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Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions

Hannah T. Osborn, Casimir C. Akoh*

University of Georgia, Department of Food Science and Technology, Food Science Building, Athens, GA 30602-7610, USA

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Abstract

The effect of emulsifier type, droplet size, and oil concentration on lipid oxidation was determined for caprylic acid/canola oil structured lipid-based emulsions. Oil-in-water emulsion samples were prepared with sucrose fatty acid ester or whey protein isolate and 10 or 30% oil, and then homogenized at 1000 or 10,000 psi to form different particle sizes. The peroxide values, anisidine values, and TOTOX values of emulsions stored at 50 °C were measured over time. Decreasing oil concentrations led to an increase in total oxidation. Whey protein isolate had a significant antioxidant effect on the oxidation rates compared to sucrose fatty acid esters. Particles size had no effect on lipid oxidation in structured lipid-based oil-in-water emulsions.

Keywords: Structured lipids; Oil-in-water emulsions; Lipid oxidation; Particle size; Whey protein isolate; Peroxide value; Anisidine value; TOTOX value

1. Introduction

Structured lipids (SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids, or to change the position of existing fatty acids on the glycerol backbone. This ability to combine the beneficial characteristics of component fatty acids into one triacylglycerol (TAG) molecule enhances the role that fats and oils play in food, nutrition, and health applications (Osborn & Akoh, 2002).

In the past, long chain triacylglycerols (LCTs), which are metabolized slowly in the body, were incorporated into emulsions for total parenteral nutrition (TPN) and enteral administration as a source of energy and essential fatty acids. Later, a physical blend of medium chain triacylglycerols (MCTs) and LCTs was proposed because the MCTs would be readily metabolized in the body for quick energy. More recently, SLs were designed to provide simultaneous delivery of beneficial long chain fatty acids (LCFAs) at a slower rate and medium chain fatty acids (MCFAs) at a quicker rate (Akoh, 2002; Babayan, 1987).

The combination of an increased absorption rate and beneficial component fatty acids in one TAG should make SLs very attractive to the medical community and functional food manufacturers. However, the food industry has been slow to incorporate SLs into their product formulations. Researchers have generated numerous articles on SLs and in many cases a potential food application is given for their product. However, few have taken the next step and studied how the SL actually behaves when used in a particular food application. An opportunity for more studies on the kinetics, physical properties, and functionality of food systems containing structured lipids definitely exists.

Lipid oxidation is a major cause of quality deterioration in many natural and processed foods (McClements & Decker, 2000). It can alter the flavour and nutritional quality of foods and produce toxic compounds (Min & Boff, 2002). Akoh and Moussata (2001) reported that unmodified canola oil, in the bulk phase, was more stable to oxidation than a canola oil/caprylic acid SL.

* Corresponding author. Tel.: +1-706-542-1067; fax: +1-706-542-1050.

E-mail address: cakoh@arches.uga.edu (C.C. Akoh).

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However, lipids often exist as emulsifier-stabilized dispersions in foods. Therefore, it is important to examine the oxidation properties of SLs in this medium.

Previous studies have highlighted the importance of the molecular environment in determining the oxidative stability of an oil-in-water (o/w) emulsion. Emulsifiers play a role in the oxidative stability of oil droplets (Fomuso, Corredig, & Akoh, 2002). Droplet charge, which is determined by the surface-active components in foods, affects the rate of lipid oxidation in an o/w emulsion (Mancuso, McClements, & Decker, 1999; Mei, McClements, Wu, & Decker, 1998; Mei, McClements, & Decker, 1999). The physical characteristics of the droplets may also affect the oxidation kinetics in o/w emulsions and vary considerably in foods, depending on their concentrations, size, and physical state. Understanding the kinetics of oxidation in o/w emulsions of varying oil droplet concentrations is critical in the food industry, where emulsified systems range from fruit beverages (<1% oil) to mayonnaise (> 80% oil). Typical food emulsions contain particle sizes ranging from 0.2 to 100 μm. A recent review of o/w emulsions indicated the need for more basic studies on the influence of droplet characteristics on lipid oxidation (McClements & Decker, 2000). Therefore, the objective of this study was to determine the effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in model o/w emulsions formulated with a canola oil/caprylic acid SL.

