Data was collected using Trios software (TA Instruments, New Castle, DE, USA).

Oleogel and emulgel samples were first placed onto the Peltier plate and cooled to the starting temperature of 0 °C (± 0.1 °C) for 10 min. Heating–cooling sweeps were performed

between the temperature of 0 – 60 °C at a rate of 2 °C min^{-1} with a fixed frequency of 1 Hz and 2% strain. This was done to help evaluate the formation process for each gel. Changes in both the storage modulus (G′) and loss modulus (G″) were evaluated as a function of temperature. All measurements were taken in triplicate.

2.6.3 Morphology and polymorphism

The morphology of oleogels and emulgels were characterized using polarized light microscopy by observing the crystalline microstructure of samples (Willett & Akoh, 2019). An Olympus BX40 microscope (Olympus America, Center Valley, PA, USA) was used at magnifications of 40, 100, 200, and 400x. Preparation of microscope slides consisted of heating samples to 60 °C and adding 1 drop of melted sample between a stationary and moving glass plate. The samples were then crystallized by storing at 4 °C overnight. Images were captured using an iDu Optics® LabCam™ (iDu Optics, New York, NY, USA) with an attached iPhone 6S (Apple, Cupertino, CA, USA), and examined with ImageJ software (National Institute of Health, LOCI, University of Wisconsin). All micrographs were taken in triplicate.

The polymorphism of samples was determined using X-ray diffraction (XRD) with a Bruker D8 Advance X-ray powder diffractometer (Billerica, MA, USA). Samples were first annealed with parameters set forth elsewhere (Willett & Akoh, 2019). Annealed samples were stored at -80 °C until analysis. XRD operating conditions included Co Kα radiation (λ = 1.79037 Å), voltage 35 kV, amperage 40 mA, scanning rate of 0.2° s^{-1}, and a diffraction angle (2θ) range from 10 – 40°. Samples were analyzed in triplicate and short d-spacings (Å) of the crystalline structures were determined using EVA-diffraction software (Billerica, MA, USA).

2.7. Statistical analysis

Statistical analysis of results was conducted using JMP® software (version 15, SAS Institute, Inc., Cary, NC, USA). Results were expressed as mean values ± standard deviation (SD) of triplicate experiments. Tukey's honest significant difference (Tukey's HSD) test was used to determine differences between all experimental results for different sample types through all tests and the level of significance ($p < 0.05$) among them.

3. Results and discussion

3.1. Oxidative stability of oleogels and emulgels

The PV and *p*-AV of oleogel and emulgel samples on each tested day are shown in Fig. 1. Additionally, changes in ω-3 FA composition for oleogel and emulgel samples are displayed in Table 1. The ω-3 FA composition focused mainly on EPA and DHA contents and starting levels of EPA and DHA were in line with reported values for the *Schizochytrium spp.* algal oil from the company's certificate of analysis. EPA and DHA content in Table 1 is also presented as normalized % and data from oleogels were compared against oil as control while emulgels were compared against emulsion as control.

The OIT values determined with DSC are found in Table 2 and correlate with the results of PV and *p*-AV and the change in ω-3 FA composition measured with GC-FID for both oleogel and emulgel samples. OIT measured with DSC has been used to evaluate antioxidant efficiency in samples as demonstrated in our previous studies and has been correlated with the oxidative stability of oil (Hyatt, Zhang, & Akoh, 2021; Zhang, Willett, Hyatt, Martini, & Akoh, 2021). For our purposes, algal oil is compared against oleogel samples while emulsion is compared against emulgel samples.

Algal oil without gelator exhibited an OIT value of 21.43 ± 0.25 min while the highest OIT value exhibited was from the 12% (w/w) monolaurin oleogel (27.03 ± 0.47 min). Table 2

shows a significant difference with Tukey's HSD test between every oleogel and algal oil by itself, suggesting that gelation helped improve the oxidative stability of algal oil present. This pattern is repeated within comparison for emulgels. Emulsion without gelator exhibited an OIT value of 20.30 ± 0.25 min while the highest OIT value was again exhibited by 12% (w/w) monolaurin emulgel (25.01 ± 0.23 min). The trend shows that increasing gelator content may improve oxidative stability as it increases OIT, and monolaurin exhibited higher OIT values than the MAG/DAG counterpart for both oleogel and emulgel samples.

These trends suggest that gelation may have a positive effect on the oxidative stability of oil, and that monolaurin as a gelator could protect against oxidation more effectively than the MAG/DAG mixture. The trends observed with OIT were also seen when examining the PV and *p*-AV results as well as the change in ω-3 FA content in Figure 1 and Table 1, respectively. The highest PV and *p*-AV results were from algal oil and emulsion without gelator for both the oleogel and emulgel comparisons, respectively. The PV and *p*-AV of algal oil after 14 days were 12.13 ± 0.287 mmol O_2/kg of oil and 227.32 ± 1.57 absorbance/g, respectively, while the PV and *p*-AV for emulsion were 14.45 ± 0.321 mmol O_2/kg of oil and 268.12 ± 5.07 absorbance/g, respectively. In both sample types the lowest PVs and *p*-AVs after 14 days were from the 12% (w/w) monolaurin sample gel, with values of 8.92 ± 0.292 mmol O_2/kg of oil and 182.15 ± 3.29 absorbance/g for oleogel and 11.81 ± 0.282 mmol O_2/kg of oil and 227.42 ± 4.03 absorbance/g for emulgel. The highest average decreases for PV and *p*-AV for algal oil were approximately 26.46% and 19.87%, respectively while the average decreases for the values of emulsion were approximately 18.27% and 15.18%, respectively.

Additionally, the change in ω-3 FA content seen in Table 1 agrees with the pattern discussed above. In order to better compare between gels that have less oil due to the increased

gelator content, all values were presented in concentration (mg/g) and normalized percentage. For oleogel samples the average decrease in EPA and DHA content was approximately 25.11% and 30.25%, respectively. The only significant difference in EPA protection among oleogels was seen with the 12% (w/w) monolaurin sample with an average decrease of 7.15% EPA content. While 10% (w/w) monolaurin was the second lowest, it was not statistically different than algal oil alone with an average decrease of 19.15% EPA content. The EPA content was not significantly protected by the MAG/DAG mixture in oleogel.

The lowest decrease for DHA content in oleogels was again the 12% (w/w) monolaurin sample with an average decrease of 9.82%. Multiple treatments exhibited significant differences in the change of DHA content, with the next best sample being the 12% (w/w) MAG/DAG oleogel followed closely behind by the 10% (w/w) monolaurin sample. Overall, the OIT, PV, *p*-AV, and change in FA composition agree on a trend of protective effect for both oleogels and emulgels, where 12% M > 12% MD = 10% M > 10% MD = 8% M > 8% MD > algal oil, (where M and MD stand for monolaurin and MAG/DAG, respectively).

3.2. Physical characteristics of oleogels and emulgels

Thermograms for oleogels and emulgels are shown in Fig. 2. The final melting completion temperatures for algal oil and emulsion were roughly -30.8 °C and 11.1 °C, respectively. These values are much lower than the gelators included in this experiment, with monolaurin and MAG/DAG both possessing a melting completion point at roughly 67.0 °C for gelators alone. The thermograms in Fig. 2 show the improvement made in melting points for each treatment compared to the non-gelated controls. All gels exhibited a much higher melting completion temperature when compared to bulk algal oil or emulsion alone. Additionally, increasing gelator

content seemed to have an effect, although not significant, on the melting completion and crystallization completion temperatures as well.

Increasing monolaurin content in gels exhibited higher melting completion temperature, 12% monolaurin (w/w) had the highest melting completion at roughly 59.38 °C. The same trend was seen with MAG/DAG oleogels, except that MAG/DAG oleogel exhibited an even higher melting completion point of roughly 62.19 °C. Emulgels exhibited a similar pattern, although monolaurin and MAG/DAG were reversed, with the 12% monolaurin and MAG/DAG emulgels exhibiting the highest melting completion points, at 65.62 and 60.18 °C, respectively. This pattern would suggest that increasing gelator content helps to develop more stable gels, and that MAG/DAG gelator is better at developing a physically stable oleogel while monolaurin gelator produces a more physically stable emulgel.

The SFC data shown in Fig. 3 exhibits a similar effect to the thermogram results in Fig. 2. SFC was highest at 0 °C and as temperature increases the SFC drops until the fat content in samples has melted completely. When temperature was set at 0, 30, or 60 °C, oleogels made with 12% MAG/DAG showed the highest SFC value (12.50, 10.27, and 0.46%, respectively). As the control group, algal oil exhibited SFC of 2.03% at 10 °C which decreased sharply to 0.67% at 15 °C, and was completely melted by 40 °C. This melting pattern exhibited with 12% MAG/DAG oleogel also matches the data from the thermograms shown in Fig. 2 as well.

For emulgels, the highest SFC exhibited was from 12% monolaurin with an average SFC at 0 and 30 °C of 14.93 and 10.40%, respectively. As the control group, emulsion exhibited an average SFC of 0.7% at 30 °C and was completely melted by 40 °C. Again, these results are in line with the thermograms of melting and crystallization shown in Fig. 2. The MAG/DAG oleogels seemed to be more thermally stable, while monolaurin emulgels were more stable than

MAG/DAG emulgels. Results of SFC data indicate that 12% and 10% monolaurin oleogels could be used as potential butterfat analogs, as the SFC for these samples were similar to what others have found in butterfat at certain temperatures (Zhang, Willett, Hyatt, Martini, & Akoh, 2021).

Fig. 4 shows the XRD data of oleogels and emulgels as well as bulk algal oil and emulsion alone. Algal oil sample exhibited only β′ short spacing peaks at 4.26, 3.99, 3.74, and 3.52 Å. Emulsion sample exhibited two β′ peaks at 3.75 and 3.72 Å. Monolaurin oleogels exhibited β′ short spacing peaks at approximately 4.5, 4.12, 3.86, 3.63, and 3.42 Å. MAG/DAG oleogel exhibited both strong β and β′ peaks at 4.68 and 4.64 Å (β), as well as 4.28, 4.26, 4.16, and 3.85 Å (β′). This result is in line with thermographs and SFC data as MAG/DAG oleogels were the most stable, and the β crystalline form is regarded as having the highest stability (Ribeiro et al. 2015).

Emulgels for both monolaurin and MAG/DAG exhibited strong β′ peaks as seen in XRD data shown with graphs in Fig. 4d and 4e. However, monolaurin emulgels exhibited more short spacing peaks at 4.43, 4.16, 4.18, 3.85, 3.71, and 3.67 Å, compared to MAG/DAG emulgels only exhibiting peaks at 3.95, 3.92, 3.68, and 3.71 Å. The higher number of short spacing peaks may correlate to a higher stability in product due to a more needle-like morphology (Sato & Ueno, 2005). This is supported by the morphology, which was examined with polarized light microscopy and is shown in Fig. 5 with micrographs. However, this morphology may also be attributed to platelets which are oriented with edges aligned with the glass coverslip (Blach, et al., 2016; Gravelle, Davidovich-Pinhas, Barbut, & Marangoni, 2017). This behavior has been observed previously and attributed to the crystals minimizing free energy associated with contacting the hydrophilic glass surface (Blake & Marangoni, 2015).

The micrographs shown in Fig. 5 are all at the 400x magnification level. Additionally, the micrographs have been converted to 8-bit images using ImageJ software for better visualization of crystal structures. The algal oil and emulsion alone exhibited little to no crystalline structure, while the monolaurin and MAG/DAG oleogels and emulgels exhibited a strong needle-like morphology, which could be larger platelets, with a trend that crystal clusters became denser as the gelator content increased. For oleogels, the 12% monolaurin and MAG/DAG samples exhibited the highest density of crystals within a given micrograph, however, it seemed that all of the MAG/DAG oleogels possessed a more tightly packed, smaller, needle-like morphology than the monolaurin oleogel counterparts. This would also agree with previous data on physical structure as it suggests the more densely packed morphology is why MAG/DAG oleogels exhibited a higher physical stability.

The smaller more needle-like structure of MAG/DAG oleogels relative to monolaurin oleogels may lend the gel to be more stable as they may possess a stronger oil binding ability (Sato & Ueno, 2005). Micrographs of emulgels were not as consistent as those for oleogels. Upon visual inspection it appears that the density of crystals increases as gelator content increased for both monolaurin and MAG/DAG emulgels. However, there doesn't appear to be a noticeable difference in the density of crystal morphology between the two types of emulgels. The thermographs, SFC, and XRD data suggests that monolaurin emulgels are more physically stable.

Images of both oleogel and emulgel samples can be found in supplementary material Fig. S1. The rheological properties for both oleogel and emulgel samples can be seen in supplementary material Fig. S2. Additionally, the initial fatty acid profile of algal oil from GC-FID analysis of FAMEs has been included in Fig. S3. Fig. S2 depicts heating sweeps where

rheology was measured under a heating program of 0 – 60 °C. The two lines displayed are the storage (G′) modulus and the loss (G″) modulus. The cross point between the two lines is called the cross-over modulus and is indicative of a phase change state with rubbery or pseudo-elastic properties . Typically, at this point the microstructure of a compound is beginning to "flow" and possibly breakdown as well (Gonzalez-gutierrez & Scanlon, 2018). This also correlates to previous data on physical characteristics for oleogels, as the monolaurin oleogels undergo a cross-over point much earlier than the MAG/DAG oleogels. However, once again, the emulgels did not correlate as well since both monolaurin and MAG/DAG emulgels have earlier cross-over points at similar temperatures.

Overall, MAG/DAG oleogels exhibited a stronger physical stability over monolaurin oleogels. This trend was reversed with emulgels, which may be explained by the HLB values of the given gelators and the polar paradox theory, which may allow for more complete hydrogen bonding in different sample matrices (Marangoni & Garti, 2018). For oleogels, the thermograms, SFC, XRD, micrographs, and rheological data all correspond to MAG/DAG producing a more physically stable gel than the monolaurin counterpart. For emulgels, the thermograms, SFC, and XRD all correlate to monolaurin producing more stable gels, but the micrographs and rheological data does not correlate as strongly as the oleogel data.

4. Conclusions

Oleogels and emulgels were successfully developed with a novel ω-3 FA rich lipid source, *Schizochytrium spp.* algal oil. These oleogels and emulgels exhibited a higher oxidative stability than bulk oil or emulsion. In addition, the physical characteristics of these gels suggest they may be able to replace saturated fat sources in foods, pharmaceuticals, and cosmetics. Oleogels with monolaurin gelator exhibited a significantly higher oxidative stability than MAG/DAG oleogels, whereas MAG/DAG

oleogels exhibited better physical stability. Additionally, monolaurin emulgels exhibited a higher oxidative stability than MAG/DAG emulgels, while also exhibiting better physical stability. With additional research these gels could replace saturated fat sources in the future while providing a cheap, alternative source of ω-FA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

J.H. Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft; S.H. Investigation, Writing - review & editing; C. A. Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition. All authors contributed to and approved the final draft of the manuscript.

Ethics Statement

This work is an original body of research by the authors and has not been published elsewhere.

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Tables **Table 4.1** Changes in ω-3 fatty acid composition of oleogels and emulgels, with bulk oil and emulsion as controls, respectively.

