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Effect of Emulsifier on Oxidation Properties of Fish Oil-Based Structured Lipid Emulsions

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The effects of the emulsifiers lecithin, Tween 20, whey protein isolate, mono-/diacylglycerols, and sucrose fatty acid ester on oxidation stability of a model oil-in-water emulsion prepared with enzymatically synthesized menhaden oil−caprylic acid structured lipid were evaluated. Oxidation was monitored by measuring lipid hydroperoxides, thiobarbituric acid reactive substances, and the ratio of combined docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents to palmitic acid in the emulsion. After high-pressure homogenization, all emulsions, except those prepared with lecithin, had similar droplet size distributions. All structured lipid emulsions, except for the lecithin-stabilized emulsions, were stable to creaming over the 48-day period studied. Emulsifier type and concentration affected oxidation rate, with 0.25% emulsifier concentration generally having a higher oxidation rate than 1% emulsifier concentration. Overall, oxidation did not progress significantly enough in 48 days of storage to affect DHA and EPA levels in the emulsion.

KEYWORDS: Caprylic acid; emulsions; menhaden oil; oil droplet sizes; oxidation properties; structured lipid

INTRODUCTION

There is currently a high level of interest in fish oils because of their purported health benefits. Most of the benefits of fish oils can be attributed to their high docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents. Fish oils play a role in the reduction of thrombotic tendencies and hypertriacylglycerolemia (*1*, *2*). DHA levels in both the brain and the retina are crucial for proper nervous system and vision development (*3*, *4*). This is especially true in infant development, where it has been shown that sight develops slowly in infants fed conventional DHA-free infant formula (*4*, *5*). However, when premature and bottle-fed babies were given a diet enriched with DHA, their sight developed as satisfactorily as that of breast-fed babies (*5*). EPA is believed to have several health benefits on cardiovascular disease, immune disorders, inflammation, allergies, and diabetes (*6*, *7*). Due to their beneficial health properties, fish oils are highly desirable as substrates for structured lipid (SL) production.

Structured lipids containing a combination of beneficial long-chain fatty acids (LCFA) and medium-chain fatty acids (MCFA) show promise in fully optimizing the benefits of fatty acid substrates. MCFA such as caprylic acid are beneficial because they are not readily re-esterified into triacylglycerols, have higher plasma clearance, and are readily oxidized and utilized as fuel and energy (*8*). These properties make them less likely to be stored as fat and also well suited for infants and stressed adults. Menhaden oil contains a high percentage of polyunsaturated fatty acids (PUFA), mostly at the *sn*-2 position. Transesterification of caprylic acid and menhaden oil using a 1,3-specific lipase results in an SL with DHA and EPA mostly at the more easily absorbed *sn*-2 position and caprylic acid at the *sn*-1 and -3 positions (*9*).

One of the main problems faced in the application of fish oils to food is their high susceptibility to oxidation. Studies done on the oxidative stability of menhaden oil-based SL have shown them to be less stable to oxidation than unmodified menhaden oil (*10*). However, most of this research has focused on their oxidative properties as bulk oils rather than in oil droplets dispersed in an aqueous phase. As lipids exist in many food systems in the form of oil-in-water emulsions, it is important to examine their oxidation properties in this medium. The presence of emulsifiers in emulsions gives them a role in the oxidative stability of oil droplets (*11*). Controlling emulsifier type, location, and concentration at the oil−water interface can increase the oxidative stability of emulsified menhaden oil (*12*). Oil droplet size is also an important consideration in controlling oxidation of lipids in emulsions; smaller droplets have an increased surface area and are more prone to oxidation than larger droplets (*13*). The interfacial membranes of emulsion droplets may impact lipid oxidation by acting as a physical barrier between the constituents of lipid oxidation (*14*). Emulsion droplets with thick interfacial membranes seem to hinder the interactions of prooxidants with the lipid (*15*). Surfactant type, headgroup size, and charge have been shown to influence oxidation properties of emulsion droplets (*15*, *16*).