2. Materials and methods

2.1. Materials

Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%) was purchased from Sigma Chemical Company (St. Louis, MO). A sn-1,3 specific immobilized lipase originating from *Rhizomucor miehei* (IM 60), was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (#27361) was provided by Land O'Lakes (St. Paul, MN). Ryoto sugar ester (S-1670) was supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). The sucrose fatty acid ester contained mainly stearic acid and consisted of approximately 75% monoester and 25% di-, tri-, or polyester. All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Structured lipid production

The SL was produced according to the apparatus set-up and optimal conditions previously reported (Xu, Fomuso, & Akoh, 2000) for reacting canola oil and caprylic acid in a packed bed bioreactor: substrate flow rate, 1 ml/min; temperature, 60 °C; substrate mole ratio 1:5 (canola oil:caprylic acid); water content, 0.20% added. The product was purified by short-path distillation (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; pressure, <0.01 Torr. The purified SL product contained the following fatty acids (mol%): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0, 47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters (Fomuso & Akoh, 1996).

2.3. Emulsion preparation

SL-based o/w emulsions (10 and 30% oil) were prepared in 10 mM phosphate buffer solutions (pH 7.0). Whey protein isolate (WPI) and Ryoto sugar ester (SFE) were used at a 0.5% (w/w) level to stabilize the o/w emulsions. The o/w emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 1000 psi or 10,000 psi in an attempt to create different particle sizes. In order to prepare o/w emulsions with intermediate and/or mixed particle sizes, equal volumes of the two previously processed emulsions were combined and passed through the homogenizer once, without pressure, to ensure adequate mixing. All samples were held on ice during processing. Sodium azide (1 mM) was added to the final emulsion volume to slow microbial growth.

2.4. Particle size distribution

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

2.5. Oxidation experiments

Five ml aliquots of the o/w emulsions were stored in covered test tubes and allowed to oxidize in a covered water bath held at 50 °C. Duplicate 5 ml o/w emulsion samples were taken periodically to determine the hydroperoxide and aldehyde levels present in the oil. Oil was extracted from the o/w emulsions by adding iso-octane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected and the solvent was evaporated under nitrogen. After solvent removal, the oil samples were weighed in order to accurately determine oxidation progression in each sample. Peroxide values (PV) were determined using the International Dairy Foundation method described in detail by Shantha and Decker (1994). Anisidine values (AnV) were determined according to the AOCS Official Method Cd 18-90 (AOCS, 1998). The TOTOX value was calculated as:

TOTOX value = 2(PV) + AnV (Shahidi & Wanasundara, 2002).

2.6. Statistical analysis

All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (2001) software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) were determined by least squares means comparisons.

3. Results

3.1. Particle size analysis

The particle size of each o/w emulsion prepared was evaluated by integrated light scattering (Fig. 1). The 10% oil emulsions stabilized by SFE produced bimodal droplet size distributions (Fig. 1A) under the 1000 psi and mixed processing conditions. This finding is similar to that observed previously for o/w emulsions prepared with 1% SFE and may be a result of the different ester sizes in the sucrose fatty acid ester (Fomuso et al., 2002). Processing the SFE emulsion (10% oil) at higher pressure (10,000 psi) resulted in a monomodal size distribution. The 30% oil emulsions stabilized by SFE and the o/w emulsions stabilized by whey protein isolate at both 10 and 30% oil showed a monomodal size distribution. Average particle diameters ($D_{3,2}$) of the o/w emulsions ranged from 0.26 to 2.69 μm and are listed in Table 1.

By varying the processing conditions, significantly ($P < 0.05$) different particle sizes were produced in the SFE stabilized o/w emulsions at both oil concentration levels (10 and 30%). However, WPI o/w emulsions responded differently to processing. At 10% oil, no changes in particle size were observed across the three processing conditions ($P < 0.05$). The WPI emulsion, containing 30% oil, had significantly ($P < 0.05$) larger particle diameters when processed at 10,000 psi than at 1000 psi or mixed counterparts (Table 1).