Time (day)	Sample	EPA				DHA			
		Concentration (mg/g)		Normalized %		Concentration (mg/g)		Normalized %	
		OG[1]	EG[2]	OG[1]	EG[2]	OG[1]	EG[2]	OG[1]	EG[2]
0	Control	65.81 ± 0.15	67.63 ± 1.25	100	100	354.14 ± 6.90	354.35 ± 6.82	100	100
	8% M[3]	64.99 ± 1.20	65.60 ± 2.52	100	100	328.59 ± 5.34	326.01 ± 11.28	100	100
	10% M	63.30 ± 0.57	64.25 ± 2.03	100	100	323.76 ± 7.79	315.84 ± 7.94	100	100
	12% M	62.25 ± 2.35	62.82 ± 1.74	100	100	315.10 ± 6.97	311.72 ± 6.90	100	100
	8% MD[4]	64.75 ± 2.72	65.88 ± 1.88	100	100	317.65 ± 3.20	323.33 ± 5.82	100	100
	10% MD	62.30 ± 2.83	63.50 ± 0.63	100	100	310.61 ± 6.26	304.92 ± 4.34	100	100
	12% MD	62.45 ± 2.10	62.13 ± 2.68	100	100	309.04 ± 12.78	304.87 ± 3.74	100	100
4	Control	61.52 ± 4.12	57.18 ± 3.79	93.48 ± 6.26^{a}	84.55 ± 5.60^{a}	328.37 ± 15.27	323.50 ± 9.01	92.72 ± 4.31^{a}	91.29 ± 2.54^{a}
	8% M	60.44 ± 8.28	57.81 ± 7.16	92.30 ± 12.74^{a}	88.13 ± 10.91^{a}	315.39 ± 21.31	322.13 ± 7.24	95.98 ± 6.49^{a}	98.81 ± 2.22^{a}
	10% M	58.98 ± 1.13	59.75 ± 1.32	93.18 ± 1.79^{a}	93.01 ± 2.05^{a}	312.58 ± 5.23	311.78 ± 1.95	96.55 ± 1.62^{a}	98.71 ± 0.62^{a}
	12% M	61.11 ± 1.13	58.89 ± 2.60	98.17 ± 1.82^{a}	93.74 ± 4.14^{a}	313.09 ± 5.54	307.35 ± 6.88	99.36 ± 1.76^{a}	98.59 ± 2.21^{a}
	8% MD	61.39 ± 2.11	59.29 ± 1.40	94.81 ± 3.26^{a}	89.97 ± 2.13^{a}	302.13 ± 35.79	308.07 ± 11.22	95.11 ± 11.27^{a}	95.28 ± 3.47^{a}
	10% MD	58.22 ± 4.17	57.21 ± 1.26	93.45 ± 6.69^{a}	90.09 ± 1.98^{a}	299.46 ± 21.90	297.63 ± 10.29	96.41 ± 7.05^{a}	97.61 ± 3.37^{a}
	12% MD	59.68 ± 6.37	57.30 ± 2.10	95.56 ± 10.20^{a}	92.26 ± 3.38^{a}	293.77 ± 65.39	293.32 ± 3.77	95.06 ± 21.16^{a}	96.21 ± 1.24^{a}
7	Control	53.40 ± 1.49	50.22 ± 2.59	81.14 ± 2.26^{b}	74.26 ± 3.83^{c}	293.97 ± 9.53	284.71 ± 8.02	82.78 ± 2.69^{b}	80.35 ± 2.26^{e}
	8% M	56.22 ± 2.49	55.56 ± 2.01	86.51 ± 3.83^{b}	84.70 ± 3.06^{b}	291.50 ± 3.42	282.66 ± 14.20	89.46 ± 1.04^{a,b}	86.70 ± 4.36^{d}
	10% M	57.41 ± 3.02	57.98 ± 0.40	90.70 ± 4.77^{a}	90.24 ± 0.62^{a}	297.38 ± 2.67	288.76 ± 4.31	91.85 ± 0.82^{a,b}	91.43 ± 1.36^{b,c}
	12% M	60.25 ± 5.67	58.44 ± 1.25	96.79 ± 9.11^{a}	93.03 ± 1.99^{a}	297.94 ± 4.66	288.85 ± 1.85	94.56 ± 1.48^{a}	92.66 ± 0.59^{b}
	8% MD	57.82 ± 5.44	54.54 ± 3.44	89.30 ± 8.40^{a,b}	82.79 ± 5.22^{b}	290.27 ± 7.64	286.77 ± 5.99	91.38 ± 2.41^{a,b}	88.69 ± 1.85^{c,d}
	10% MD	59.15 ± 5.13	55.01 ± 1.01	94.94 ± 8.23^{a}	86.63 ± 1.59^{a,b}	292.82 ± 10.91	289.05 ± 6.60	94.27 ± 3.51^{a}	94.79 ± 2.04^{a}
	12% MD	58.78 ± 4.23	57.30 ± 1.59	94.12 ± 6.77^{a}	92.23 ± 2.56^{a}	294.33 ± 13.96	288.87 ± 4.59	93.95 ± 6.46^{a}	94.75 ± 1.51^{a}
10	Control	51.57 ± 1.64	49.21± 1.28	78.36 ± 2.49^{c}	72.76 ± 1.89^{e}	265.53 ± 4.78	254.82 ± 7.24	74.98 ± 1.35^{d}	71.91 ± 2.04^{d}
	8% M	52.34 ± 3.41	52.72 ± 4.63	80.54 ± 5.25^{b,c}	80.37 ± 7.06^{c,d}	282.91 ± 5.14	257.12 ± 4.81	86.10 ± 1.56^{b,c}	78.87 ± 1.48^{b,c}
	10% M	53.86 ± 4.93	54.92 ± 2.88	85.24 ± 7.79^{b}	85.48 ± 4.48^{b}	286.33 ± 3.79	259.63 ± 3.24	88.44 ± 1.17^{a,b}	82.20 ± 1.03^{a,b}
	12% M	58.71 ± 2.85	56.72 ± 3.71	94.31 ± 4.58^{a}	90.29 ± 5.91^{a}	291.63 ± 6.28	261.21 ± 9.73	92.55 ± 1.99^{a}	83.80 ± 3.12^{a}
	8% MD	52.01 ± 4.22	49.03 ± 3.95	80.32 ± 6.52^{b,c}	74.42 ± 6.00^{e}	265.18 ± 9.27	252.77 ± 3.52	83.48 ± 2.92^{c}	78.18 ± 1.09^{c}
	10% MD	51.82 ± 3.76	50.17 ± 2.67	83.18 ± 6.04^{b}	79.01 ± 4.20^{d}	269.74 ± 3.84	254.19 ± 2.60	86.84 ± 1.24^{b,c}	83.36 ± 0.85^{a}
	12% MD	52.05 ± 2.13	51.62 ± 3.06	83.35 ± 3.41^{b}	83.08 ± 4.93^{b,c}	277.56 ± 4.21	255.93 ± 3.63	89.81 ± 1.36^{a,b}	83.95 ± 1.19^{a}
14	Control	49.28 ± 2.18	48.89 ± 1.83	74.89 ± 3.31^{b}	72.29 ± 2.71^{c}	247.03 ± 2.62	223.01 ± 4.96	69.75 ± 0.74^{d}	62.93 ± 1.40^{c}
	8% M	49.72 ± 1.19	50.95 ± 0.96	76.50 ± 1.83^{b}	77.67 ± 1.46^{b,c}	269.93 ± 2.67	225.72 ± 2.99	76.22 ± 0.81^{c}	69.24 ± 0.92^{a,b}
	10% M	51.18 ± 6.01	52.04 ± 1.31	80.85 ± 9.49^{a,b}	81.01 ± 2.04^{a,b}	272.98 ± 2.59	226.45 ± 1.13	84.32 ± 0.80^{b}	71.70 ± 0.36^{a,b}
	12% M	57.80 ± 3.25	55.81 ± 3.06	92.85 ± 5.22^{a}	88.84 ± 4.87^{a}	284.46 ± 3.63	229.44 ± 5.78	90.18 ± 1.15^{a}	73.60 ± 1.85^{a}
	8% MD	47.11 ± 4.37	46.14 ± 3.47	71.58 ± 6.75^{b}	70.04 ± 5.27^{c}	243.20 ± 11.26	220.33 ± 7.78	76.56 ± 3.54^{c}	68.14 ± 2.41^{b}
	10% MD	47.41 ± 1.48	46.34 ± 2.47	76.10 ± 2.38^{b}	72.98 ± 3.89^{b,c}	251.69 ± 9.96	211.21 ± 4.84	81.03 ± 3.21^{b,c}	69.27 ± 1.59^{a,b}
	12% MD	48.03 ± 4.02	47.06 ± 1.34	76.91 ± 6.44^{b}	75.74 ± 2.16^{b,c}	264.23 ± 5.16	213.50 ± 4.65	85.50 ± 1.67^{a,b}	70.03 ± 1.53^{a,b}

[1]Denotes oleogel

[2]Denotes emulgel

[3]Denotes monolaurin gels

[4]Denotes MAG/DAG gels

[a,b,c,d,e] Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Table 4.2 Oxidation induction time measured with differential scanning calorimetry for oleogel and emulgel samples, with bulk oil and emulsion as controls, respectively.

Oxidation Induction Time (OIT)		
Sample	**Oleogel**	**Emulgel**
Algal Oil	21.43 ± 0.25^{e}	NA
Emulsion	NA	20.31 ± 0.25^{f}
8% Monolaurin	24.67 ± 0.21^{c}	21.33 ± 0.11^{e}
10% Monolaurin	$26.73 \pm 0.38^{a,b}$	24.4 ± 0.13^{b}
12% Monolaurin	27.03 ± 0.47^{a}	25.01 ± 0.23^{a}
8% MAG/DAG	23.07 ± 0.15^{d}	21.81 ± 0.25^{e}
10% MAG/DAG	24.47 ± 0.16^{c}	22.47 ± 0.21^{d}
12% MAG/DAG	25.97 ± 0.22^{b}	23.27 ± 0.20^{c}

^{a,b,c,d,e} Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Figures

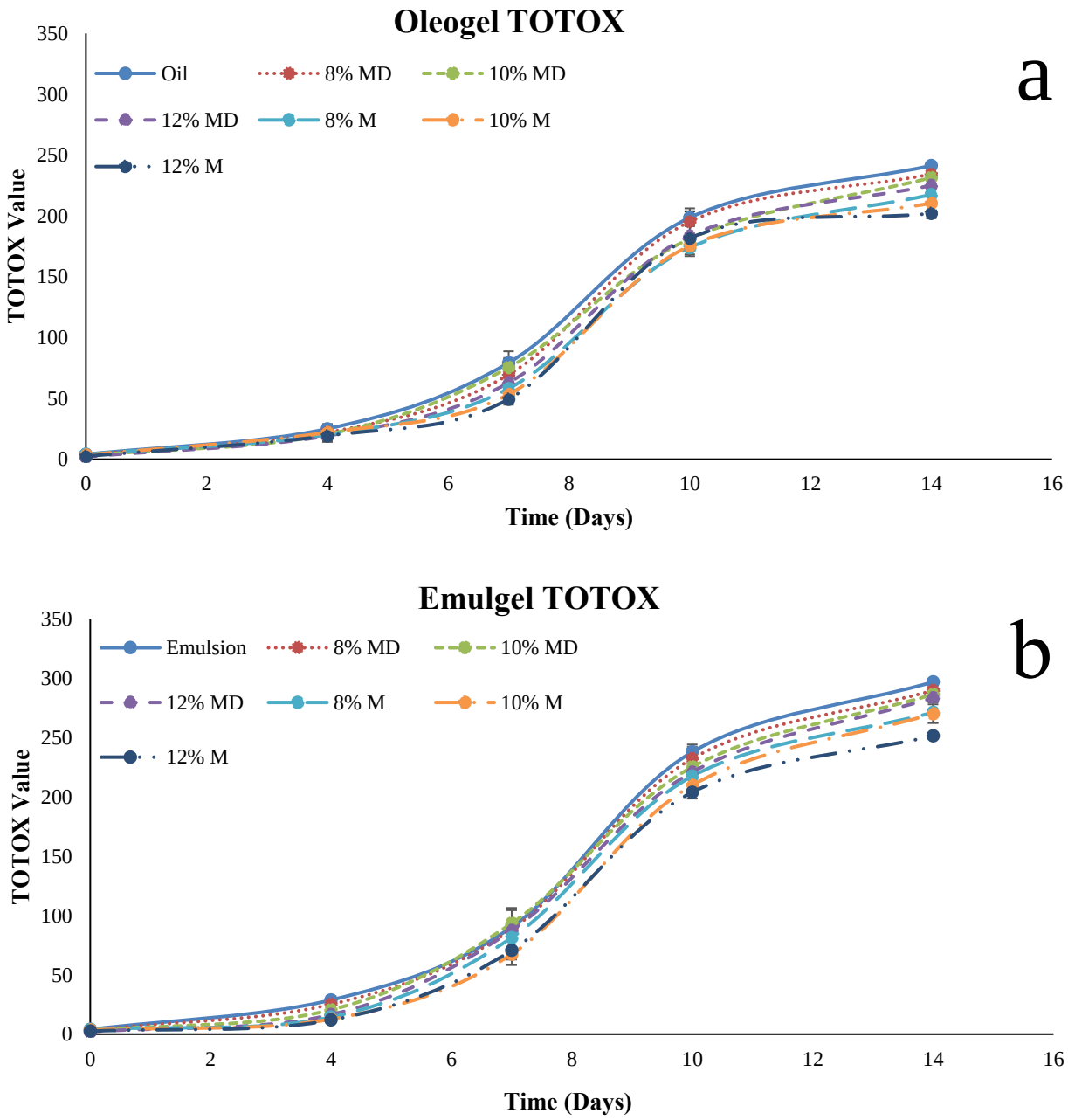
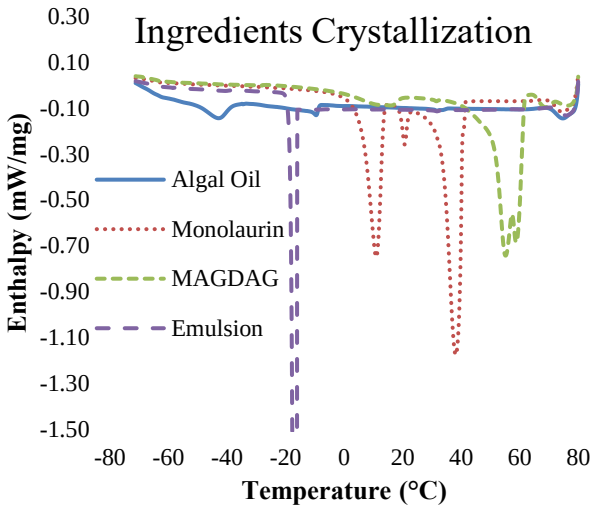


Figure 4.1 Graphs depict (a) TOTOX* values of oleogel samples and bulk oil as well as (b) TOTOX* values of emulgel and emulsion during the 14-day accelerated oxidation study.

*TOTOX value = 2(PV) + *p*AV, PV = peroxide value, *p*AV = *p*-Anisidine value.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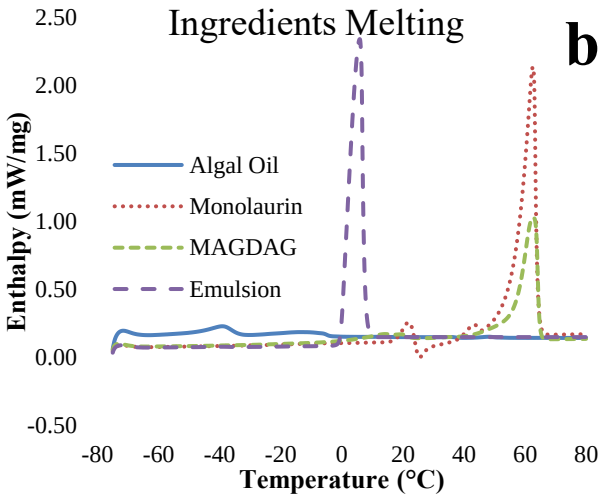
Enthalpy (mW/mg)
Temperature (°C)
Algal Oil
Monolaurin
MAGDAG
Emulsion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Temperature (°C)
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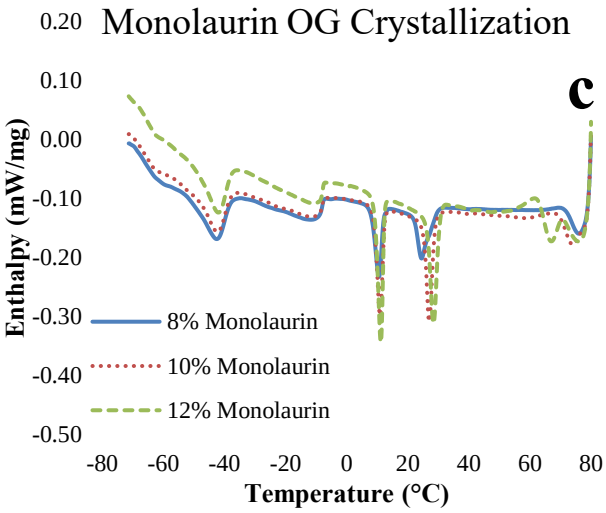
Monolaurin
MAGDAG
Emulsion


