Characterization and comparison of oleogels and emulgels prepared from Schizochytrium algal oil using monolaurin and MAG/DAG as gelators

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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.

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 also studied to determine if any protective effect was achieved. 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$^{(r)}$ 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 decreased concentrations as well.

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 dynes/cm, 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 degC 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$^{(r)}$ Polystat$^{(r)}$ digital refrigerated circulating water bath (Cole-Parmer, Vernon Hills, IL, USA) set at 1 degC. 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 degC (+- 0.1 degC).

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 emulsion. 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 degC 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 degC, 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). The TOTOX value on each given day was calculated using PV and *p* AV. PV and *p* AV tests were conducted in triplicate, and all reagents were prepared fresh on the day of analysis. TOTOX 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 FAMEs of 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 emulgel 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 standard curve with heptadecanoic acid (C17:0).

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 degC, 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-Geratebau 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 degC and 60 degC at intervals of 5 degC. 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 degC (+- 0.1 degC) for 10 min. Heating–cooling sweeps were performed between the temperature of 0 – 60 degC at a rate of 2 degC 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 degC and adding 1 drop of melted sample between a stationary and moving glass plate. The samples were then crystallized by storing at 4 degC overnight. Images were captured using an iDu Optics$^{(r)}$ 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 degC until analysis. XRD operating conditions included Co Kα radiation ($\lambda = 1.79037$ 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

TOTOX values were calculated for oleogel and emulgel samples by combining PV and *p* AV and reported because they better represent the overall oxidation status of oleogel and emulgel samples. These TOTOX values 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.

The OIT values determined with DSC are found in Table 2 and correlate with the results on TOTOX values 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 TOTOX results as well as the change in ω-3 FA content in Figure 1 and Table 1, respectively. The highest TOTOX value was from algal oil and

emulsion without gelator for both the oleogel and emulgel comparisons, respectively. The TOTOX value of algal oil after 14 days was 251.32 ± 1.25 while the TOTOX value for emulsion was 297.08 ± 2.69. In both sample types the lowest TOTOX value after 14 days was the 12% (w/w) monolaurin sample gel, with a value of 202.07 ± 2.33 for oleogel and 251.53 ± 1.79 for emulgel. The highest average decrease in TOTOX value for algal oil was approximately 19.59% while the average decrease in TOTOX value of emulsion was approximately 15.33%.

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, TOTOX, 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. 1 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.

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 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 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 needle like morphology is why MAG/DAG oleogels exhibited a higher physical stability.

The smaller more needle like structure of MAG/DAG 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. 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.

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Figure captions

Fig. 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

Fig. 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 EG

Fig. 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 C

Fig. 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

Fig. 5 Polarized light micrographs taken at 0 C 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

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