3.2. Primary oxidation

Lipid oxidation is accelerated by reactions that take place at the surface of o/w emulsion droplets. Based on this principle alone, the rate of lipid oxidation should increase as the droplet size decreases, because of the increased surface area that is exposed to the aqueous phase (McClements & Decker, 2000). The effect of particle size on lipid oxidation rates was explored in this study. Hydroperoxides were measured to determine the initial rate of oxidation because they are generally accepted as the first product formed by oxidation (Rossell, 1986). Particle size did not significantly affect the peroxide values of the o/w emulsions at any point in the study (Figs. 2 and 3). Roozen, Frankel, and Kinsella (1994) also found no dependence of the lipid oxidation

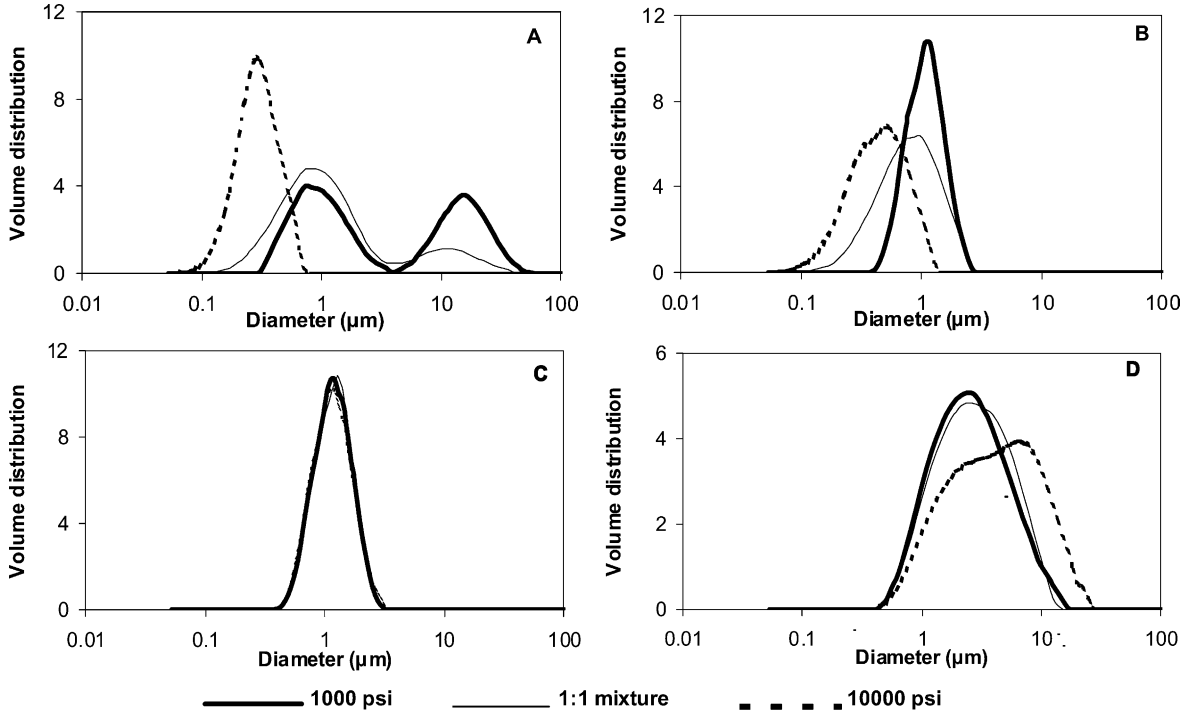

Fig. 1. Particle size distribution of structured lipid-based o/w emulsions stabilized by sucrose fatty acid ester (A = 10% oil; B = 30% oil) and whey protein isolate (C = 10% oil; D = 30% oil). Data shown are the averages of duplicate samples.

Table 1
Average apparent particle diameter ($D_{3,2}$) (μm) of structured lipid-based emulsions after six homogenizer passes[a]

Emulsifier	% Oil	Processing conditions		
		1000 psi	1:1 mixture	10,000 psi
S-1670	10	1.47±0.34a	0.83±0.21ab	0.26±0.01b
	30	1.00±0.01a	0.64±0.09b	0.37±0.03c
WPI	10	1.09±0.12a	1.09±0.02a	1.07±0.18a
	30	1.98±0.21a	2.08±0.04a	2.69±0.06b