Monolaurin OG Crystallization
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Enthalpy (mW/mg)
Temperature (°C)
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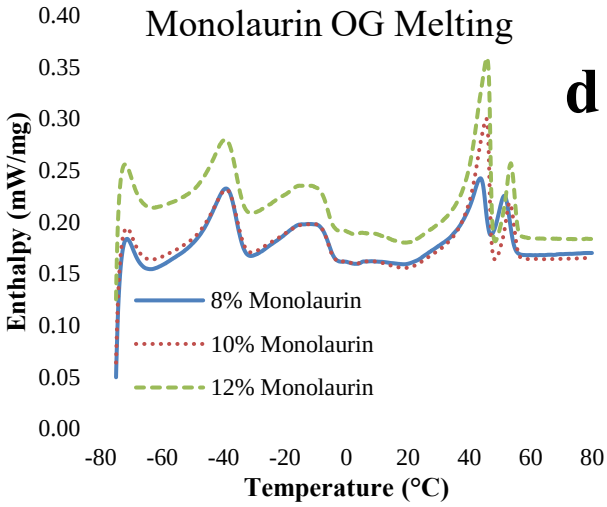
10% Monolaurin
12% Monolaurin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Temperature (°C)
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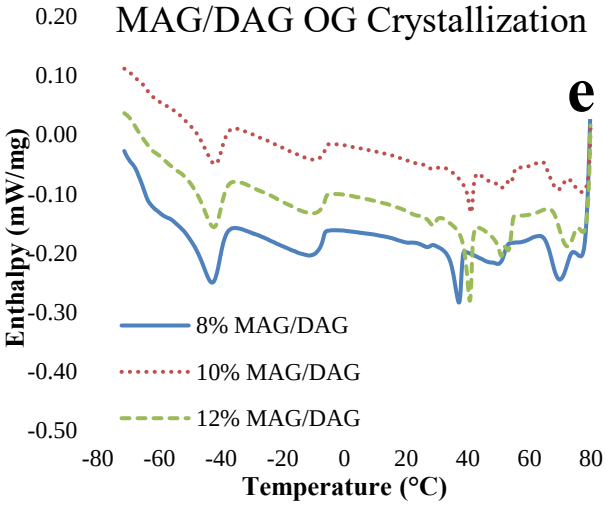
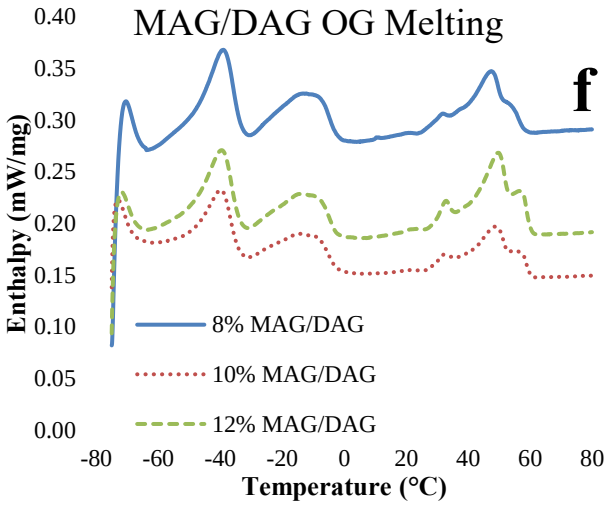
10% Monolaurin
12% Monolaurin


MAG/DAG OG Crystallization
e
Enthalpy (mW/mg)
Temperature (°C)
8% MAG/DAG
10% MAG/DAG
12% MAG/DAG


MAG/DAG OG Melting
f
Enthalpy (mW/mg)
Temperature (°C)
8% MAG/DAG
10% MAG/DAG
12% MAG/DAG

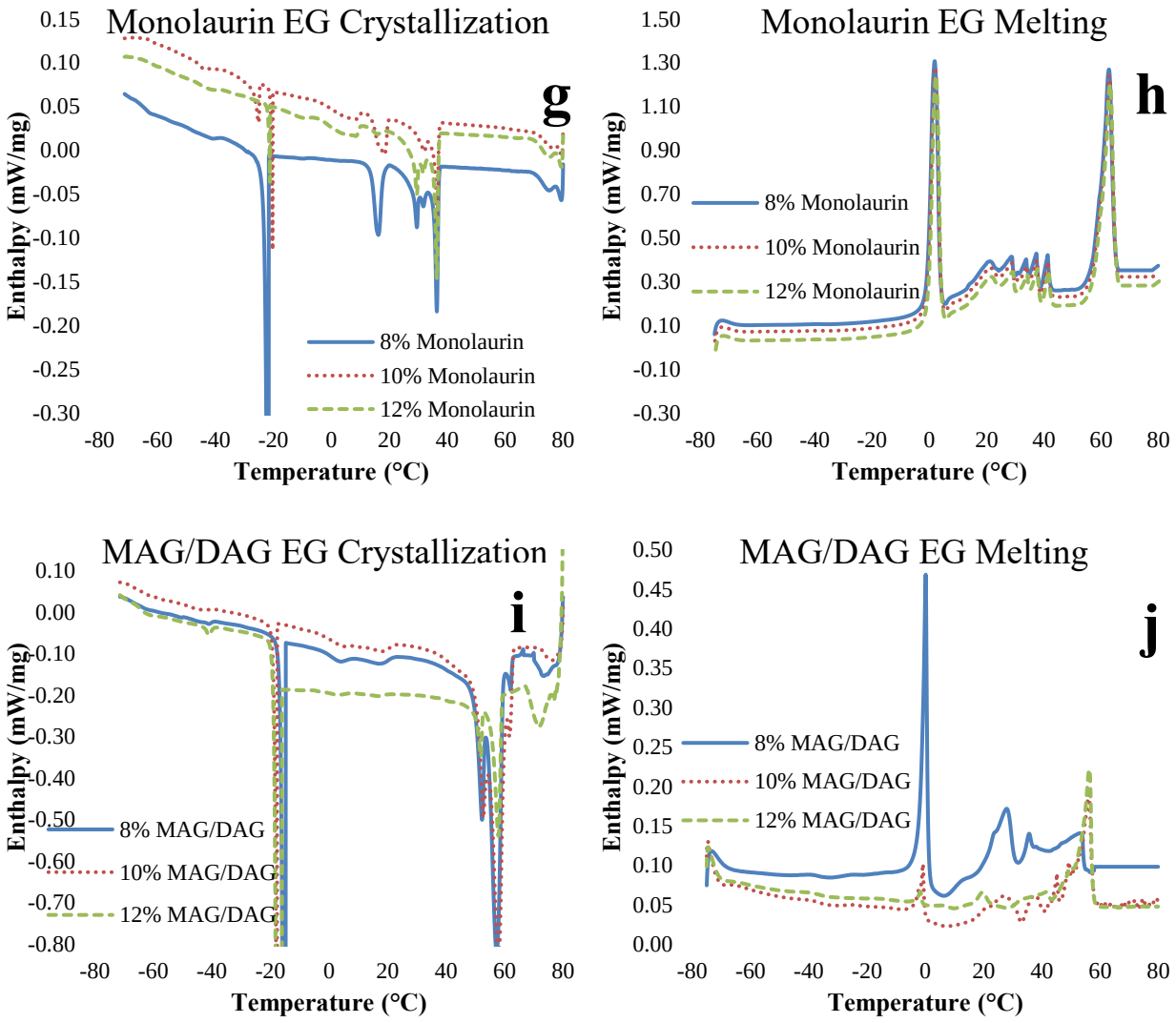


Figure 4.2 Differential scanning calorimetry (DSC) thermograms: (a) depicts crystallization of "ingredients" (algal oil, emulsion, and monolaurin and MAG/DAG gelators), (b) depicts melting of ingredients, (c) depicts crystallization of monolaurin oleogels (OGs), (d) depicts melting of monolaurin OGs, (e) depicts crystallization of MAG/DAG OGs, (f) depicts melting of MAG/DAG OGs, (g) depicts crystallization of monolaurin emulgels (EGs), (h) depicts melting of monolaurin EGs, (i) depicts crystallization of MAG/DAG EGs, and (j) depicts melting of MAG/DAG EGs.

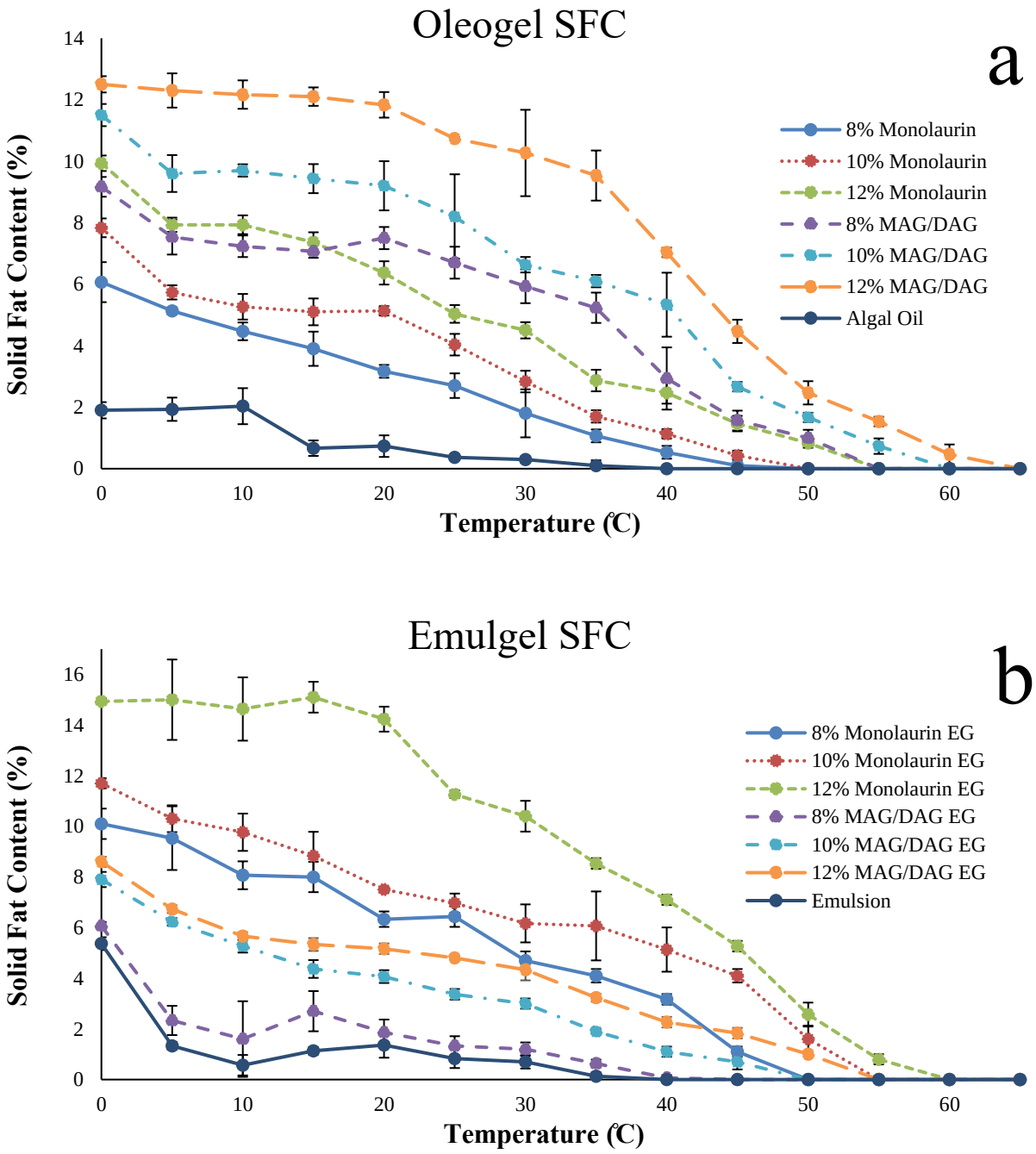
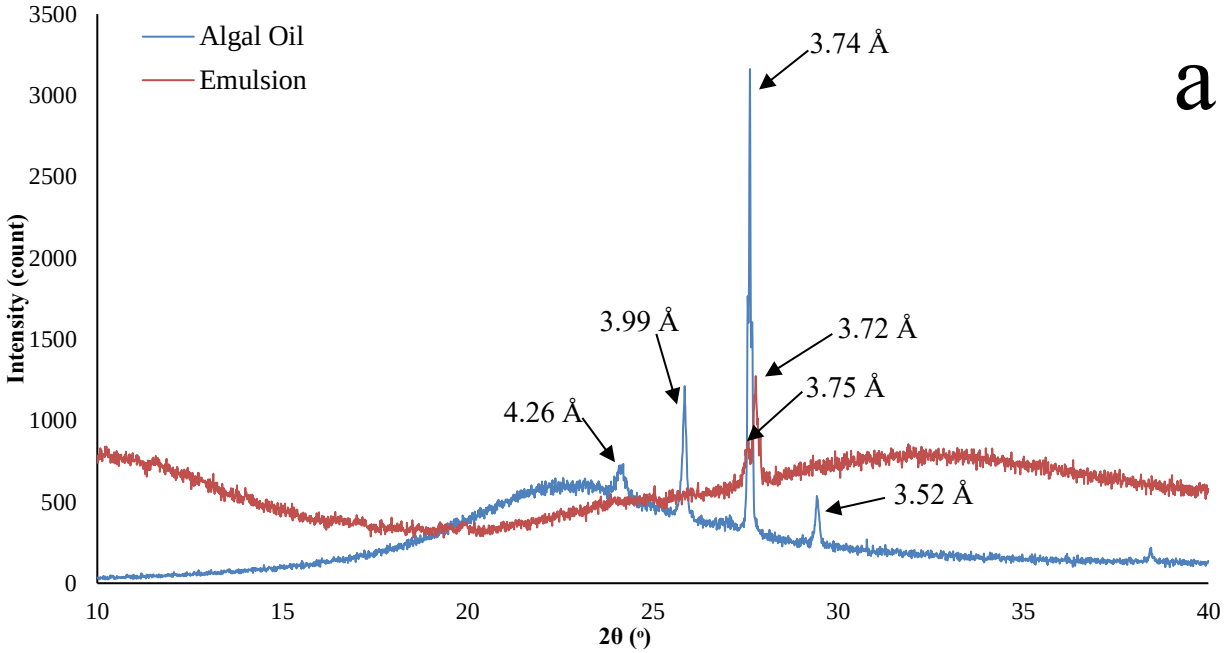
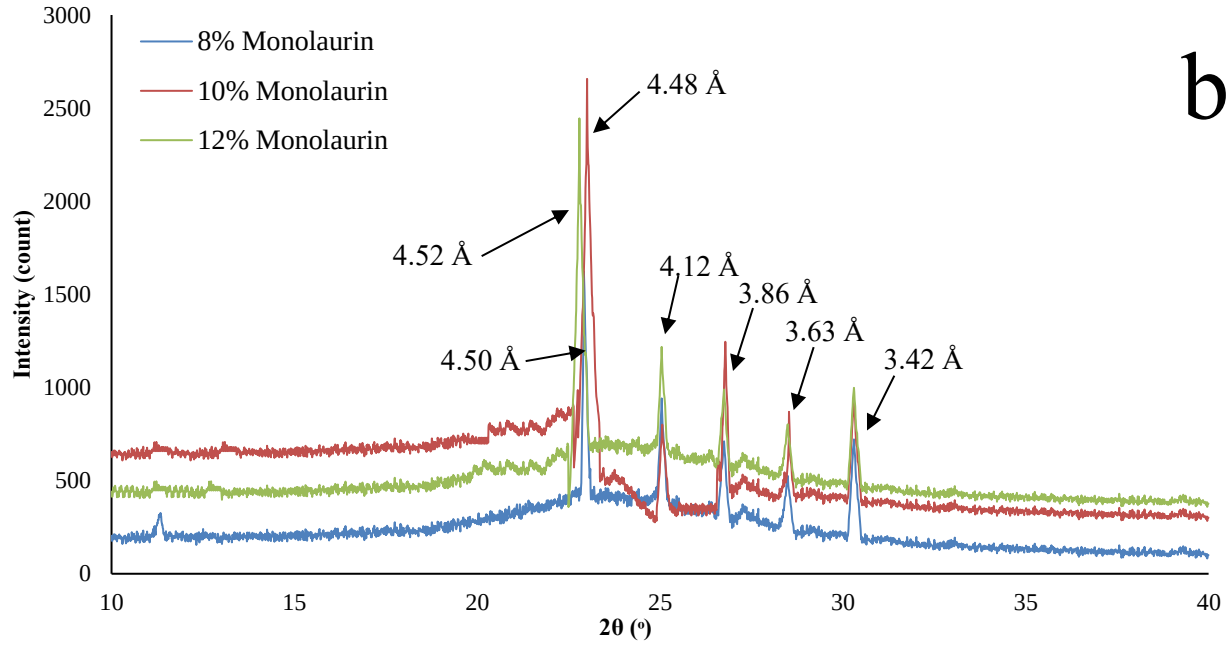
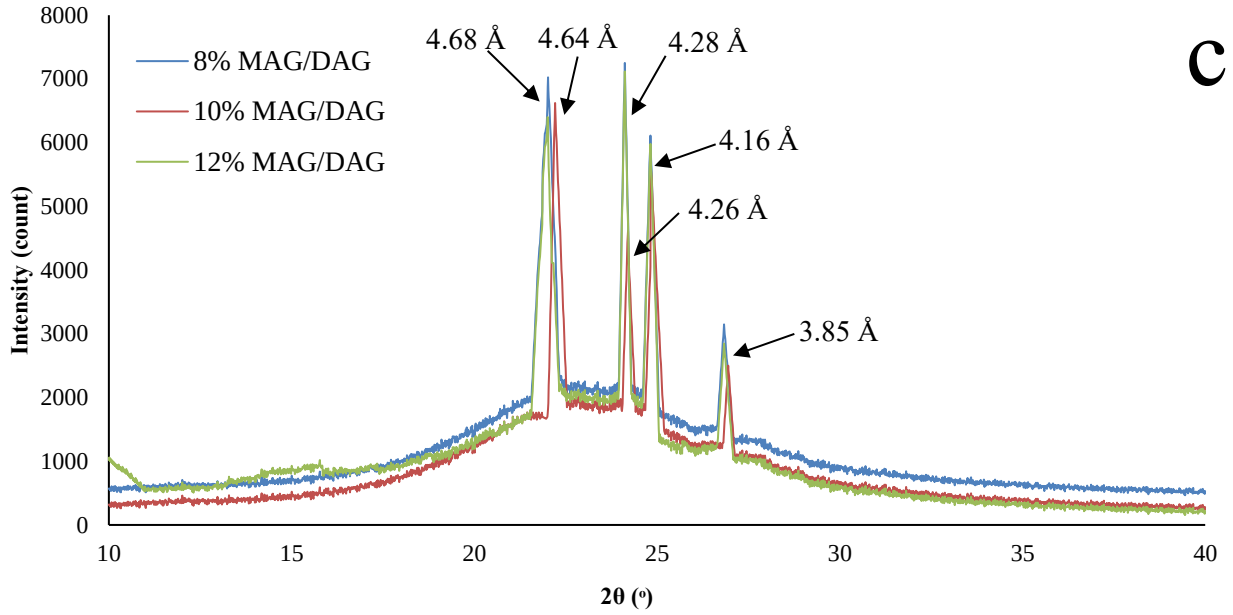
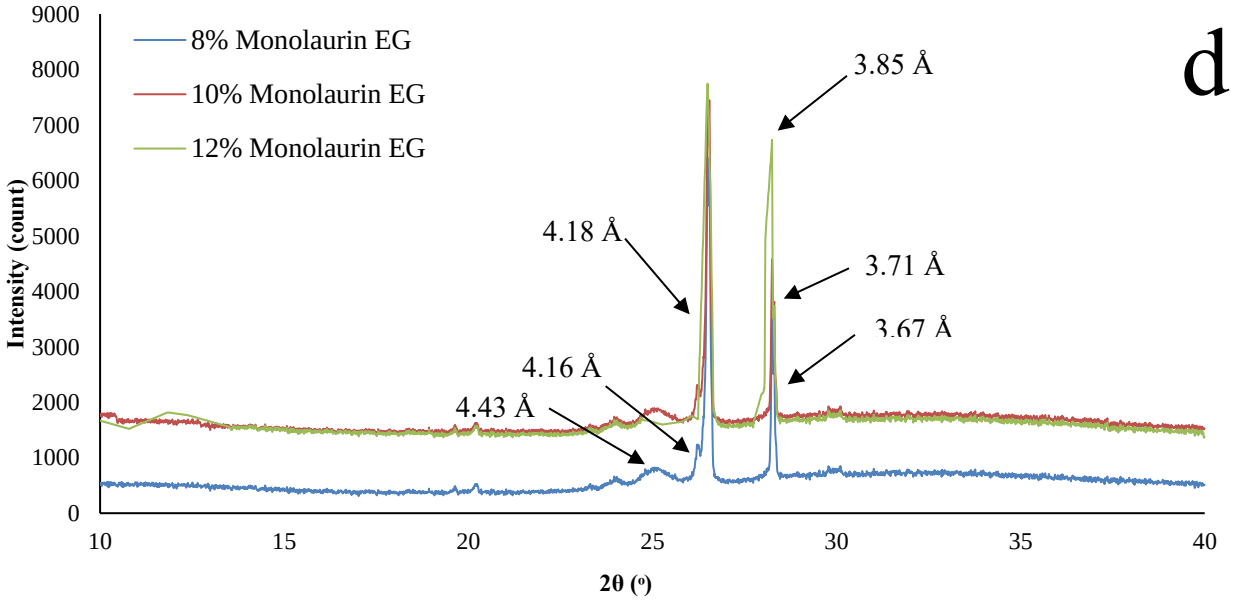