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In this paper we examined the impact of emulsifiers on the oxidation properties of an SL (menhaden oil−caprylic acid) oil-in-water model emulsion.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized (RBD) menhaden oil was obtained from Omega Protein, Inc. (Reedville, VA). Caprylic acid (99% pure) was purchased from Sigma Chemical Co. (St. Louis, MO). Immobilized Lipozyme IM from *Rhizomucor miehei* was purchased from Novo Nordisk Biochem North America, Inc. (Franklinton, NC). A commercially available whey protein isolate, *Bi*PRO, was provided by Davisco Foods International, Inc. (Le Sueur, MN). Tween 20 was purchased from Fisher Scientific (Fair Lawn, NJ). Ryoto sugar ester, that is, sucrose fatty acid ester containing mainly stearic acid (S-1670), was supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan). The sugar ester consisted of approximately 75% monoester and 25% di-, tri-, or polyester. Lecithin (LECIGRAN 5750) was a gift from Riceland Foods, Inc. (Stuttgart, AR). LECIGRAN 5750 is a defatted fine powder soy lecithin. BFP 65K [mono-/diacylglycerols, 52% (minimum) monoacylglycerols, iodine value = 65−75] was donated by American Ingredients Co. (Grandview, MO). Trichloroacetic acid and thiobarbituric acid were purchased from J. T. Baker, Inc. (Phillipsburg, NJ) and Sigma Chemical Co., respectively. Cadmium acetate and potassium iodide were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Fisher Scientific, respectively. Organic solvents were purchased from J. T. Baker Chemical Co. or Fisher Scientific.

SL Synthesis and Purification. SLs from menhaden oil and caprylic acid were synthesized using a packed bed column bioreactor as described by Xu et al. (*9*). SL products were purified using a KDL-4 (UIC Inc., Joliet, IL) short-path distillation unit under conditions previously described (*10*). Free fatty acids (FFA) content was determined according to AOCS Official Method Ca 5a-40 (*17*). Percent free fatty acids was expressed in terms of oleic acid.

Fatty Acid Composition Analysis. The fatty acid composition of SL was determined by gas chromatography according to a method previously described (*18, 19*).

Emulsion Preparation. Ten percent purified SL oil-in-water emulsions were prepared in 10 mM phosphate buffer solutions (pH 7.0). Two concentrations (0.25 and 1%) of emulsifiers, Tween 20, Ryoto sugar ester (S-1670), whey protein isolate, mono-/diacylglycerols, and lecithin were used to stabilize the emulsions. SL oil-in-water model emulsions were prepared using a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) at 50 MPa. All emulsions were passed through the homogenizer six times. Resulting emulsions were stored as 10-mL aliquots in translucent vials at 4 °C.

Particle Size Distribution. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, U.K.) using standard optical parameters.

Creaming Studies. Creaming stability was evaluated by storing emulsions in 10-mL graduated test tubes at 4 °C. The volume of cream separated out at the top was measured at 0, 2, 4, 8, 16, 32, and 48 days.

Evaluation of Lipid Oxidation. Oxidation was monitored over a 48-day period. Progress was monitored by measuring lipid peroxides (*20*), thiobarbituric acid reactive substances (TBARS) (*20*), and EPA and DHA contents in the SL. EPA and DHA contents were monitored because of their high double-bond content and greater susceptibility to oxidation. To measure EPA and DHA content, 1 mL of hexane was added to 300 μL of emulsion and thoroughly mixed. The organic phase was separated, methylated, and then analyzed for its fatty acid composition as previously described (*18, 19*). The mole percentages of EPA and DHA were determined from the fatty acid composition of the emulsion extract, then added, and expressed as a ratio of palmitic acid. Palmitic acid was used as a reference fatty acid because of its greater stability to oxidation than EPA and DHA. Sample vials were tested at 0, 2, 4, 8, 16, 32, and 48 days.

Statistics. The Statistical Analysis Systems (*21*) was used to analyze data. Data are expressed as the average of triplicate experiments. Least-squares (LS) means were used to determine significant differences. Significance was determined at $p < 0.05$.

Table 1. Fatty Acid Composition (Mole Percent) of Unmodified Menhaden Oil and Structured Lipid

fatty acid	menhaden oil[a]	structured lipid[b]
C8:0		42.6
C14:0	8.5	6.1
C16:0	19.4	13.4
C16:1	10.3	6.0
C17:0	3.9	ND
C18:0	5.4	1.0
C18:1	15.0	6.3
C18:2	3.5	1.1
C18:3	1.7	1.0
C18:4	3.6	
C20:1	1.4	
C20:5	11.9	9.3
C22:5	2.5	
C22:6	12.9	13.2

[a] Unmodified menhaden oil. [b] Structured lipid from menhaden oil and caprylic acid.

