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# Oxidative Comparison of Emulsion Systems from Fish Oil-Based Structured Lipid versus Physically Blended Lipid with Purple-Fleshed Sweet Potato (*Ipomoea batatas* L.) Extracts

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**ABSTRACT:** The effects of the purple-fleshed sweet potato extract (PFSPE) on oxidation stabilities of a model oil-in-water emulsion prepared with enzymatically synthesized fish oil−soybean oil structured lipid (SL) versus physically blended lipid (PBL) without modification were evaluated. The anthocyanins in PFSPE were analyzed and identified by HPLC-MS. The fatty acid composition of SL was similar to that of PBL, except palmitic acid (1.48 in PBL and 9.61% in SL) and linoleic acid (62.47 in PBL and 49.58% in SL). Peonidin 3-caffeoylsophoroside-5-glucoside, peonidin-3-(6′,6′-caffeoylferuloylsophoroside)-5-glucoside, peonidin-dicaffeoylsophoroside-5-glucoside, peonidin 3-(6′,6″-caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside were identified as the major anthocyanin compounds in PFSPE. Different levels (200, 500, 1000 ppm) of PFSPE were added into both SL- and PBL-based emulsions, with 200 ppm catechin as comparison. Oxidation was monitored by measuring the peroxide value and thiobarbituric acid reactive substances. The antioxidant activity of PFSPE increased with an increased concentration, the concentration of 1000 ppm showed high antioxidant ability similar to that of catechin in both PBL- and SL-based oil-in-water emulsions. It is notable that the SL-based emulsion appeared to have better oxidative stability than the PBL-based emulsion.

**KEYWORDS:** *structured lipid, anthocyanins, sweet potato (Ipomoea batatas L.), emulsion, antioxidant*

## INTRODUCTION

Structured lipids (SLs) are triacylglycerols (TAGs) that were modified to change the fatty acid composition by chemical or enzymatic process. Until now, there are many reports of SL produced by lipase-catalyzed interesterification reaction.[1−3] In most papers, polyunsaturated fatty acids were preferred as desired fatty acids in TAG structures of SLs due to their health benefits. For example, docosahexaenoic acid (DHA, 22:6) as an n-3 polyunsaturated fatty acid was studied for SL synthesis because of its benefits on human sensorial and neuronal systems.[4] Because DHA is quite sensitive to oxidation, the studies also focused on the oxidative properties of SLs containing DHA.[5,6]

Because lipids exist in food systems mainly as oil-in-water (O/W) emulsions such as milk, mayonnaise, and salad dressing, it is important to investigate the oxidative stability of O/W emulsion systems. However, most studies have focused on the SLs as fats and oils themselves rather than the emulsion systems. It is generally recognized that lipid oxidation in emulsion systems is complex due to their multiphase system and numerous other influencing factors (i.e., droplet size, emulsifier type, pH, buffer type, metal ions, temperature, etc.). Until now few researchers have studied O/W emulsions produced with SL containing DHA. Fomuso et al.[7] reported that the emulsifier type and concentration affected the oxidation rate of O/W emulsions from fish oil-based SL, in which addition of 0.25% emulsifier generally showed a higher oxidation rate than that of 1% emulsifier. In addition, Shen[8] observed that an O/W emulsion system prepared with 1,2-dioctadecanoyl-3-docosahexaenoyl glycerol oxidized more rapdily than that with 1,3-dioctadecanoyl-2-docosahexaenoyl glycerol, suggesting that DHA is more stable to oxidation when it is located at the sn-2 position of TAG. Because polyunsaturated fatty acids such as DHA are highly unstable to oxidation, antioxidant would be needed to retard the oxidation rate.

Recently, natural antioxidants from plants such as flavonoids, tocopherols, and carotenoids are widely appreciated for their putative health-promoting properties.[9,10] Anthocyanins, a group of flavonoids, are of special interest among the numerous varieties of natural antioxidants. Recently, a high level of anthocyanins in purple-fleshed sweet potato (PFSP) has been found. PFSP is a new variety of sweet potato (*Ipomoea batatas*) cultivar with reddish-purple flesh.[11] Furthermore, it was reported the anthocyanins from PSFP were acylated anthocyanins with aromatic acids,[12−14] which are more stable than other anthocyanins to pH, heat, and light.[15] Recent research[16,17] on the extracted anthocyanins from PFSP exhibited strong radical scavenging ability, yet no report on the antioxidative capacity in O/W emulsion was found.