Figure 4.3 Graphs depict solid fat content (SFC) of (a) oleogels and algal oil and (b) emulgels and emulsion over an increasing temperature program of 0 – 65℃.


Algal Oil
Emulsion
3.74 Å
3.99 Å
3.72 Å
3.75 Å
4.26 Å
3.52 Å
Intensity (count)
2θ (º)
a


8% Monolaurin
10% Monolaurin
12% Monolaurin
4.48 Å
4.52 Å
4.12 Å
3.86 Å
3.63 Å
4.50 Å
3.42 Å
Intensity (count)
2θ (º)
b


c
8% MAG/DAG
10% MAG/DAG
12% MAG/DAG
4.68 Å
4.64 Å
4.28 Å
4.16 Å
4.26 Å
3.85 Å
Intensity (count)
2θ (º)


d
8% Monolaurin EG
10% Monolaurin EG
12% Monolaurin EG
3.85 Å
4.18 Å
3.71 Å
3.67 Å
4.16 Å
4.43 Å
Intensity (count)
2θ (º)

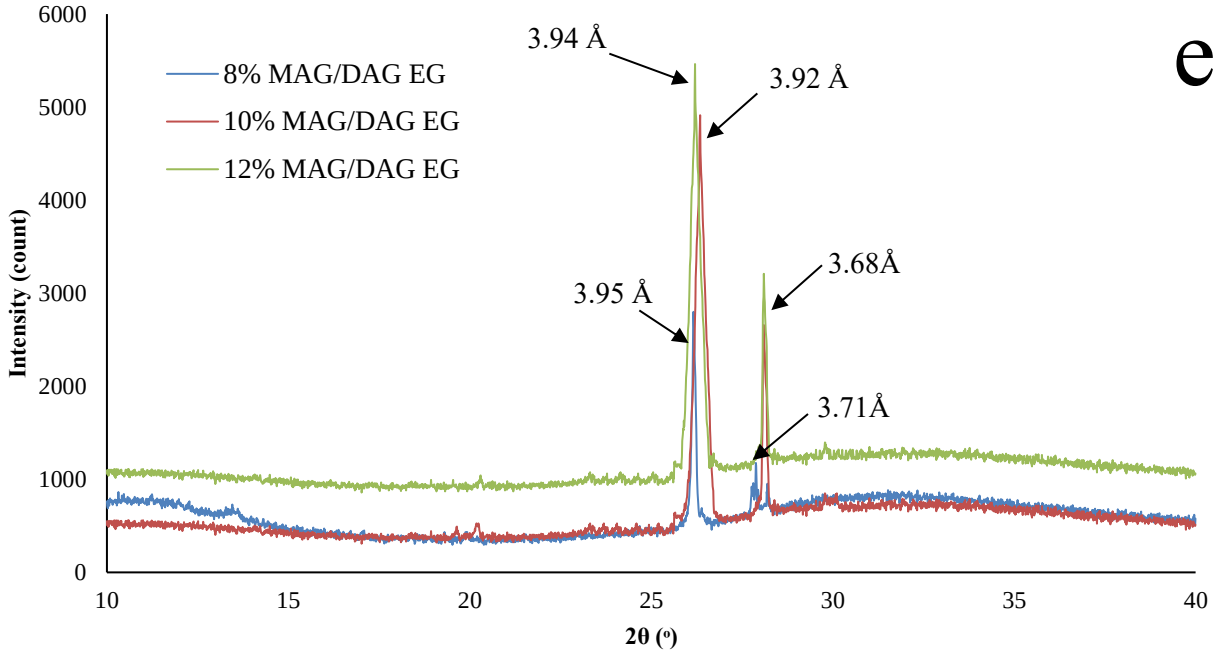
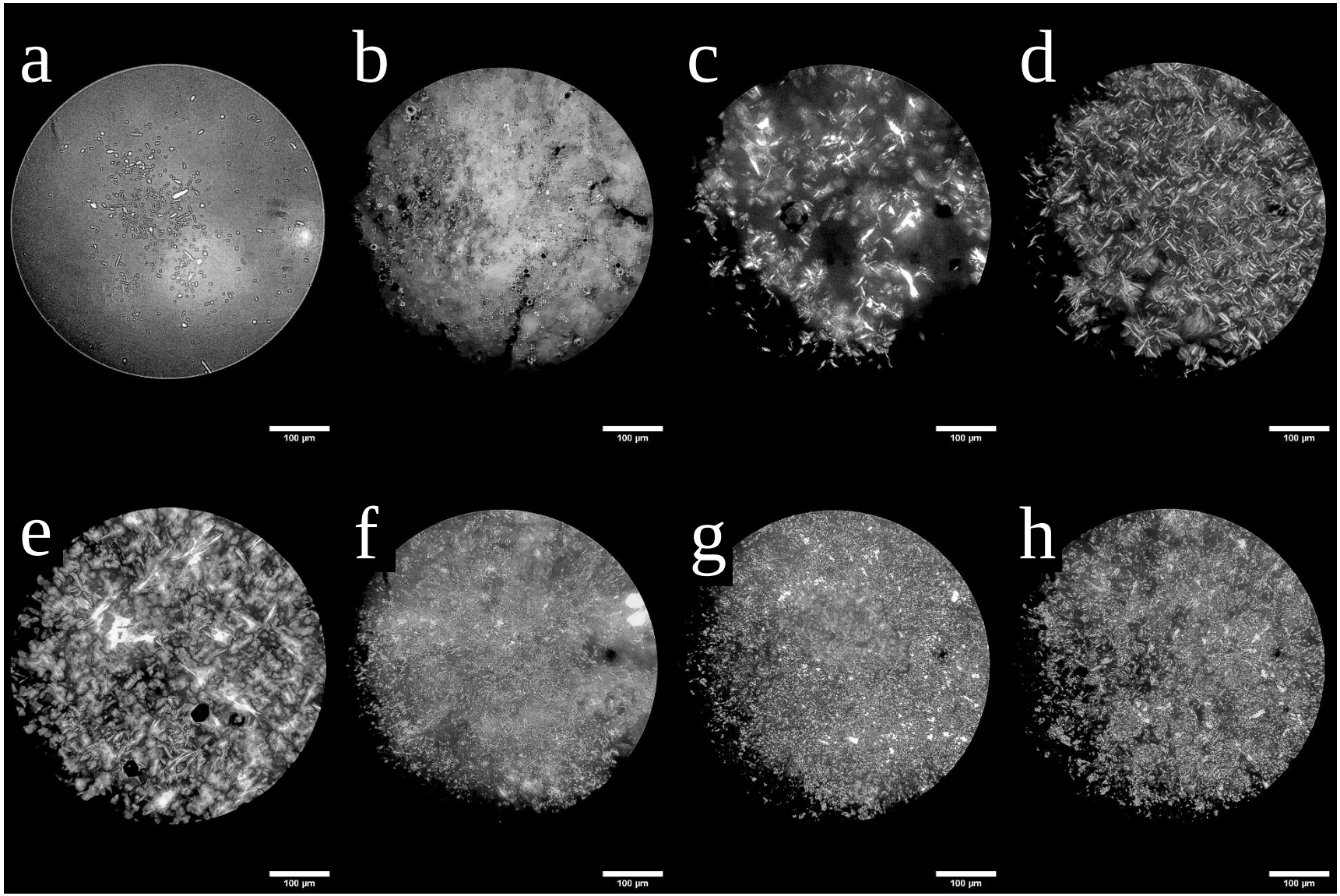


Figure 4.4 Graphs depict crystalline structures of samples determined with X-ray diffraction (XRD) for (a) algal oil and emulsion, (b) monolaurin OGs, (c) MAG/DAG OGs, (d) monolaurin EGs, and (e) MAG/DAG EGs.


a
b
c
d
e
f
g
h
100 µm

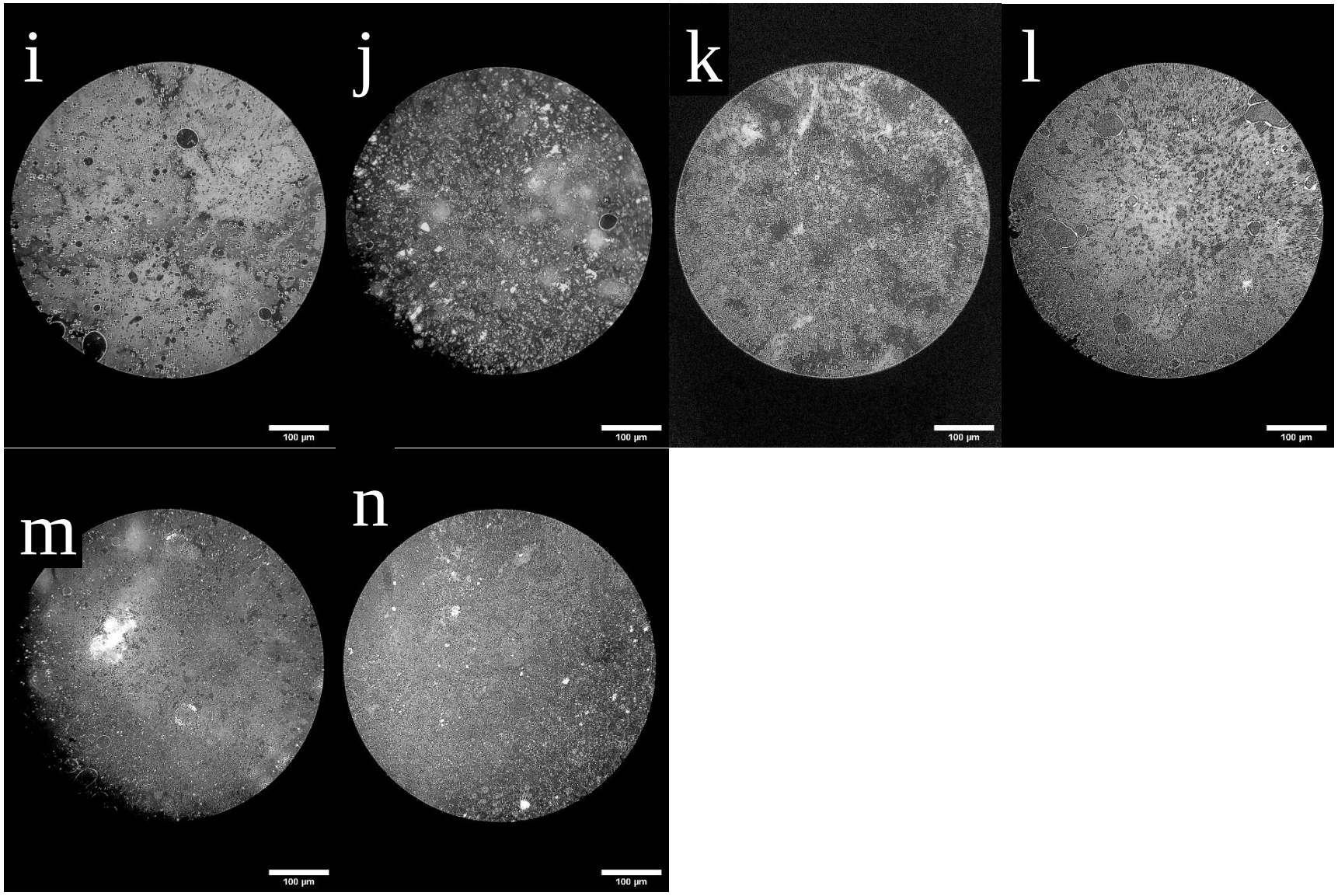

i
j
k
l
m
n
100 µm
100 µm
100 µm
100 µm
100 µm
100 µm

Figure 4.5 Polarized light micrographs taken at 0℃ with 400x magnification for (a) algal oil, (b) emulsion, (c) 8% monolaurin OG, (d) 10% monolaurin OG, (e) 12% monolaurin OG, (f) 8% MAG/DAG OG, (g) 10% MAG/DAG OG, (h) 12% MAG/DAG OG, (i) 8% monolaurin EG, (j) 10% monolaurin EG, (k) 12% monolaurin EG, (l) 8% MAG/DAG EG, (m) 10% MAG/DAG EG, and (n) 12% MAG/DAG EG.

CHAPTER 5

COMBINING ANTIOXIDANTS AND PROCESSING TECHNIQUES TO IMPROVE OXIDATIVE STABILITY OF A *SCHIZOCHYTRIUM* ALGAL OIL INGREDIENT WITH APPLICATION IN YOGURT [1]

[1] Hyatt, J. R., Zhang, S., & Akoh, C. C. Submitted to Journal of Food Chemistry, September 12, 2022.