RESULTS AND DISCUSSION

The SL used in this study was prepared using parameters obtained by response surface methodology (*9*). Free fatty acids were reduced to 0.3% after short-path distillation. The fatty acid composition of unmodified menhaden oil and the structured lipid are shown in **Table 1**. Oxidation stability was the main concern in this paper because of the high combined content of EPA and DHA (22.5%) in the SL. SL was used to produce 10 emulsions using five different emulsifiers listed above at two concentrations, 0.25 and 1%. Differences in oxidative behavior among emulsifiers can depend on the surface charge of emulsion droplets, with decreasing rates in the order of anionic, nonionic, and cationic surfactants (*22*, *23*).

We evaluated the particle size distribution of all SL emulsions prepared. Emulsifier concentration and type did not affect the emulsification process for whey protein isolate, Tween 20, and mono-/diacylglycerols. Most emulsions had a monomodal size distribution with an average droplet diameter of ∼0.30−0.37 μm (**Figures 1** and **2**). A bimodal droplet size distribution was observed for emulsions prepared with 1% sucrose fatty acid ester (**Figure 1B**) and lecithin (**Figure 2B**). Emulsions prepared with sucrose fatty acid ester were characterized by oil droplets of diameter ∼0.23 μm, with a small population of droplets of diameter >20 μm. This could be representative of the different ester sizes in the sucrose fatty acid ester. Emulsions prepared with a low concentration of lecithin (0.25%) showed a monomodal size distribution with an average diameter of 0.34 μm. At higher concentration (1%) lecithin emulsions showed a bimodal distribution with a population of particles with a larger diameter of 4.7 μm. At high emulsifier concentration, surfactant molecules may exist as (aggregates) micelles, liposomes, or a separate lamellar mesophase (*24*, *25*). These structures have been shown to be of different size distributions as measured by nuclear magnetic resonance (NMR) and electron microscopy (*25*). By using high homogenization pressures and six homogenizer passes, we were able to maintain droplet size distributions at similar levels. This is essential so that comparisons between emulsifiers can be carried out without a surface area bias.

Creaming studies were performed on the emulsions to serve as an indicator of emulsion stability. During the 48-day period studied, all but the lecithin-stabilized emulsions were stable to creaming. Maximum creaming for both 0.25 and 1% lecithin was observed on the fourth day. Maximum creaming was 15 and 4.7% for 0.25 and 1% lecithin emulsions, respectively. It has been shown that phospholipid-stabilized emulsions are

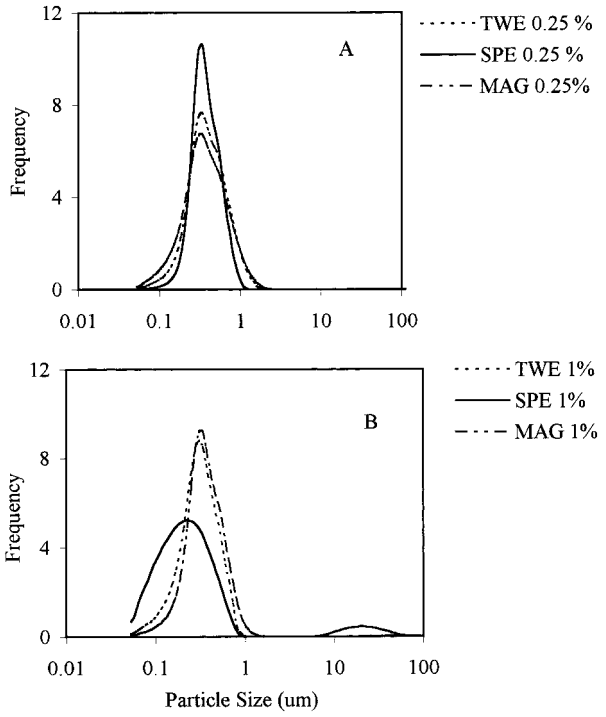

Figure 1. Particle size distribution of nonionic emulsifiers in structured lipid emulsions after six homogenizer passes: (A) emulsifier concentration at 0.25%; (B) emulsifier concentration at 1%. TWE, Tween 20; SFE, sucrose fatty acid ester; MAG, mono-/diacylglycerols. Data shown are the average of three replicate samples.