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

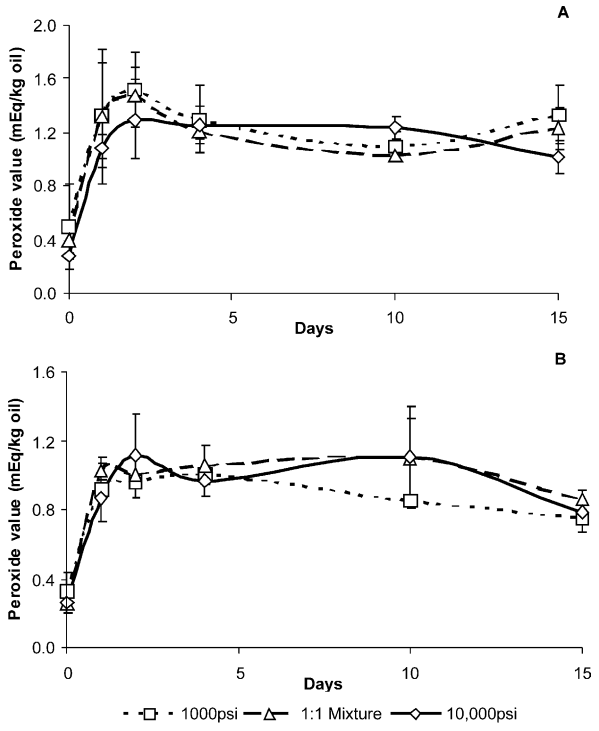

Fig. 2. The effect of particle size on lipid peroxides over time in structured lipid-based o/w emulsions stabilized by sucrose fatty acid ester (A = 10% oil; B = 30% oil). Data shown are the average of duplicate samples held at 50 °C.

rate on droplet size. Because limited amounts of hydroperoxides were available in the systems (Day 0 PV ~0.3 meq/kg oil), they may have all been present at the droplet surface in every o/w emulsion system studied. This may explain why changing the droplet size did not affect the oxidation rates (McClements & Decker, 2000).

Emulsifier type significantly affected hydroperoxide formation on days 1, 2, 3, and 10 ($P < 0.05$). SFE emulsions initially showed a rapid increase in peroxides that peaked by day 2 and generally remained constant for the rest of the study (Fig. 2). However, the PV increase of WPI emulsions was more gradual throughout the study, with the exception of the 30% oil mixed system, which peaked on day 4 (Fig. 3). This antioxidant effect of WPI has been previously reported

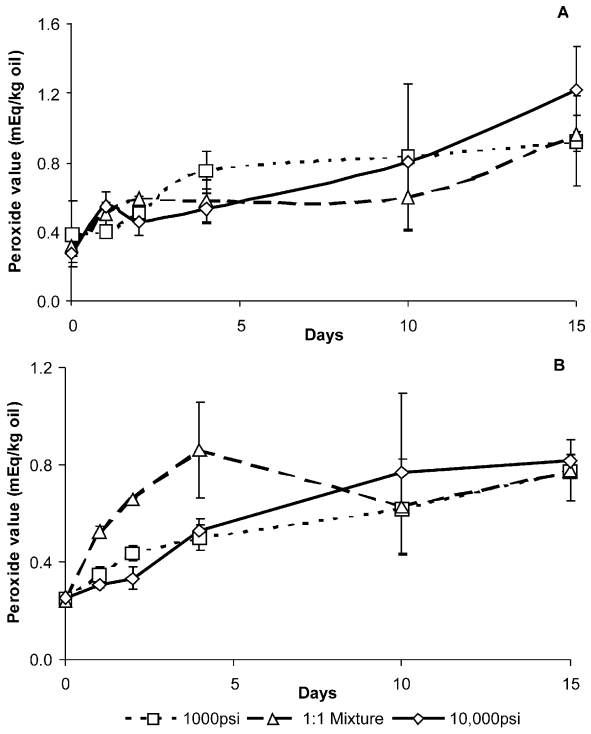

Fig. 3. The effect of particle size on lipid peroxides over time in structured lipid-based o/w emulsions stabilized by whey protein isolate (A = 10% oil; B = 30% oil). Data shown are the average of duplicate samples held at 50 °C.

(Fomuso et al., 2002). It was hypothesized that whey proteins inhibit lipid oxidation by inactivating peroxyl radicals (Tong, Sasaki, McClements, & Decker, 2000). On the final day of the study, there was no significant difference between the peroxide levels of the WPI and SFE emulsions ($P < 0.05$).