Abstract

The physicochemical properties of ω-3 FA-rich ingredients produced using processing combinations of gelation, microencapsulation, and antioxidant addition were assessed. All ingredients (bulk algal oil, AO; oleogel, OG; microencapsulation, ME; and a microencapsulated oleogel, MEOG) used *Schizochytrium spp.* algal oil and either a combination of α, β, γ, and δ tocopherols (TOC) or 1-*o*-galloylglycerol (GG) as antioxidant. Highest performing ingredients for each processing type were selected to develop yogurts and compared against a butterfat yogurt (BF) and a store-bought yogurt product for physicochemical properties. Yogurt developed with MEOG with GG as antioxidant exhibited average peroxide values (PV) and *p*-Anisidine values (*p*-AV) after 24 day storage of 7.17 mmol O_2/kg of oil and 118.85 abs/g, respectively. These results were similar to a store-bought yogurt made with a saturated fat source, which exhibited values of 6.83 mmol O_2/kg of oil and 117.95 absorbance/g for PV and *p*-AV, respectively, after 24 days of storage at 4°C.

Keyword: Antioxidant; Gelation; Microencapsulation; Lipid oxidation; Yogurt.

1. Introduction

Omega-3 fatty acids (ω-3 FA) are of particular interest to researchers and the food industry due to their associated benefits such as reducing the risk of heart disease and stroke (He, 2009). However, ω-3 FA are typically underutilized in food products due to their high susceptibility to lipid oxidation (Galano et al., 2015). Issues with lipid oxidation can be resolved by the addition of antioxidants, but physical state limitations are another critical point limiting the use of ω-3 FA in foods (Lucca & Tepper, 1994). Most food products utilize saturated fat sources for their production, which are solid at ambient temperature, whereas ω-3 rich-FA lipid sources are typically liquid even at refrigeration temperatures. Overcoming physical state limitations can be accomplished by various processing steps such as gelation and microencapsulation (Co & Marangoni, 2012; Bakry et al., 2016). Gelation can be used to develop oleogels (an edible type of organogels) which have been proven to improve oxidative stability while overcoming physical limitations of oils rich in polyunsaturated fatty acids (PUFA). Microencapsulation can also be used to overcome physical limitations of PUFA-rich lipid sources while also improving oxidative stability (Tamjidi, Nasirpour, and Shahedi, 2012).

When processing techniques are used to improve the physical properties of ω-3 FA-rich lipid sources, there is also a noticeable improvement in the oxidative stability after processing (Willett & Akoh, 2019a; Hyatt, Zhang, & Akoh, 2022). Moreover, when two processing techniques are combined there is an additional increase in oxidative stability (Willett & Akoh, 2019b). This is most likely due to improvements in conditions associated with oxidation, such as light, heat, and oxygen accessibility. In theory, combining the use of antioxidants with processing techniques could further improve the oxidative stability of the final product. We wanted to develop an ω-3 FA-rich lipid ingredient utilizing a novel lipid source, *Schizochytrium spp.* algal oil, and apply it in a food product to obtain comparable

physical characteristics and oxidative stability to a similar food product made with a traditional saturated FA source.

Antioxidants for this study were selected from previous research based on the highest performing antioxidants in some model systems (Hyatt, Zhang, & Akoh, 2021). The antioxidants chosen include a mixture of α, β, γ, and δ tocopherols (TOC) as well as an enzymatically synthesized novel compound, 1-*o*-galloylglycerol (GG) which was utilized in a previous study (Zhang & Akoh, 2020). Evidence showed that GG performed exceptionally well in non-polar media (Zhang, Willett, Hyatt, Martini, & Akoh, 2021), and TOC performed better in a polar environment (Hyatt, Zhang, & Akoh, 2021). Due to the method of microencapsulation used, TOC may outperform GG at the interface of the polar and non-polar phase in the double emulsion method required for microencapsulations. From our previous study, GG is expected to outperform TOC in samples without water present.

The objectives of this study include developing a food product using an ω-3 FA lipid source in place of a saturated fat and understanding how well antioxidants may function in conjunction with processing techniques. Very little research has been conducted on the combination of antioxidants with processing techniques to improve the oxidative stability of lipids. The results from this study should provide information on how well a susceptible PUFA could be protected in a food product. Knowledge gained could be utilized in the food, cosmetic, and pharmaceutical industries. Comparing the physicochemical properties of developed ingredients using different combinations of processing techniques and antioxidants could enhance the use of underutilized, sustainable, ω-3 FA lipid sources in the future.

To understand the physical properties of differently processed ingredients, the rheology and thermal behavior of samples were compared against butterfat (BF) . Developed yogurt products were compared in terms of viscosity and rheology with yogurt made with BF as control and a store-bought

yogurt. The oxidative stability of the developed ingredients were compared by measuring peroxide value (PV), *p*-Anisidine value (*p*-AV), oxidation induction times (OIT), and the change in FA composition. Tests were completed at multiple time points (0, 4, 7, 10, and 14) over the course of a 14-day accelerated oxidation study. The oxidative stability of developed yogurt products were compared against both controls previously mentioned with an accelerated oxidation by measuring PV, *p*-AV, and SPME (solid phase microextraction)-GC/MS. Developed yogurt products were also compared against each other for physical characteristics including whey separation and water holding capacity (WHC).

2. Materials and Methods

2.1. Chemicals, reagents, starter culture, and store-bought yogurt

Algal oil was purchased from Baoding Faithful Industry Co. (Baoding, China). Monolaurin was purchased from Inspired Nutrition (Salem, OR, USA). Tween® 80, Span 80, and sodium alginate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Calcium chloride was obtained from VWR International, LLC (West 141 Chester, PA, USA). All other reagents and solvents were of analytical or HPLC grades and were purchased from Fisher Chemical (Fair Lawn, NJ, USA), Sigma-Aldrich Chemical Co. and J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). Traditional yogurt starter culture consisted of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and was obtained from Bacillus Bulgaricus (Hoboken, NJ, USA). Developed yogurt products used UHT skimmed milk from Natrel (Mont-Laurier, QC, Canada). White mountain whole milk Bulgarian yogurt was purchased from a local Kroger (Athens, GA, USA) for the purpose of using a similar store-bought product for comparison with developed yogurts. All materials mentioned were used without further purification steps.

2.2. Preparation of oleogel and microencapsulation

Oleogel preparation followed parameters set forth elsewhere with modifications (Hyatt, Siyu, & Akoh, 2022). Oleogels were developed in triplicate by dissolving monolaurin at a concentration of 12% (w/w) in 10 g of algal oil at 90 °C. Gel mixtures were stirred constantly for 10 min until fully dissolved and then transferred to Ace Glass vials purchased from VWR™ (Radnor, PA, USA). Samples were flushed with nitrogen using an Organomation 12-position N-EVAP (Organomation Associates, Inc., Berlin, MA, USA) and placed at 4 °C to develop the gel network and to store for further analysis. Samples using TOC or GG as antioxidants included algal oil (AO-TOC and AO-GG), oleogel (OG-TOC and OG-GG), microencapsulated (ME-TOC and ME-GG), and microencapsulated oleogel (MEOG-TOC and MEOG-GG). In these samples antioxidants were added to the initial mixture of oil and gelator.

Preparation of the microencapsulated samples followed parameters set forth elsewhere using a modified double emulsion method (Sagiri et al., 2014). First, an oil-in-water emulsion was produced by mixing 0.5 g of sodium alginate and 20 g of deionized (DI) water at 25°C using a U.S. Solid 600W Ultrasonic homogenizer (U.S. Solid, Cleveland, OH, USA) with a 13 mm probe for 10 min with a 3 sec on/off pulse method at 80% power. Then 0.4 g of calcium carbonate was added and further homogenized for 2 min. After homogenization, 0.5 g of Span 80 and 5 g of the internal phase, either bulk oil or oleogel with and without antioxidants, were added and homogenized for 5 min. The resulting emulsion was used to form a double emulsion. The emulsion was homogenized further in an ice bath for 5 min to form a thick emulsion. This emulsion was then added to 60 mL of algal oil that was kept at 0 °C (external phase) and homogenized for 5 min. 5 mL of acidified oil (4.5 mL of algal oil mixed with 0.5 mL glacial acetic acid) was added to the external phase while stirring to induce ionic crosslinking and gelation of the alginate layer to form microcapsules. The formed microcapsules were washed

with 0.5 M calcium chloride containing 1% Tween 80, and then washed with water. Microcapsules were stored at 4°C until further analysis.

2.3. Encapsulation efficiency

Encapsulation efficiency (EE) was determined as previously described (Ifeduba & Akoh, 2015). EE was determined to evaluate the captured oil of microencapsulated samples, as a higher EE shows a higher % of internal phase captured within microcapsules. Solvent extractable surface oil and total oil of the microcapsules were determined and EE (%) was calculated using the following formula adapted from (Ifeduba & Akoh, 2015):

$$EE\ (\%) = \left(\frac{mass\ of\ encapsulated\ core\ material}{mass\ of\ active\ material}\right) \times\ 100$$

In order to determine solvent extractable oil, 0.25 g of microencapsulated product was added to 2.5 mL of hexane and vortexed for 3 min. The mixture was centrifuged for 5 min at 1000 rpm. The organic layer was collected, filtered through an anhydrous sodium sulfate, and then transferred to a pre-weighed round bottom flask. A vacuum-rotary evaporator at 60 °C was used to evaporate the solvent. Solvent extractable oil was then calculated as w/w % of suspension.

To quantify total amount of oil, 5 mL of 5 M HCl was added to 0.5 g of microcapsules, agitated at 60°C for 3 h, and then cooled to room temperature. The mixture was transferred to a separatory funnel and extracted twice with 5 mL hexane. The extracted organic layer was filtered through an anhydrous sodium sulfate column and transferred to a pre-weighed round bottom flask. Solvent was removed at 60°C using a rotary evaporator. Total oil was calculated as w/w % of suspension. Results of both EE and leaching can be found in Table S1. All experiments were performed in triplicate and reported as mean ± standard deviation (SD).

2.4. Internal phase leaching

Leaching of the internal phase was studied to determine the stability of the microcapsules. Less internal phase leakage is typically correlated to a more stable microencapsulated product. Following the method from our previous study (Willett & Akoh, 2019b), microcapsules were first wiped to remove traces of oil/moisture on the surface. Then, 0.5 g of microcapsule sample was weighed on a filter paper and placed in a Fisher Scientific Isotemp™ 500 series gravity convection oven (Fischer Scientific, Waltham, MA, USA) set at 37°C, and the leakage was visually monitored for 2 h. Images were taken at the start and end point to subjectively inspect samples for leaching (Fig. S1).

To quantify the amount of leaching, another method was selected (Sagiri et al., 2014; Brodenave, Janaswamy, & Yao, 2014). Briefly, 0.1 g of microcapsules was soaked in 1 mL of DI water for 1 h at 37°C. The mixture was centrifuged at 2000 rpm for 10 min. The supernatant was then collected and dried at 55°C for 48 h. The dried supernatant was weighed and leaching (%) was calculated as follows:

$$Leaching\ (\%) = \frac{dried\ supernatant\ wt\ (g)}{microcapsule\ initial\ wt\ (g)} \times 100$$

2.5. Initial accelerated oxidation study

For accelerated oxidation test, 10 g of each sample were placed into Reacti-vials™ within a Reacti-Therm™ heating and stirring module (Thermo Fisher Scientific, Waltham, MA, USA) fitted with three aluminum heating blocks. The sample types are listed as follows: BF, bulk algal oil (AO), algal oil with TOC (AO-TOC), algal oil with GG (AO-GG), oleogel (OG), oleogel with TOC (OG-TOC), oleogel with GG (OG-GG), microcapsule (ME), microcapsule with TOC (ME-TOC), microcapsule with GG (ME-GG), microencapsulated oleogel (MEOG), microencapsulated oleogel with TOC (MEOG-TOC), and microencapsulated oleogel with GG (MEOG-GG). These abbreviations can also be found in Table S2.

The Reacti-Therm™ module was set at 40 °C, and samples were removed for testing on days 4, 7, 10, and 14 with an initial test on day 0. 40 °C was selected to allow structure of oleogels to stay intact. PV of samples were assessed according to the AOCS Official Method Cd 8b-90 (American Oil Chemists' Society, 2011). *p*-AV were assessed on the same days as PV using the AOCS Official Method Cd 18–90 (American Oil Chemists' Society, 2011). PV and *p*-AV tests were conducted in triplicate, and all reagents were prepared fresh on the day of analysis. Results were reported as mean ± SD.

Fatty acid composition was measured for samples on days 4, 7, 10, and 14 with an initial test on day 0. This was done in order to track the change in fatty acid profile over the accelerated oxidation study. An aliquot of each sample was removed from sample vials on test days and centrifuged at 2,000 rpm for 20 min. The required amount of oil (50 mg) was extracted from the resulting supernatant to test the oxidation of oil in sample matrix. The change in fatty acid composition was determined by following AOAC Official Method 996.01 (Satchithanandam, Fritshce, & Rader, 2001) to prepare fatty acid methyl esters (FAMEs) and analyzed using an Agilent 6890 N GC system with an FID detector (Agilent, Santa Clara, CA, USA) and a Supelco SP-2560 capillary column (100 m x 0.25 mm ID, 0.20 µm film) (Sigma-Aldrich Co., St. Louis, MO, USA). GC analysis followed procedure set forth previously (Ifeduba and Akoh, 2013). Briefly, 1 µL of sample was injected at a split ratio of 5:1, the carrier gas (He) flow was 1.1 mL min^{-1} and the detector temperature was 250 °C. The oven was held at 140 °C for 5 min, then increased to 240 °C at a rate of 4 °C min^{-1} and held for 15 min. FAMEs analysis was conducted in triplicate for each sample, and results were expressed as average mg/g concentrations and normalized percentages for notable fatty acids and calculated using an internal standard, heptadecanoic acid (C17).

2.6. Oxidation induction time (OIT) measured with differential scanning calorimeter (DSC)

The oxidative stability of samples were measured using a 204F-1 Phoenix differential scanning calorimeter (Netzsch-Garätebau GmbH, Selb, Germany) to determine the oxidation induction time (OIT). The test followed parameters in previous reports (Hyatt, Zhang, & Akoh, 2021; Zhang, Willett, Hyatt, Martini, & Akoh, 2021) with slight modifications for both sample types. Bulk oil and oleogel samples were analyzed using 10 ± 0.5 mg aliquots placed in aluminum crucibles with pierced caps against a pierced blank empty crucible. Microencapsulated samples were analyzed against a pierced blank crucible which held an equivalent amount of water to offset interference caused by water present in the sample (Pollastri, Porter, McIntosh, & Simon, 2000). The modified DSC parameters used during OIT measurements were as follows: samples were heated from 40 to 105 °C at a rate of 20 °C min^{-1} under constant nitrogen flow at 50 mL min^{-1}. At 105 °C, after a 3 min stabilization, gas flow was switched to oxygen at 50 mL min^{-1}. The OIT of the sample was calculated as the onset time of the exothermic peak subtracted from stabilization time (3 min) and heating time (2.5 min). All experiments were carried out in triplicate and results were reported as mean ± SD.

2.7 Physical properties of developed ingredients

2.7.1 Thermal behavior

The DSC was also used to analyze the thermal behavior of the developed ingredients following AOCS Official Method Cj 1-94 (American Oil Chemists' Society, 2011). The onset and completion temperatures for melting were measured using Proteus thermal analysis software (Netzsch-Gerätebau GmbH, Selb, Germany). Prior to experimentation microcapsule samples were wiped with filter paper to remove traces of oil or moisture on the surface. Microcapsule samples were then heated from -60 to 120 °C at a rate of 20 °C/min, according to (Willett &

Akoh, 2019b). All experiments were conducted in triplicate and results were reported as mean ± SD.

2.7.2 Rheological properties

The rheological properties of ingredients and yogurts were analyzed using an HR-2 Discovery Hybrid Rheometer (TA Instruments, New Castle, DE, USA). A parallel plate (diameter 40 mm, gap of 1 nm) was used for the measurements. Temperature was controlled with a Peltier Plate Temperature System (TA Instruments, New Castle, DE, USA). Data was collected using Trios software (TA Instruments, New Castle, DE, USA). Results were obtained and analyzed using parameters described in a previous study (Willett & Akoh, 2019a). All experiments were conducted in triplicate.