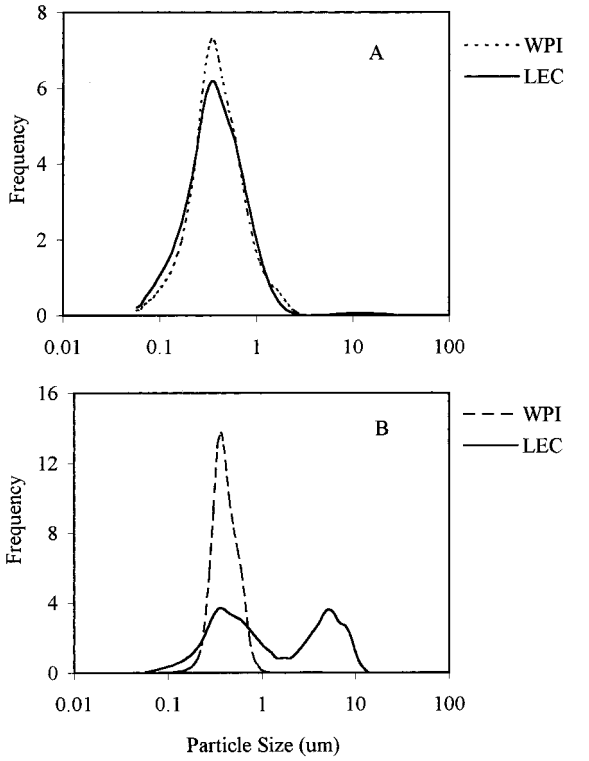

Figure 2. Particle size distribution of ionic emulsifiers in structured lipid emulsions after six homogenizer passes: (A) emulsifier concentration at 0.25%; (B) emulsifier concentration at 1%. WPI, whey protein isolate; LEC, lecithin. Data shown are the average of three replicate samples.

unstable in the presence of electrolytes (*26*, *27*). The phosphate buffer may have destabilized the lecithin-based emulsions in this study.

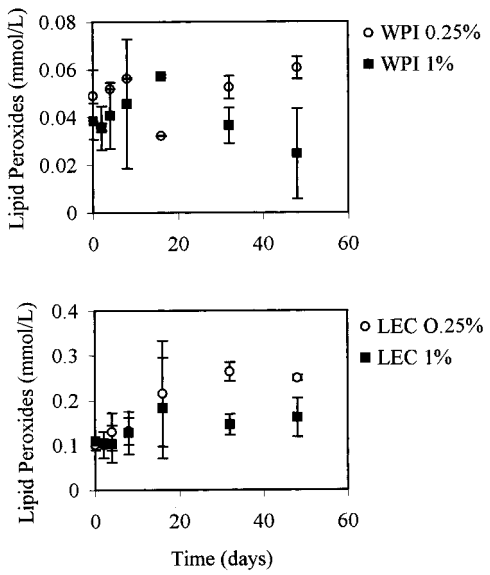

Figure 3. Changes in lipid peroxides with time in structured lipid emulsions containing ionic emulsifiers at 0.25 and 1% emulsifier concentrations. (See **Figure 2** for abbreviations.) Data shown are the average of three replicate samples. Error bars on chart represent standard deviation.