The peroxide values were significantly affected by oil concentration on days 1, 2, and 15 of the study. The 10% emulsions had significantly higher amounts of hydroperoxides on these days compared to the 30% emulsions (Figs. 2 and 3). This finding is similar to that previously reported for safflower oil-in-water emulsions (Sims, Fioriti, & Trumbetas, 1979). One possible cause of this increase is that the number of radicals generated per droplet increased as the droplet concentration decreased (McClements & Decker, 2000). Additionally, at higher oil concentrations, more unsaturated fatty acids may have moved into the interior of the oil droplets, and therefore became less accessible to direct interaction with the prooxidants in the aqueous phase (McClements & Decker, 2000).

3.3. Secondary oxidation

The peroxides in oxidized oil are transitory intermediates that decompose into various carbonyl and other compounds (Rossell, 1986). Measuring secondary oxidation products is important in the determination of

lipid oxidation in food products for human consumption, because they are generally odour-active, whereas primary oxidation products are colourless and flavourless. The AnV test was utilized to determine the level of aldehydes, principally 2-alkenals and 2,4-alkadienals, present in the emulsified oil (Shahidi & Wanasundara, 2002). Significant correlations between AnV and flavour acceptability scores of soybean oil have been reported in the past (List, Evans, Kwolek, Warner, & Boundy, 1974). Unlike hydroperoxides, aldehydes do not decompose rapidly, thus allowing the past history of an oil to be determined with the AnV (Shahidi & Wanasundara, 2002). Therefore, the low initial AnV for the o/w emulsions (~1.9) indicates that the acidolysis reaction, distillation process, and homogenization did not cause oxidative damage to the SL.

Emulsifier type had a significant effect on the AnV on days 1, 2, 10, and 15. The SFE emulsions had significantly ($P < 0.05$) higher AnV on those days (Fig. 4). This was expected because of the delayed development of hydroperoxides in the WPI emulsions. The hydroperoxides must first be present before they can decompose into secondary products. Again, this result demonstrates that WPI is functional in o/w emulsions as an antioxidant. The AnV for WPI emulsions did not change significantly between 0 and 15 days at 50 °C (Fig. 5). The ability of WPI to inactivate peroxyl radicals in the emulsified oil and thus prevent the development of secondary oxidation products may be responsible for the negligible changes in aldehydes measured during this study.

The oil concentration effect was significant near the end of the study on days 10 and 15. The AnV were relatively stable for the WPI emulsions throughout the study (Fig. 5). Oil concentration effect was more pronounced in the SFE emulsions (Fig. 4). Similar to the hydroperoxide results, the 10% SFE emulsions were significantly more oxidized than their 30% counterparts by the end of the study. Particle size did not significantly affect the AnV at any time, which is in agreement with the hydroperoxide results obtained in this study.

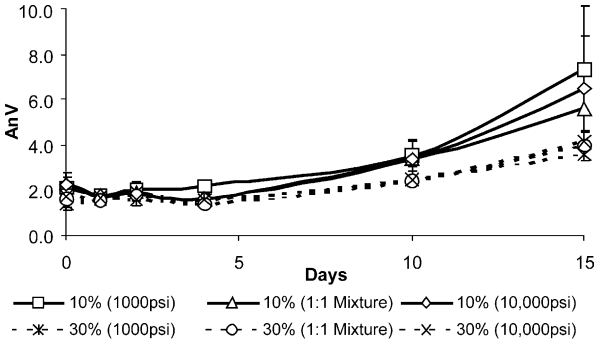

Fig. 4. The effect of oil concentration on the anisidine value over time in structured lipid-based o/w emulsions stabilized by sucrose fatty acid ester. Data shown are the average of duplicate samples held at 50 °C.