2.8 Formulation of omega-3 (ω-3) yogurt

Yogurts were made with ultra-high temperature (UHT) skim milk, yogurt starter culture, and the experimental ingredients which performed best over the initial 14-day accelerated oxidation study. For control, BF and bulk algal oil without antioxidant were used to develop yogurts. The most oxidatively stable combination of antioxidant and processing method was chosen from each processed sample type. These included the following: AO-GG, OG-GG, ME-TOC, and MEOG-GG.

Manufacturer's instructions were used to prepare yogurts with minor changes to include the incorporation of developed experimental ingredients. Initially, 100 mL of skim milk were heated to 100 °C and the ingredients were added at a 10% (w/w) concentration. 10% (w/w) concentration for the fat content was selected as it mirrored the store-bought product. After the mixture reached 100 °C it was cooled down to approximately 43 °C, at which point yogurt starter culture was added at 0.48% (w/w) according to the manufacturer's instructions. Yogurt samples

were then placed into an incubator between 40-43 °C and allowed to sit for 8 h. Once yogurt samples were set, the liquid whey on the yogurt surface was carefully removed and weighed before samples were stored at 4 °C.

2.9 Physical properties of yogurts

2.9.1. Whey separation

The spontaneous separation of whey from developed yogurts during fermentation was measured as described in previous research (Ifeduba & Akoh, 2015, Lucey, Munro, & Singh, 1998). The following formula was used to calculate the degree of whey separation:

$$\text{Whey separation (\%)} = \left(\frac{Weight\ of\ whey\ (g)}{Total\ weight\ of\ yogurt\ (g)}\right) \text{x } 100$$

2.9.2. Water holding capacity (WHC)

Water holding capacity (WHC) of yogurt samples was determined according to a method described elsewhere with minor changes (Tamjidi, Nasirpour, and Shahedi, 2012). Ten grams of yogurt was centrifuged for 20 min at 1500 rpm. The resulting supernatant (whey) was removed and weighed and WHC was calculated using the following formula:

$$\text{WHC (g/kg)} = 1000 - \left(\left[\frac{\text{Weight of whey (g)}}{\text{Total weight of yogurt (kg)}}\right] \text{x } 1000\right)$$

All tests conducted for physical properties of yogurts were completed in triplicate and were reported as mean ± SD.

2.9.3. Apparent viscosity

In addition to the rheological properties of yogurts, the apparent viscosity of developed yogurts was measured using the same HR-2 Discovery Hybrid Rheometer. Apparent viscosity of yogurts were evaluated at 25 °C over increasing shear rate (El-Messery, Aly, Lopez-Nicolas, Sanchez-Moya, & Ros, 2021). All measurements were taken in triplicate.

2.10 Oxidative stability of ω-3 yogurts

Lipid oxidation in yogurts was monitored by measuring PV and *p*-AV over 24 days of storage at 4 °C. A similar testing protocol was used for the ingredients, and testing was conducted on days 0, 6, 12, 18, and 24. On days 0, 12, and 24, qualitative analysis of the oxidation products and the relative amount of a selected malodourous compound was conducted using GC-MS with SPME protocol adapted from previous research with some adaptations for SPME protocol (Marsili & Laskonis, 2014).

A 5890 Hewlett-Packard gas chromatograph connected with a 5971 Hewlett-Packard mass spectrometer was used for the GC-MS-SPME analysis (Hewlett-Packard, Palo Alto, CA, USA). The fiber material of the SPME fiber was Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). Sample preparation consisted of 10 g of yogurt placed inside of a SPME sample vial with an agitating stir-bar heated to 60 °C for 1 h prior to exposure to the SPME fiber for adsorption of volatile components for 30 min. After adsorption the SPME fiber was placed inside the GC inlet for desorption and GC-MS analysis. A 30 m × 0.25 mm with 0.25 μm DB-5MS column (Agilent, Santa Clara, CA, USA) was used.

Initial oven temperature was 40 °C for 3 min, followed by a heating ramp at 10 °C min^{-1} to 270 °C and held for 3 min. Carrier gas consisted of helium at a flow rate of 1.0 mL min^{-1} through the column, and the inlet was set to a 5:1 split. Tentative identification of compounds was accomplished using a National Institute of Standards and Technology (NIST) library and qualitative data on amounts of selected compounds were expressed as average arbitrary area units × 10^3 ± SD and calculated based on relative peak area as conducted in previous research (Moran et al., 2022). All measurements were taken in triplicate.

2.11. Statistical analysis

Statistical analysis of results was conducted using JMP® software (version 15, SAS Institute, Inc., Cary, NC, USA). Results were expressed as mean values ± standard deviation (SD) of triplicate experiments. Tukey's honest significant difference (Tukey's HSD) test was used to determine differences between all experimental results for different sample and the level of significance ($p < 0.05$) among them.

3. Results and discussion

3.1. Microencapsulation stability

A visualization of leaching of internal phase after a 2 h period in a 37 °C oven is shown in Fig. S1. Samples which contained encapsulated oleogel showed signs of leakage, such as loss of volume and drying out, but not as obvious as in samples containing encapsulated algal oil. Samples with algal oil alone appeared to have noticeably changed colors to a burnt tint, possibly signifying that the internal phase has leached and oxidized on the outside surface. The results could be attributed to the increased stability of the oleogel within the microencapsulation and is supported by data from the EE and leaching tests. The results also indicated that the gelation alone may not be enough to stabilize AO.

Table S1 shows the results of EE and leaching for ingredients using the microencapsulation processing technique. ME and MEOG with both antioxidants are included for each sample type. Addition of antioxidants did not show a significant effect on the EE or leaching as expected. However, the difference between processing techniques was significant for both EE and % leaching. Microencapsulation of AO alone, resulted in an average EE of 90.61% and an average % leaching of 17.69%, whereas the microencapsulated oleogel presented average values of 98.16% and 8.64%, for EE and % leaching, respectively. Inclusion of gelation on algal oil prior to encapsulation increased the average EE by 7.55% and reduced leaching by 9.05%.

These results are in-line with those from previous studies that compared encapsulated bulk oils against encapsulated oleogels (Sagiri et al., 2014; Willett & Akoh, 2019b). This is possibly due to the algal oil being liquid at ambient temperature as others have observed in previous studies (Sagiri et al., 2012). As stated by others, the internal phase of microencapsulation affects the leaching as a solid internal phase is more stable and can prevent leaching as pressure builds from the alginate particles (Badve, Sher, Korde, & Pawar, 2007).

3.2. Initial accelerated oxidation

PV and *p*-AV results of different ingredients on each day are presented in Fig. 1. Changes in ω-3 FA composition for samples are shown in Table 1. The ω-3 FA composition focused on EPA and DHA contents. EPA and DHA levels were initially in line with reported values for the *Schizochytrium spp.* algal oil from the company's certificate of analysis. FA content in Table 1 is presented as normalized % and (mg/g) concentration, and data from ingredients are compared against bulk algal oil as a control group.

The OIT values determined with DSC are presented in Fig. 1 and they correlated with some exceptions with the results of PV and *p*-AV and the change in ω-3 FA composition measured with GC-FID. Bulk algal oil without antioxidant or processing exhibited an OIT value of 21.50 ± 0.29 min while the highest OIT value observed was for the ingredient (66.61 ± 3.01 min). Data from Table 1 shows that a significant difference was observed between AO and multiple ingredients by day 14 for degradation of EPA and DHA content. Every ingredient exhibited a higher normalized percentage of EPA and DHA by the end of the accelerated oxidation test than AO alone, but MEOG-TOC and MEOG-GG were significantly better for EPA and DHA. While OG, OG-GG and OG-TOC were also significantly better for protecting DHA.

This may provide evidence that oleogel with antioxidant helps to slow down oxidation progress better than microencapsulation with the same type/amount of antioxidant. However, the results of OIT may disagree and seems to suggest that microencapsulation with antioxidant protects better than gelation and antioxidant. Through the addition of antioxidant and a combination of two processing techniques the oxidative stability can be further improved. The best ingredient in terms of slowing oxidation of EPA and DHA was from MEOG-GG which contained normalized percentages of 81.64 ± 1.79 and 80.48 ± 2.42 for EPA and DHA, respectively. These findings from OIT and FA content overtime are supported by the PV and *p*-AV in Fig. 1. AO and BF were included in the PV and *p*-AV as controls. The PV of algal oil on day 14 was 15.33 ± 1.04 mmol O_2/kg of oil while BF was 8.50 ± 0.51 mmol O_2/kg of oil. The lowest PV at day 14 of any sample was that of MEOG-GG at 7.22 ± 0.53 mmol O_2/kg of oil. According to the data from PV, OIT, and FAMEs analysis the MEOG-GG sample slowed the oxidation of EPA and DHA more than others and outperformed the butterfat sample.

The results of *p*-AV were similar to algal oil and BF with a day-14 value of 264.98 ± 3.88 and 149.96 ± 4.29 abs/g, respectively. However, MEOG-GG was not lower than BF with a *p*-AV of 154.76 ± 3.11 abs/g on day-14. This value was not a statistically significant difference. The trends followed by other samples seem to suggest that the addition of antioxidant provides a stronger protective effect in preventing oxidation of FA, but the combination of processing techniques coupled with antioxidants provided the most significant advantage for slowing oxidation.

Two ingredient samples, MEOG-TOC and MEOG-GG exhibited significant differences in EPA content compared to AO alone. Five ingredient sample types exhibited significant differences against algal oil for change in DHA content, namely: OG, OG-TOC, OG-GG,

MEOG-TOC, and MEOG-GG. This result suggests that OG without antioxidant protected DHA better than MEOG without antioxidant, but the day-14 normalized value of DHA content for MEOG was lower than OG. This was only due to a larger SD. MEOG was not significantly different according to the results of Tukey's HSD test. The combination of microencapsulation and gelation had a slower oxidation rate according to OIT, PV, and *p*-AV than either gelation or microencapsulation processing technique alone.

Overall, according to the results of OIT, PV, *p*-AV, and change in FA composition, the trend in increased oxidative stability seems to be MEOG-GG > BF > MEOG-TOC > OG-GG = ME-TOC > MEOG > ME-GG > OG-TOC > AO-GG > AO-TOC > ME = OG > AO. The addition of antioxidant had greater protective effect than processing techniques without antioxidant. Based on these results the processing techniques with the best oxidative stability were selected for development of yogurt. MEOG-GG, OG-GG, ME-TOC, and AO-GG were selected along with AO and BF. GG acted as a stronger antioxidant in samples where it was expected to interact with oil phase, and TOC provided better protection in the microencapsulation samples as it may be interacting at the interface with the polar water phase better than GG. These results support the mechanism proposed by the polar paradox theory (Porter, 1980).

3.3. Physical properties of ingredients

Table 2 shows the onset and completion melting points observed for different ingredient types during analysis of thermal properties. AO was used as a control group and exhibited a melting completion temperature of approximately -3.50 ± 0.01 °C. This is to be expected as the FA content of algal oil indicated that it is in a liquid state at temperatures well below ambient conditions. Gelation was successful in converting algal oil into a solid at ambient temperatures,

and OG exhibited a melting completion temperature at approximately 58.58 ± 0.42 °C. However, microencapsulation may have been more useful in developing a solid fat ingredient under ambient conditions as ME exhibited a melting completion temperature of approximately 68.62 ± 3.19 °C. MEOG had the highest melting completion temperature at 71.41 ± 2.29 °C but was not significantly different from ME sample type. However, ME and MEOG both had significantly higher melting completion temperatures than OG, but OG was significantly higher than AO.

These results agreed with the rheological data shown in Fig. S2. Within Fig. S2 the cross point between the two lines is called the cross-over modulus and is indicative of a phase change state with rubbery or pseudo-elastic properties. The cross point of OG occurred at approximately 49.39 °C which falls just short of the exhibited melting completion point shown in Table 2. The cross point for ME sample was approximately 69.19 °C and the cross-point of MEOG was not reached before the final temperature. These values agree with the results of the thermal properties shown in Table 2, and they provide evidence to support the theory that a combination of processing techniques may help in achieving a more physically stable product. The rheology of BF was used as a control and exhibited a cross-point at 24.49 °C. These results show that the developed ingredients from the selected processing techniques enhanced the physical stability of solid fat ingredients from bulk liquid algal oil. They had a higher melting range than typical BF. This allows for the use of these novel ingredients in yogurts in place of traditional saturated fats.

3.4 Physicochemical properties of developed yogurts

3.4.1 Physical properties of yogurts

The separation of whey from yogurt, also known as wheying-off, is defined as the loss of whey from the network which becomes visible as surface whey on top of yogurt. The resulting visible whey is considered a source of negative consumer opinion as it leads consumers to believe a

product is deficient (Lee & Lucey, 2010). Spontaneous whey separation is related to an unstable network and can be due to an increase in the rearrangements of the gel matrix, induced by damage to a weak gel network, or by the instability of internal matrix caused by ingredients (Lucey, Munro, & Singh, 1998). Data from Table 3 shows the WHC and whey separation for the yogurts made with selected ingredients. Algal oil alone in yogurt showed the largest amount of % whey separation, at approximately 2.06 ± 0.12%, which is expected due to the physical state of algal oil.

The gelation and microencapsulated ingredients used in yogurt lowered the whey separation to 1.41 ± 0.11% and 0.97 ± 0.11%, respectively. Microencapsulation was more efficient in preventing the loss of lipids than gelation, but it was not significantly different than BF, which exhibited a whey separation of 0.72 ± 0.15%. However, the combination of both processing techniques (gelation and microencapsulation) did provide a significantly lower whey separation. Indeed, MEOG yogurt exhibited the lowest value of whey loss at 0.46 ± 0.09%. These results were similar to the WHC results shown in Table 3.

WHC can be used to determine the mechanical stability of yogurt under stress. The higher the WHC of a given sample, the higher the physical stability of yogurt. Again, algal oil alone had the lowest results at 532.93 ± 21.57 g/kg while BF as an ingredient exhibited the highest WHC at 740.22 ± 20.49 g/kg. Gelation, microencapsulation, and a combination of the two processing techniques all showed significantly improved WHC over algal oil alone. MEOG had the highest WHC among the developed ingredients at 712.41 ± 13.64 g/kg. While this result was lower than the WHC of BF yogurt, it wasn't significantly different. This observation supports the results seen in the % whey separation where MEOG sample may be able to act as a lipid ingredient with a physical stability similar to BF or other saturated fat sources.

Fig. 2 and Fig. S2 show the viscosity and the rheology of yogurt samples, respectively. In Fig. 2 the viscosity of yogurts is shown as a logarithmic scale of viscosity (Pa·s) over a changing shear rate (1/s). As the profile for the viscosity is similar in every yogurt sample, it's important to focus on the starting viscosity at the starting shear rate. The viscosity of yogurt products would be lowered in physically unstable samples compared to more physically stable samples. The highest viscosities observed were from the MEOG yogurt, BF yogurt, and the store-bought yogurt, at 3.42, 3.39, and 2.76 Pa·s, respectively. These results show that the MEOG can be used to develop yogurt product with comparable viscosities as saturated fat sources or products currently on the market.

The combination effect that processing techniques have on the use of algal oil as a lipid ingredient can be seen in the starting viscosity of other samples. Algal oil alone and algal oil with antioxidant exhibited almost identical starting viscosities, 0.852 and 0.873 Pa·s, which are to be expected since the antioxidant would have little effect on the physical properties of agal oil. The use of processing techniques increased the starting viscosities higher than oil without processing as ME and OG starting values were 1.506 and 2.738 Pa·s, respectively. Overall BF and MEOG exhibited the highest starting viscosities. This further supports the idea that the combination of two processing techniques could be used to develop an ω-3 FA-rich lipid ingredient that is comparable to traditional saturated fats in physical characteristics.

The rheological profiles of yogurts shown in Fig. S2 suggest that the physical differences in ingredients may have an effect on the rheology of developed products. While the rheological profiles for developed yogurts and the store-bought product were similar, there were some noticeable differences in each processing technique. Algal oil alone had the lowest starting loss and storage moduli, and over the course of increasing temperatures the two moduli were closer

than any other sample. This may show that the physical stability of algal oil yogurt was lower than every other, sample type tested. While ME and OG samples exhibited similar profiles to each other it may be important to note that the starting values were still low compared to MEOG, BF, and store-bought yogurt. MEOG and BF were similar, and both exhibited a wider range of variation in multiple runs, as shown by the larger SD bars, compared to the store-bought product.

This may be evidence of better quality control that the market product has than the laboratory developed yogurt products herein, but the similar rheological profiles and viscosity data indicate that a combination of processing techniques can be effective enough to replace saturated fat in a food product such as yogurt. This is supported by the improvement over algal oil alone and the similarity to BF seen in WHC and % whey separation by ME, OG, and MEOG ingredients. The combination of processing techniques and MEOG, can be used to replace saturated fat in a yogurt product, and could potentially replace saturated fats in other food, pharmaceutical, or cosmetic products.