Hydroperoxides are primary oxidation products that have a shorter half-life than secondary oxidation products. Jacobsen et al. (*28*) have shown that higher emulsifier concentrations in mayonnaise led to lower lipid peroxide values but did not seem to significantly affect oxidative flavor deterioration. We measured peroxide values on the same time scale as for the creaming test (**Figures 3** and **4**). Lower emulsifier levels (0.25%) showed significantly ($p < 0.05$) higher oxidation levels than emulsions prepared with 1% emulsifier; however, whey protein isolate did not show any significant differences with concentration (**Figure 3**). Smaller droplet sizes led to higher oxidation rates because of increased surface area (*13*). Because droplet sizes were about the same for most emulsions, it may be deduced that emulsifier concentration, rather than droplet size distribution, caused changes in oxidation properties. At higher surfactant concentrations, the packing of surfactant molecules at the oil−water interface is tighter; hence, the membrane acts as an efficient barrier to the diffusion of lipid oxidation initiators into the oil droplets (*29*). The presence of oligo- or multilayers of surfactants at the oil/water interface at high surfactant concentration may play a role in reducing the entry of prooxidants into the oil droplets. Previous research has shown that interfacial thickness provided by Brij 700 is able to act as a physical barrier that separates lipid substrate from prooxidants in the aqueous phase (*16*). This may be the principle behind higher oxidation rates for 0.25% emulsifier concentration. Peroxide values for Tween 20 (**Figure 4**) showed a significant difference in oxidation between high and low emulsifier levels, whereas TBARS did not show any differences. For all emulsifiers, substantial changes in lipid peroxides were seen within the first 4 days. Lipid peroxide values increased progressively as time increased. No significant trend was observed for whey protein isolate stabilized SL emulsions. This was true for both low and high concentrations. This trend was not observed in the TBARS test results. On all days tested, statistical evaluations ($p < 0.05$) showed that emulsifier type also significantly affected lipid peroxide levels. On day 48, the highest lipid peroxide values were observed for sucrose fatty acid ester stabilized emulsions (nonionic emulsifiers). The lowest values were seen with whey protein isolate stabilized emulsions followed by the lecithin-

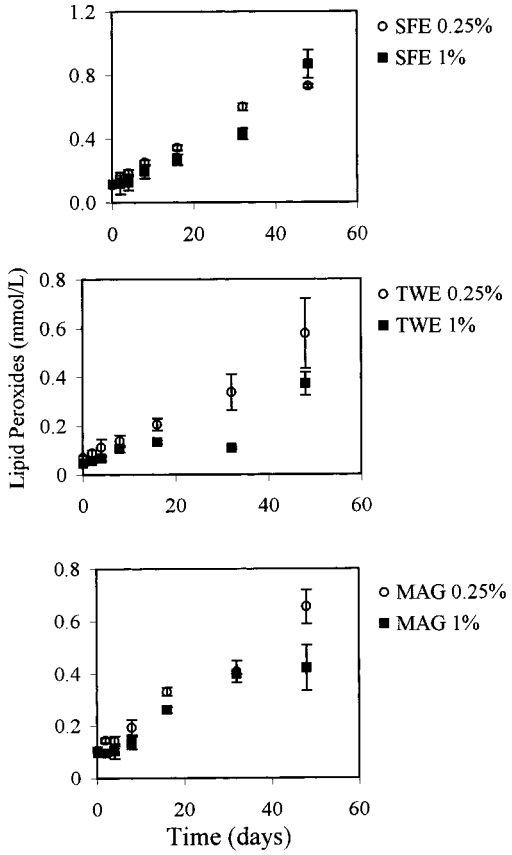

Figure 4. Changes in lipid peroxides with time in structured lipid emulsions containing nonionic emulsifiers at 0.25 and 1% emulsifier concentrations. (See **Figure 1** for abbreviations.) Data shown are the average of three replicate samples. Error bars on chart represent standard deviation.

stabilized emulsions (ionic emulsifiers). Whey proteins have been reported to inhibit lipid oxidation, which may account for their low peroxide values (*30*). They are thought to inhibit oxidation by chelation of iron and copper and by inactivation of peroxyl radicals (*12*, *30*).

TBARS are secondary oxidation products formed from the breakdown of oxidized polyunsaturated fatty acids. It has been shown that flavor threshold values correlate well with TBARS results of some vegetable oils (*31*). Oxidation products of SL emulsions in the presence of emulsifiers at different concentrations were evaluated, and the results shown in **Figures 5** and **6**. For Tween 20 stabilized emulsions, concentration had no effect on TBARS, whereas for all other emulsions, 0.25% emulsifier had significantly more TBARS than the higher concentration level of 1%. On all days tested, statistical evaluations ($p < 0.05$) showed that emulsifier type significantly affected the TBARS value. After the first 2 days of incubation, the highest TBARS were observed for 0.25% mono-/diacylglycerols, 0.25% Tween 20, 1% mono-/diacylglycerols, and 1% Tween 20. On day 48, TBARS values for 1% Tween 20, sucrose fatty acid ester, whey protein isolate, mono-/diacylglycerols, and 0.25% lecithin were significantly lower than those for 1% mono-/diacylglycerols, Tween 20, whey protein isolate, and sucrose fatty acid ester. The highest and lowest TBARS values were obtained for 0.25% mono-/diacylglycerols and 1% lecithin, respectively. Lecithin-stabilized emulsions may have had lower TBARS values because of the lower specific surface area that characterized the emulsions and the droplet stabilization which resulted in creaming.