The main objective of the study in this paper was to compare the oxidative properties of O/W emulsion prepared by SL versus physically blended lipid (PBL) from fish oil (FO) and

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soybean oil (SO). SL was produced from interesterification reaction between FO and SO catalyzed by immobilized lipase (Lipozyme RM IM), whereas the O/W emulsion system was prepared with a sophorolipid as an emulsifier. Sophorolipid was a mixture of macorolactone and free acid structures that are acetylated to the sophorose ring.[18] So far, few studies have compared such composition of SL and PBL in O/W emulsion systems. Besides, the composition of the selected anthocyanins in PFSP extracts (PFSPE) was studied, and the antioxidative capacity in O/W emulsion systems was evaluated by measuring lipid peroxides and thiobarbituric acid reactive substances.

## MATERIALS AND METHODS

**Materials.** Fish oil comprising 26.86% DHA was provided from Ilshin. Co. (Chungju, Korea). Soybean oil and purple-fleshed sweet potatoes were both purchased from a local market (Daejeon, Korea). PFSP were dried at 35 °C in an oven for 24 h and then ground into powder by a blender. Lipozyme RM IM from *Rhizomucor miehei* was obtained from Novozymes A/S (Copenhagen, Denmark). Gallic acid propyl ester was obtained from Fluka Chemie (St. Louis, MO). Ammonium thiocyanate, barium chloride dihydrate, hydrogen peroxide, iron(II) sulfate heptahydrate, trichloroacetic acid, bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane, and catechin (≥95%) were obtained from Sigma Chemical Co.(St. Louis, MO). All solvents were of HPLC grade and obtained from Fisher Scientific (Norcross, GA). Sophorolipid for emulsion preparation was provided by Dr. Solaiman from the U.S. Department of Agriculture (Pennsylvania).

**Synthesis of Structured Lipids.** FO and SO (4 g/12 g; 1:3 w/w) were added in a 250 mL flask with a screw cap. Lipozyme RM IM from *R. miehei* (10% by weight of total substrates) was added for synthesis of SL. The reaction mixture was incubated in an orbital-shaking water bath for 6 h at 55 °C. After incubation, Lipozyme RM IM was removed by filtering with a vacuum filter. The obtained reaction product was stored at −20 °C until analysis. In addition, FO and SO mixture without reaction was also prepared as PBL. Such SL and PBL were used for further O/W emulsion preparation.

**Analysis of Fatty Acid Composition.** PBL and SL were analyzed for fatty acid composition. Samples were saponified with 0.5 N NaOH in methanol (1.5 mL) for 5 min at 100 °C. After cooling to room temperature, the saponified sample was mixed with $BF_3$ methanol (2 mL) for methylation and heated for 3 min at 100 °C. After cooling, iso-octane (2 mL) and saturated NaCl (1 mL) were added and vortexed. Then the upper iso-octane layer was collected. A Hewlett-Packard 6890 gas chromatograph (GC) equipped with a capillary column (SP-2560, 100 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, PA) and a flame ionization detector (Agilent Technologies, Little Falls, DE) was used. The oven was set at 150 °C and held for 5 min, then increased to 220 °C at the rate of 4 °C/min, and finally held again for 30 min. The temperatures of injector and detector were set at 250 and 280 °C, respectively, and nitrogen was used for carrier gas. Duplicate analyses were performed. Fatty acid composition at the sn-2 position was analyzed by pancreatic lipase hydrolysis as described previously.[19] Each oil sample (7 mg) was placed in a test tube, and 1 M Tris-HCl buffer (7 mL), 0.05% bile salts (1.75 mL), 2.2% $CaCl_2$ (0.7 mL), and pancreatic lipase (7 mg) were added. After thorough mixing, the sample was incubated in a water bath at 37 °C for 3 min with vigorous vortexing for 1 min. The enzymatic hydrolysate was extracted by diethyl ether twice and then separated on thin-layer chromatography with developing solvent consisting of hexane/diethyl ether/acetic acid = 50:50:1 (v/v/v). The band corresponding to 2-monoacylglcyerol was scraped, and the fatty acids were analyzed by GC after methylation as described previously.