3.4.2 Chemical properties of yogurts

The results of PV and *p*-AV over the course of 24 days storage at 4 °C for developed yogurts and store-bought product can be seen in Fig.1. The highest PV from yogurts came from AO which exhibited a PV of 14.01 ± 0.98 mmol O_2/kg of oil on day 24, whereas the lowest PV was from the store-bought product at 6.83 ± 0.57 mmol O_2/kg of oil. MEOG-GG was the next lowest with a PV of 7.17 ± 0.76 mmol O_2/kg of oil. While MEOG-GG did not perform as well as the store-bought product it was the closest and was not a statistically different. Yogurt developed with BF was less oxidatively stable than the MEOG-GG yogurt with a PV of 8.17 ± 0.29 mmol O_2/kg of oil at the end of storage.

AO without processing but with added antioxidant, AO-GG, did show a significant improvement over AO alone for PV. It was not as effective as combining processing techniques with antioxidants. MEOG-GG was the highest performing combination among the developed ingredients used in yogurt products. These results were similar to those seen for the *p*-AV of yogurt products as well. However, one difference that was noticeable was that ME-TOC yogurt product gave the highest *p*-AV value even more than algal oil alone. ME-TOC had a *p*-AV of 209.84 ± 1.49 absorbance/g while algal oil alone exhibited a *p*-AV of 188.29 ± 2.04 absorbance/g on day 24.

MEOG-GG, once again, had the best oxidative stability of ingredient samples with a *p*-AV of 118.85 ± 0.98 abs/g and was close to identical when compared with store-bought product, which exhibited a *p*-AV of 117.95 ± 4.13 abs/g. The general trends for PV and *p*-AV results observed in yogurt samples agreed with the results of the GC-MS-SPME shown in Table 4. Tentative identification was completed with NIST database to identify oxidation products including the volatile malodourous compound, 1-penten-3-one, that was previously identified as a source of fishy smell with negative consumer perception (Grosch, 1987; Marsili & Laskonis, 2014)

Along with 1-penten-3-one, 4-hydroxy-2-nonenal (4-HNE), and 4-hydroxy-2-hexanal (4-HHE) were also tentatively identified. These compounds have been observed previously as oxidation products for highly unsaturated FA (Ismail, Bannenberg, Rice, Schutt, & MacKay, 2016). Algal oil yogurt without antioxidant contained the highest amount of the three volatile compounds at the end of the 24-day period, with 7.72 ± 0.74, 21.15 ± 2.19, and 19.97 ± 2.61 (relative abundance × 10^3) for 1-penten-3-one, 4-HNE, and 4-HHE, respectively. A significant difference in malodorous content for yogurts with developed ingredients can be noticed at the

start of the storage period, which supports previous results stating that microencapsulation or other processing techniques may also provide malodorous content masking qualities (Bakry et al., 2016).

The content of 4-HNE and 4-HHE over the 24-day storage period was significantly lower for the best performing sample, MEOG-GG, compared with the AO yogurt. Results of MEOG-GG yogurt on day 24 were 4.07 ± 0.43, 14.56 ± 1.61, and 13.67 ± 1.32 (relative abundance × 10^3) for 1-penten-3-one, 4-HHE, and 4-HNE, respectively. This evidence supports the data from PV and *p*-AV of yogurts that the combination of gelation and microencapsulation with antioxidant addition could be used to successfully develop an ω-3 PUFA-rich ingredient which could be used to replace saturated fats in food products.

4. Conclusions

Using a combination of processing techniques with the addition of antioxidants, an ω-3 PUFA-rich food ingredient was produced and compared with butterfat for physicochemical characteristics. The rheology and thermal properties results showed that a combination of oleogelation and microencapsulation gave a product with similar physical properties to BF. Combining processing techniques and addition of antioxidants resulted in a highly improved and oxidatively stable ingredient. The accelerated oxidation study measured with PV, *p*-AV, fatty acid profile, and OIT revealed that the highest performing ingredient, MEOG-GG, was significantly different from BF in terms of oxidative stability. Yogurts developed with optimal ingredients for each processing type exhibited rheological properties and viscosities similar to a yogurt developed with BF and a store-bought yogurt. The oxidative stability of developed yogurts showed improvement over yogurt with bulk AO alone, and the highest performing yogurt samples were not significantly different from yogurt produced with BF and store-bought

yogurt. Overall, MEOG-GG achieved results which were either significantly better or similar to an on the market yogurt product, which used saturated fat as the lipid source. The results of our study indicate that a combination of processing techniques with the addition of antioxidants has potential to produce ω-3 FA-rich lipid ingredients that can replace traditional saturated FA sources in foods, cosmetics, and pharmaceuticals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution Statement

J.H. Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft; S.H. Investigation, Writing - review & editing; C. A. Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition. All authors contributed to and approved the final draft of the manuscript.

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Tables

Table 5.1 Changes in ω-3 fatty acid composition of ingredient samples with bulk algal oil as control.

Time (day)	Sample	EPA		DHA	
		Concentration (mg/g)	Normalized %	Concentration (mg/g)	Normalized %
0	AO	65.53 ± 1.40	100	356.43 ± 5.74	100
	AO-TOC	65.43 ± 1.75	100	353.17 ± 5.96	100
	AO-GG	65.12 ± 1.02	100	351.36 ± 6.25	100
	OG	62.32 ± 3.21	100	326.22 ± 6.11	100
	OG-TOC	63.12 ± 2.84	100	327.68 ± 7.58	100
	OG-GG	63.65 ± 2.16	100	325.34 ± 7.19	100
	ME	62.12 ± 1.85	100	331.28 ± 7.28	100
	ME-TOC	62.65 ± 2.03	100	333.46 ± 6.71	100
	ME-GG	63.45 ± 1.52	100	332.49 ± 6.37	100
	MEOG	63.19 ± 0.98	100	324.35 ± 7.94	100
	MEOG-TOC	64.87 ± 1.76	100	322.16 ± 6.23	100
	MEOG-GG	63.72 ± 2.98	100	326.51 ± 7.16	100
4	AO	61.76 ± 1.66	94.25 ± 2.53[a]	316.87 ± 11.84	88.90 ± 3.32[a]
	AO-TOC	62.06 ± 1.97	94.85 ± 3.01[a]	321.52 ± 9.52	91.04 ± 2.69[a]
	AO-GG	62.97 ± 1.43	96.69 ± 2.19[a]	323.18 ± 10.23	91.98 ± 2.91[a]
	OG	60.84 ± 1.01	97.63 ± 1.62[a]	304.63 ± 7.17	93.38 ± 2.19[a]
	OG-TOC	60.91 ± 2.08	96.49 ± 3.29[a]	308.71 ± 11.71	94.21 ± 3.57[a]
	OG-GG	61.64 ± 2.25	96.84 ± 3.53[a]	311.46 ± 10.97	95.73 ± 3.37[a]
	ME	60.12 ± 3.12	96.78 ± 5.02[a]	301.22 ± 12.64	90.93 ± 3.99[a]
	ME-TOC	60.84 ± 2.41	97.11 ± 3.84[a]	304.98 ± 14.39	91.46 ± 4.32[a]
	ME-GG	61.55 ± 1.59	97.01 ± 2.51[a]	305.74 ± 8.15	91.96 ± 2.45[a]
	MEOG	61.12 ± 2.45	96.72 ± 3.88[a]	309.26 ± 13.57	95.34 ± 4.18[a]
	MEOG-TOC	61.85 ± 2.84	95.34 ± 4.38[a]	314.52 ± 11.76	96.67 ± 3.65[a]
	MEOG-GG	62.16 ± 2.47	97.56 ± 3.88[a]	317.28 ± 10.52	97.17 ± 3.22[a]
7	AO	49.51 ± 2.42	75.55 ± 3.69[a]	288.27 ± 6.16	80.88 ± 1.73[c]
	AO-TOC	52.28 ± 2.36	79.90 ± 3.61[a]	290.63 ± 12.75	82.29 ± 3.61[b,c]
	AO-GG	53.79 ± 2.49	82.60 ± 3.82[a]	292.71 ± 9.54	83.31 ± 2.72[b,c]
	OG	59.17 ± 4.58	94.95 ± 7.35[a]	291.14 ± 5.47	89.25 ± 1.67[a,b,c]
	OG-TOC	60.82 ± 4.38	96.06 ± 6.94[a]	296.26 ± 10.82	90.41 ± 3.31 [a,b,c]
	OG-GG	61.14 ± 5.24	94.94 ± 8.24[a]	301.73 ± 11.78	92.74 ± 3.62 [a,b,c]
	ME	56.61 ± 6.16	91.13 ± 9.92[a]	285.41 ± 8.19	86.15 ± 2.47 [a,b,c]
	ME-TOC	57.81 ± 5.14	92.27 ± 8.20[a]	291.15 ± 15.81	87.31 ± 4.74 [a,b,c]

	ME-GG	58.64 ± 5.39	92.42 ± 8.49[a]	294.77 ± 13.64	86.15 ± 4.11 [a,b,c]
	MEOG	58.79 ± 4.82	93.03 ± 7.63[a]	298.27 ± 9.96	91.96 ± 3.08[a,b]
	MEOG-TOC	60.44 ± 5.27	93.17 ± 8.12[a]	303.82 ± 9.84	94.31 ± 3.05[a]
	MEOG-GG	61.77 ± 5.67	96.94 ± 8.89[a]	308.91 ± 8.53	94.61 ± 2.61[a]
10	AO	44.27 ± 4.84	67.56 ± 7.39[b]	260.41 ± 10.74	73.06 ± 3.01[c]
	AO-TOC	50.42 ± 3.95	77.06 ± 6.04[a,b]	264.87 ± 6.93	74.99 ± 1.96[c]
	AO-GG	53.63 ± 3.14	82.36 ± 4.82[a,b]	269.29 ± 9.71	76.67 ± 2.76[b,c]
	OG	51.19 ± 4.42	82.14 ± 7.09[a,b]	275.72 ± 11.52	84.52 ± 3.53[a]
	OG-TOC	52.96 ± 3.28	83.90 ± 5.17[a,b]	279.91 ± 9.14	85.42 ± 2.79[a]
	OG-GG	54.47 ± 3.37	85.58 ± 5.29[a,b]	283.11 ± 6.75	87.02 ± 2.07[a]
	ME	48.48 ± 4.74	78.04 ± 7.63[a,b]	274.13 ± 8.21	82.75 ± 2.48[a,b]
	ME-TOC	50.13 ± 4.61	80.02 ± 7.36[a,b]	277.87 ± 5.43	83.33 ± 1.63[a,b]
	ME-GG	52.21 ± 5.34	82.29 ± 8.42[a,b]	279.24 ± 7.74	83.98 ± 2.33[a,b]
	MEOG	52.94 ± 5.19	83.78 ± 8.21[a,b]	277.81 ± 10.67	85.65 ± 3.29[a]
	MEOG-TOC	55.17 ± 3.87	85.05 ± 5.97[a,b]	279.68 ± 7.52	86.81 ± 2.33[a]
	MEOG-GG	56.69 ± 3.42	88.97 ± 5.37[a]	285.19 ± 8.51	87.34 ± 2.61[a]
14	AO	41.62 ± 2.22	63.51 ± 3.38[c]	242.14 ± 12.52	67.93 ± 3.51[d]
	AO-TOC	43.35 ± 3.19	66.25 ± 4.87[b,c]	246.87 ± 10.90	69.90 ± 3.09[c,d]
	AO-GG	47.26 ± 2.61	72.57 ± 4.01 [a,b,c]	250.79 ± 11.32	71.38 ± 3.22[b,c,d]
	OG	43.97 ± 2.72	69.66 ± 4.63 [a,b,c]	251.83 ± 9.05	77.20 ± 2.77[a,b,c]
	OG-TOC	45.83 ± 3.02	72.61 ± 4.78 [a,b,c]	254.96 ± 10.77	77.80 ± 3.29[a,b,c]
	OG-GG	48.09 ± 3.30	75.55 ± 5.18 [a,b,c]	257.64 ± 6.96	79.19 ± 2.14[a,b]
	ME	42.04 ± 1.59	67.68 ± 2.55 [a,b,c]	246.31 ± 8.34	74.35 ± 2.52[a,b,c]
	ME-TOC	45.71 ± 2.43	72.96 ± 3.87 [a,b,c]	255.33 ± 9.87	76.57 ± 2.96[a,b,c,d]
	ME-GG	44.02 ± 3.27	69.66 ± 5.15 [a,b,c]	248.06 ± 10.35	74.61 ± 3.11[a,b,c,d]
	MEOG	45.43 ± 2.33	71.89 ± 3.68[a,b,c]	247.89 ± 11.51	76.42 ± 3.55[a,b,c,d]
	MEOG-TOC	50.12 ± 2.12	77.26 ± 3.27[a,b]	255.21 ± 10.12	79.22 ± 3.17[a,b]
	MEOG-GG	52.02 ± 1.14	81.64 ± 1.79[a]	262.78 ± 7.92	80.48 ± 2.42[a]

[a,b,c,d] Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Table 5.2 Thermal behavior of different processing sample types with algal oil as control.

Sample	Onset (°C)	Completion (°C)	Peak Enthalpy (mW/mg)
AO	-43.40 ± 1.61^{c}	-3.50 ± 0.01^{c}	0.23 ± 0.02^{b}
OG	24.98 ± 2.03^{b}	58.58 ± 0.42^{b}	0.36 ± 0.02^{b}
ME	52.62 ± 4.06^{a}	68.62 ± 3.19^{a}	1.20 ± 0.15^{a}
MEOG	57.09 ± 1.05^{a}	71.41 ± 2.29^{a}	1.47 ± 0.24^{a}

[a,b,c] Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

Table 5.3 Physical properties for developed yogurt products with butterfat and algal oil as controls.

Sample	Water holding capacity (WHC, g/kg)	Whey separation (%)
BF	740.22 ± 20.49^{a}	0.72 ± 0.15^{c}
AO	532.93 ± 21.57^{c}	2.06 ± 0.12^{a}
OG	610.76 ± 18.80^{b}	1.41 ± 0.07^{b}
ME	637.28 ± 11.71^{b}	0.97 ± 0.11^{c}
MEOG	712.41 ± 13.64^{a}	0.46 ± 0.09^{d}

[a,b,c] Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation

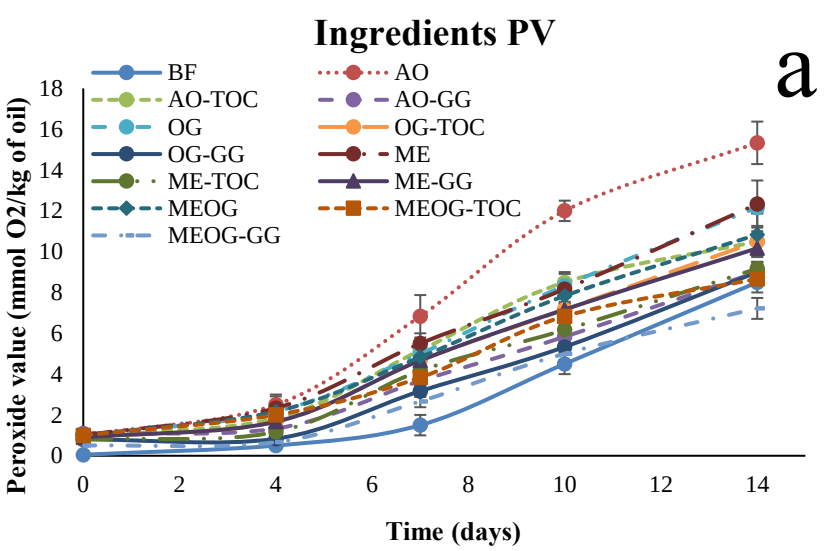
Table 5.4 Analysis of malodorous compound and oxidation products with developed yogurts using GC-MS-SPME