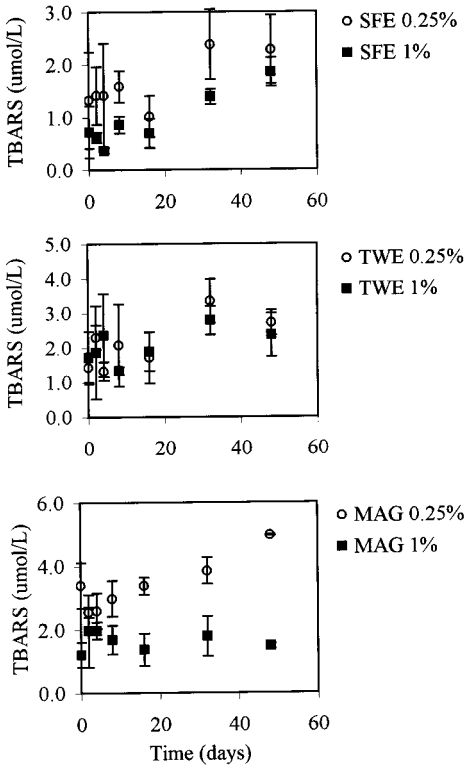

Figure 5. Changes in thiobarbituric acid reactive substances (TBARS) with time in structured lipid emulsions with nonionic emulsifiers at 0.25 and 1% emulsifier concentrations. (See **Figure 1** for abbreviations.. Data shown are the average of three replicate samples. Error bars on chart represent standard deviation.

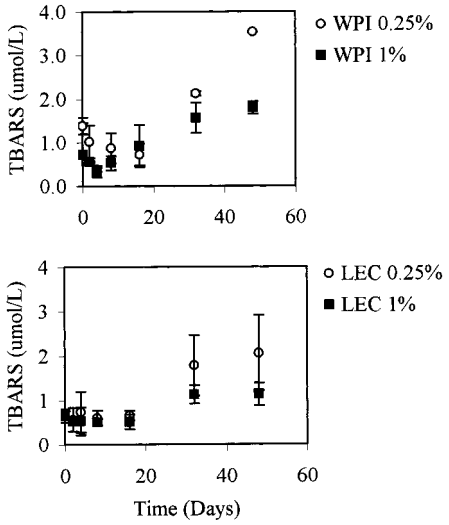

Figure 6. Changes in thiobarbituric acid reactive substances (TBARS) with time in structured lipid emulsions with ionic emulsifiers at 0.25 and 1% emulsifier concentrations. (See **Figure 2** for abbreviations.) Data shown are the average of three replicate samples. Error bars on chart represent standard deviation.

For each of the time periods studied, we measured the ratio of EPA and DHA to palmitic acid in the emulsions. These values were used as markers of oxidation progress. We would expect to see this ratio decrease with an increase in oxidation of EPA and DHA. In this study, there was no significant decrease (data not shown) in the amount of EPA and DHA present over the time period studied. Despite differences shown in TBARS and peroxide values, in the time frame studied, oxidation levels were

not high enough to make a significant ($p < 0.05$) difference in the amount of EPA and DHA present.

Lipid oxidation in model oil-in-water emulsions prepared with SL appeared to be influenced by emulsifier type and concentration. The higher concentration of emulsifier (1%) consistently led to lower oxidation rates compared to 0.25% emulsifier. Droplet size distribution was not influenced by emulsifier type. All but the lecithin emulsions were stable to creaming during the 48-day test period. The potential to use SL in mainstream food emulsions appears great and needs to be investigated. The results of this study will help in optimizing formulations and understanding the complexity of lipid oxidation in real food emulsions prepared with menhaden oil structured lipid.

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