3.4. Total oxidation

The TOTOX value combines evidence about the past history and present state of an oil, and is used frequently in the food industry (Shahidi & Wanasundara, 2002). Fig. 6 illustrates the effect of emulsifier type on the total oxidation of the canola oil SL-based o/w emulsions. This effect was highly significant ($P < 0.01$) on all days of analysis after day 0. On day 15 of the study, the mean differences in total oxidation between SFE and WPI emulsions were 4.99 and 2.46 for 10 and

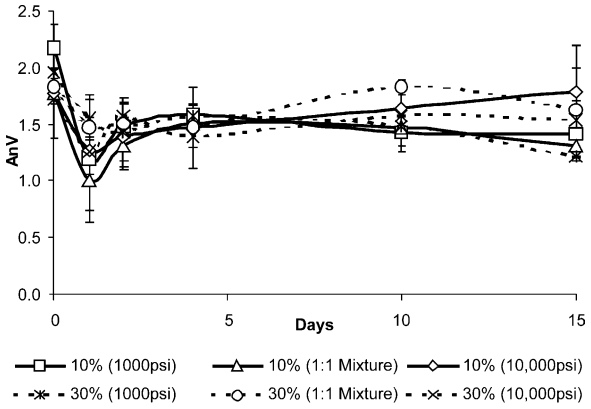

Fig. 5. The effect of oil concentration on the anisidine value over time in structured lipid-based o/w emulsions stabilized by whey protein isolate. Data shown are the average of duplicate samples held at 50 °C.

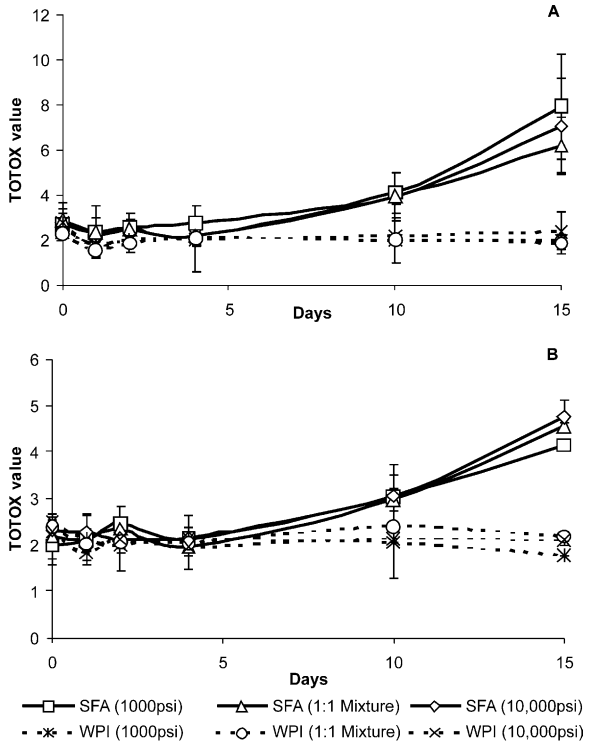

Fig. 6. The effect of emulsifier type (SFE = sucrose fatty acid ester; WPI = whey protein isolate) on total oxidation over time in structured lipid-based o/w emulsions (A = 10% oil; B = 30% oil). Data shown are the averages of duplicate samples held at 50 °C.

30% oil emulsions, respectively. Again, this result demonstrates the antioxidant properties of WPI. The concentration effect was also significant on days 2, 4, and 15. By day 15, the mean TOTOX values for the 10% SFE emulsions were nearly twice that of the 30% SFE emulsions (7.06 and 4.48, respectively). Data, from a study on palm oil quality that included both sensory and chemical analyses, showed that oil samples with a TOTOX value of 3.48 corresponded to an overall quality rating of 3.3 on a scale of 1 to 5 (1 = very poor; 5 = very good). However, a second oil with a TOTOX value of 8.68 was given an overall quality rating of 1.3 (poor) by the same sensory panel (Idris, Abdullah, & Halim, 1992). Rossell (1986) deems oil samples with a TOTOX value above 10 unacceptable. Therefore, antioxidants and/or cold storage may be necessary to slow oxidative deterioration in food o/w emulsions stabilized by SFE, especially when low concentrations of oil are used in the product formulation. Particle size did not significantly affect the TOTOX values throughout the study.

4. Conclusion

Lipid oxidation was significantly affected by oil concentration and emulsifier type. A decrease in the oil concentration led to an increase in total oxidation. WPI had a significant antioxidant effect on the oxidation rates compared to SFE. Clearly, lipid oxidation in SL-based o/w emulsions is a highly complex area, in which oil concentration and emulsifier type play an important role. Further studies on the influence of ingredients and antioxidant types are needed to expedite the incorporation of structured lipids into product formulations by the food industry.

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