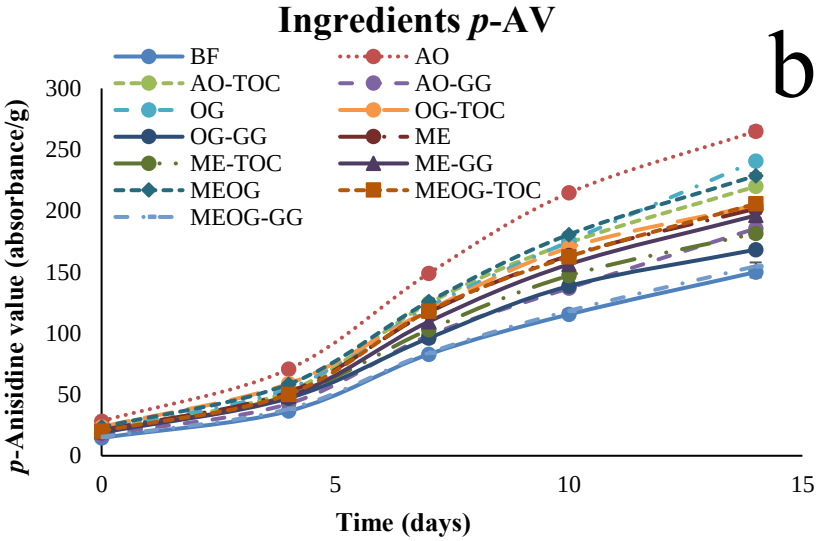
Time (days)	Sample	Compounds[1]		
		1-penten-3-one	4-hydroxy-2-hexanal (4-HHE)	4-hydroxy-2-nonenal (4-HNE)
0	AO	3.62 ± 0.31^{a}	5.32 ± 0.62^{a}	4.83 ± 0.5^{a}
	AO-GG	2.81 ± 0.25^{b}	5.67 ± 0.68^{a}	4.54 ± 0.55^{a}
	OG-GG	$2.42 \pm 0.28^{b,c}$	5.01 ± 0.63^{a}	4.70 ± 0.48^{a}
	ME-TOC	$2.12 \pm 0.22^{b,c}$	5.12 ± 0.69^{a}	4.72 ± 0.62^{a}
	MEOG-GG	2.01 ± 0.25^{c}	4.94 ± 0.66^{a}	4.78 ± 0.40^{a}
12	AO	5.63 ± 0.62^{a}	12.06 ± 1.03^{a}	13.77 ± 1.37^{a}
	AO-GG	$4.98 \pm 0.51^{a,b}$	$10.78 \pm 1.12^{a,b}$	$11.21 \pm 1.19^{a,b}$
	OG-GG	$4.86 \pm 0.56^{a,b}$	9.32 ± 1.06^{b}	9.98 ± 1.15^{b}
	ME-TOC	3.89 ± 0.40^{b}	$10.11 \pm 0.94^{a,b}$	$11.46 \pm 0.86^{a,b}$
	MEOG-GG	3.77 ± 0.43^{b}	8.19 ± 0.92^{b}	8.40 ± 0.92^{b}
24	AO	7.72 ± 0.74^{a}	21.15 ± 2.19^{a}	19.97 ± 2.61^{a}
	AO-GG	5.78 ± 0.53^{b}	$16.87 \pm 1.79^{a,b}$	$16.02 \pm 1.89^{a,b}$
	OG-GG	$5.34 \pm 0.56^{b,c}$	15.44 ± 1.68^{b}	14.84 ± 1.74^{b}
	ME-TOC	4.16 ± 0.48^{c}	19.68 ± 0.66^{a}	17.73 ± 1.02^{a}
	MEOG-GG	4.07 ± 0.43^{c}	14.56 ± 1.61^{b}	13.67 ± 1.32^{b}

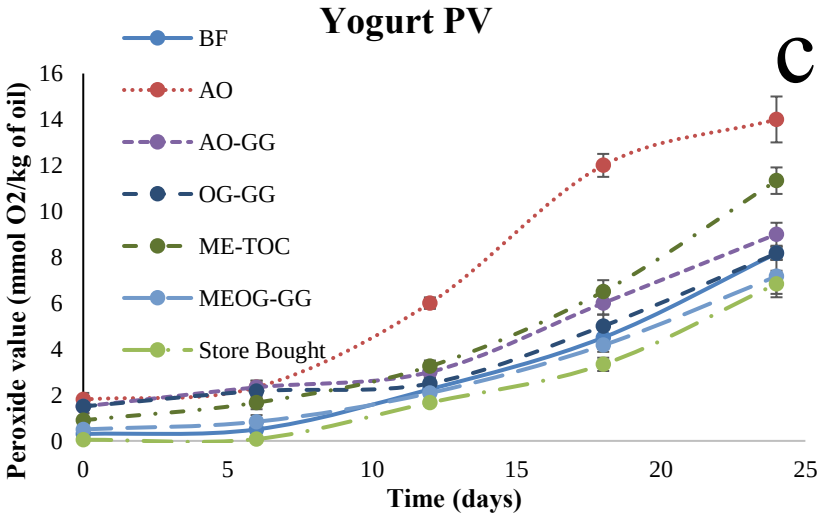
[1]Relative abundance (arbitrary area units $\times 10^3$) of tentatively identified volatile compounds

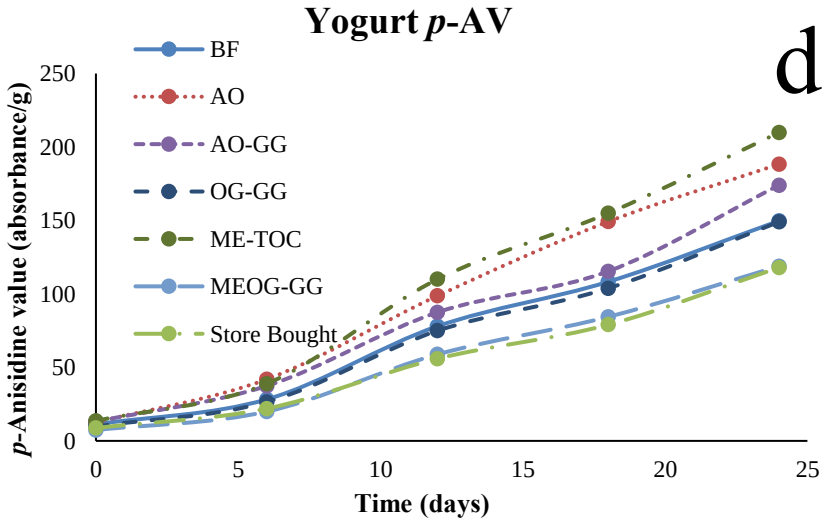
^{a,b,c} Different letters indicate significant statistical difference at $p < 0.05$

Results are expressed as means (n=3) ± standard deviation


Ingredients PV
a
BF
AO
AO-TOC
AO-GG
OG
OG-TOC
OG-GG
ME
ME-TOC
ME-GG
MEOG
MEOG-TOC
MEOG-GG
Peroxide value (mmol O2/kg of oil)
Time (days)


Ingredients p-AV
b
BF
AO
AO-TOC
AO-GG
OG
OG-TOC
OG-GG
ME
ME-TOC
ME-GG
MEOG
MEOG-TOC
MEOG-GG
p-Anisidine value (absorbance/g)
Time (days)


Yogurt PV
c
BF
AO
AO-GG
OG-GG
ME-TOC
MEOG-GG
Store Bought
Peroxide value (mmol O2/kg of oil)
Time (days)


Yogurt p-AV
d
BF
AO
AO-GG
OG-GG
ME-TOC
MEOG-GG
Store Bought
p-Anisidine value (absorbance/g)
Time (days)

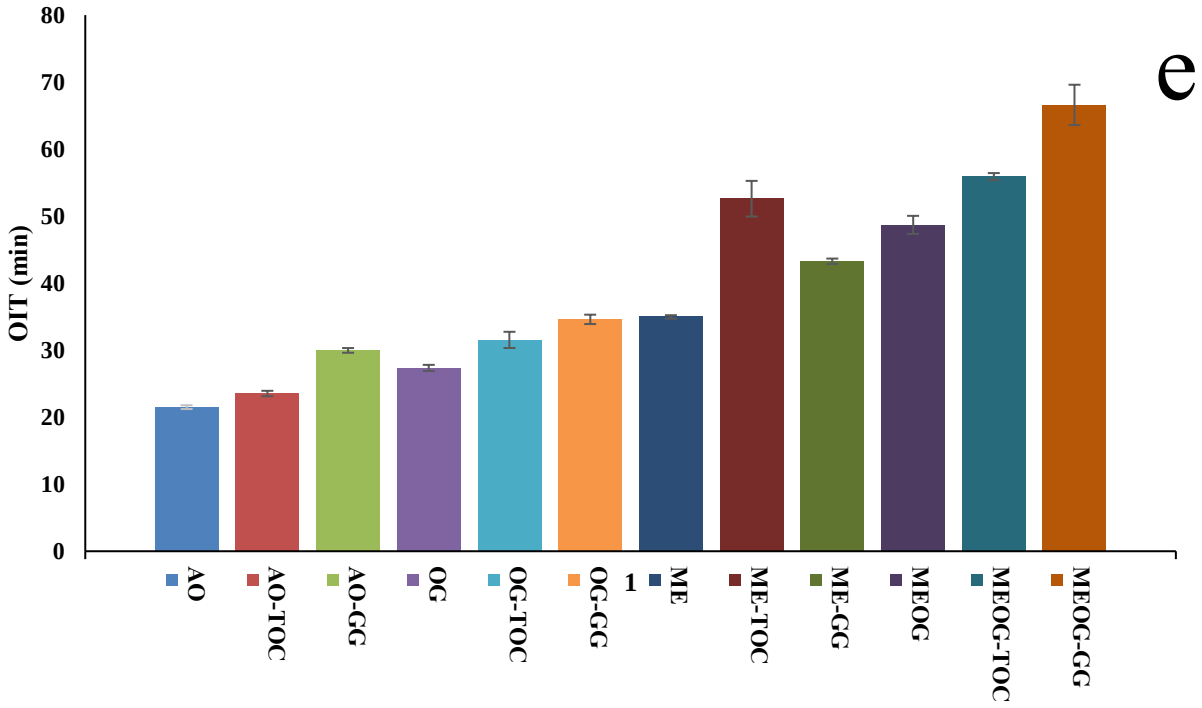

e
OIT (min)
80
70
60
50
40
30
20
10
0
AO
AO-TOC
AO-GG
OG
OG-TOC
OG-GG
1
ME
ME-TOC
ME-GG
MEOG
MEOG-TOC
MEOG-GG

Fig. 5.1

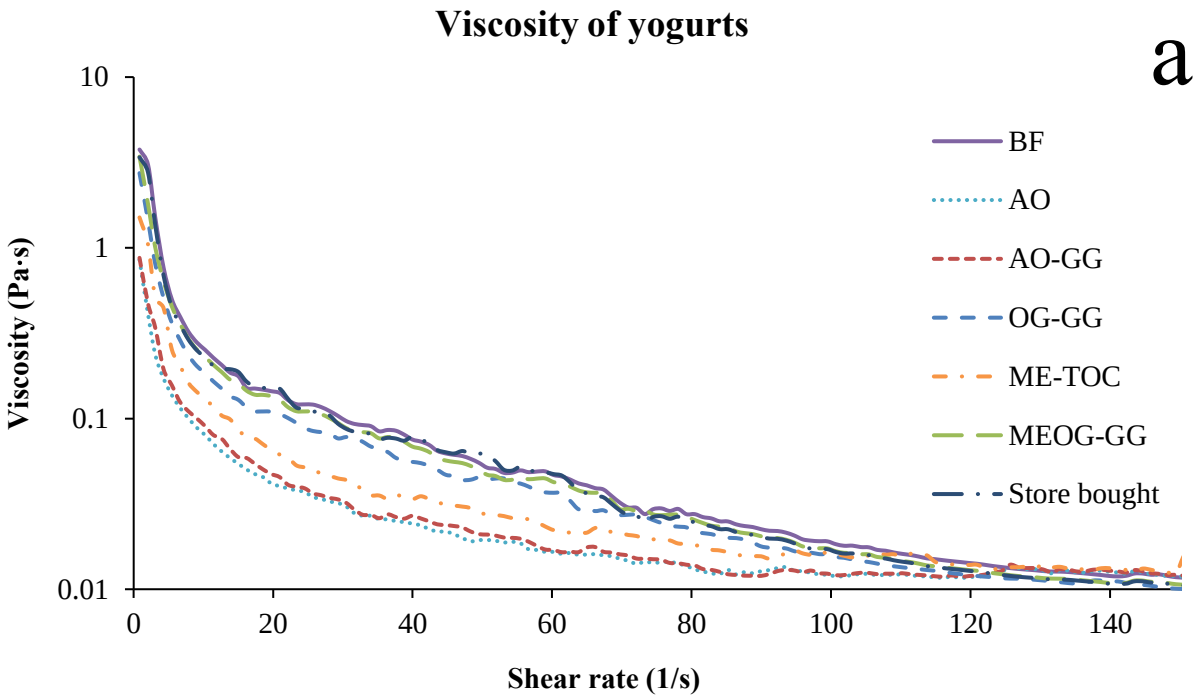

Viscosity of yogurts
a
10
1
0.1
0.01
Viscosity (Pa·s)
0
20
40
60
80
100
120
140
Shear rate (1/s)
BF
AO
AO-GG
OG-GG
ME-TOC
MEOG-GG
Store bought

Fig. 5.2

CHAPTER 6

CONCLUSIONS

The efficacy of different antioxidants and combinations were evaluated in both bulk soybean oil and O/W emulsions. The hydrophilic (1-o-galloylglycerol, GG) and the more lipophilic (tocopherol mixture, TOC) antioxidants performed best in lipophilic media and hydrophilic media, respectively. The effects of gelation on the physicochemical properties of *Schizochytrium sp.* algal oil were evaluated. Gelation was shown to improve the physical characteristics of a liquid bulk oil for possible use as a saturated fat replacement. Lipid oxidation was slower with gelation than bulk oil alone. The antioxidants which previously performed best (GG and TOC) were used in combination with microencapsulation and gelation processing techniques to develop ingredients which could be utilized in products. Experimental yogurts were developed with these ingredients and they showed oxidation rates similar to a commercial store-bought yogurt product.

The highest performing antioxidant in bulk soybean oil was GG. After an accelerated oxidation period of 30 days at 90 °C, bulk soybean oil with GG had a TOTOX value of 206.82 compared to bulk oil alone at 232.09. TOC performed best in O/W emulsions with a TOTOX value at the end of accelerated oxidation of 196.72 compared to O/W emulsion alone at 352.88. Gelation improved the physical characteristics of *Schizochytrium sp.* algal oil and a 12% (w/w) gel using monolaurin as gelator (12% M) improved the SFC enough so it's similar to butterfat (BF) at similar temperatures (4.5% SFC at 30 ℃). Additionally, gelation improved the melting point of *Schizochytrium sp.* algal oil (59.38 ℃ with 12% M) and became solid at ambient temperatures

(≈ 20 – 24 °C). The chemical characteristics of algal oil were also improved after gelation with slower oxidation rates.

Oleogels and emulsion gels (emulgels) were prepared with monlaurin.12% M oleogel and emulgels had much lower PV and *p*-AV at the end of accelerated oxidation. The PV and *p*-AV of algal oil after 14 days were 12.13 ± 0.29 mmol O_2/kg of oil and 227.32 ± 1.57 absorbance/g, respectively, while the PV and *p*-AV for emulsion were 14.45 ± 0.321 mmol O_2/kg of oil and 268.12 ± 5.07 absorbance/g, respectively. 12% M had PV and *p*-AV values of 8.92 ± 0.30 mmol O_2/kg of oil and 182.15 ± 3.29 absorbance/g for oleogel and 11.81 ± 0.28 mmol O_2/kg for oil and 227.42 ± 4.03 absorbance/g for emulgel. Ingredients were developed using combinations of GG and TOC as antioxidants with microencapsulation and gelation processing techniques. The combination of GG with microencapsulation of a developed gel (MEOG-GG) was the highest performing ingredient with the slowest oxidation rate and desirable physical traits.

MEOG-GG was used with a yogurt starter culture and skim milk to develop a yogurt that outperformed yogurt made with BF and was similar to store-bought yogurt product. Store-bought yogurt exhibited a PV 6.83 ± 0.57 mmol O_2/kg while MEOG-GG was 7.17 ± 0.76 mmol O_2/kg at the end of a 24-day storage period at 4 °C. This trend was similar to that seen with *p*-AV as MEOG-GG had *p*-AV of 118.85 ± 0.98 abs/g while store-bought yogurt exhibited a *p*-AV of 117.95 ± 4.13 abs/g at the end of 24 days of storage. These results indicate that a combination of processing techniques with the addition of antioxidants has potential to produce ω-3 FA-rich lipid ingredients that can replace traditional saturated FA sources in foods products.

Suggestions for future work:

1. Incorporate more antioxidant compounds of different varieties (coumarins, benzimidazoles, flavones, phenols, and anthocyanins) in combination with processing techniques such as gelation and microencapsulation with different ω-3 rich FA sources (salmon oil, menhaden oil, cod liver oil, chia seed oil, flaxseed oil, and walnut oil) to determine their efficacy in preventing lipid oxidation in a variety of ingredients and food products.
2. Utilize newly developed ingredients in multiple food product types to measure efficacy in preventing lipid oxidation and include sensory evaluations in experimental design.