**HPLC Analysis of TAG Profile and Neutral Lipids.** Separation of TAG species from SO, FO, PBL, and SL was performed by reversed-phase HPLC (RP-HPLC) according to the method of Zhu et al.[20] with modification. Sample (1 mg/mL) was dissolved in acetone. After filtration with a disposable syringe filter (0.5 μm), 20 μL of the prepared sample was manually injected into a Nova-Pak C18 column (150 × 3.9 mm i.d.;Waters, Milford, MA) connected to an evaporative light scattering detector (Sedere, Alfortville, France), which was operated at 55 °C. The elution solvent consisted of (A) acetonitrile and (B) acetone with the following gradient: hold at 10% of B at initial 10 min, raise to 45% of B in 20 min, then at 50 min arrive at 80% of B, and reach 10% of B in the last 5 min to equilibrate the column for the next injection. The flow rate was 1 mL/min.

Separation of TAG, diacylglycerol (DAG), monoglycerol (MAG), and free fatty acid (FFA) was performed by HPLC with a Hypersils BDSCPS column (250 × 4.6 mm i.d., Thermo Electron Corp., U.K.) and Sedex 75 ELSD (Afortvill, France) operated at 40 °C with nitrogen as nebulizing gas at a pressure of 2.2 bar. After each sample (1 mg/mL) was dissolved in hexane and filtered with a disposable syringe filter (0.5 μm), 10 μL of the prepared sample was manually injected. The elution solvent consisted of (A) 0.4% acetic acid in hexane and (B) 0.4% acetic acid in methyl *tert*-butyl ether with the following gradient: hold at 0% of B at initial 5 min, then increase to 80% in 10 min, isocratic for 2 min, then decrease to 0% in 5 min, and isocratic for 5 min to equilibrate the column for the next injection. The flow rate was 1 mL/min.

**Determination of Total Anthocyanins in Purple-Fleshed Sweet Potato.** PFSP powder was extracted twice with a 70% ethanol solution and again once with a 95% ethanol solution under reflux. After filtration, the combined extract solutions were concentrated by a rotary vacuum evaporator and then further dried by a vacuum freeze-dryer (SFDSF06, Samwon Freezing Engineering Co., Korea). The sticky dried PFSP extract (PFSPE) was stored at −40 °C until analysis, avoiding light.

The total anthocyanins were measured as described by Ribereau-Gayon[21] and Lapornik.[22] PFSPE solution (1 mL) was pipetted into two tubes with 1 mL of 0.01% HCl solution in 95% ethanol added. After that, 10 mL of 2% aqueous HCl solution was added into the first tube (A1) and 10 mL of solution at pH 3.5 (prepared from 0.2 M $Na_2HPO_4$ and 0.1 M citric acid) into another tube (A2). Both samples were measured under 520 nm against blank.

$$\text{content of total anthocyanins (mg/L)} = (\text{A1} - \text{A2}) \times f$$

$$f = 396.58$$

**LC-MS Analysis of Purple-Fleshed Sweet Potato Extract.** The sample was dissolved in 0.1% formic acid in water for LC-MS. The LC-MS analysis was carried out according to ref 23 with a modification, using an Agilent 1200 HPLC (Agilent, Santa Clara, CA) equipped with a diode array detector (set at 520 nm) and a linear ion trap quadruple LC-MS/MS (Applied Biosystems, Forster City, CA) with a quaternary pump. The column was a 250 × 4.6 mm i.d., Synergi 4 μ POLAR-RP 80A (Phenomenex, Torrance, CA) equipped with a Security Guard AQ C18 (4 × 3.0 mm i.d.; Phenomenex) and a thermostat column compartment. The operating flow rate was 1.0 mL/min with an injection volume of 20 μL by an autosampler. The elution solvent was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with the gradient program described as follows: 0−5 min, isocratic at 5% B; 5−11 min, 5−8% B; 11−15 min, isocratic at 8% B; 15−19 min, 8−10% B; 19−22 min, 10−13% B; 22−25 min, isocratic at 13% B; 25−30 min, 13-15% B; 30−35 min, 15−18% B; 35−38 min, isocratic at 18% B; 38−42 min, 18−20% B; 42−45 min, isocratic at 20% B; 45−48 min, 20−25% B; 48−50 min, isocratic at 25% B; 50−60 min, 25−5% B. The MS parameters for the analysis were as follows: ion spray voltage, 4000 V; ion source temperature, 550 °C. Mass spectra were acquired using electrospray ionization in the positive ionization (PI) mode over the range of $m/z$ 100−1300.

**Preparation of Emulsion and Oxidation Study.** O/W emulsions (10% oil by weight) from SL (SL-emulsion) and PBL (PBL-emulsion) were prepared in bis[2-hydroxyethyl]imino-tris-[hydroxymethyl]methane buffer solution (pH 7), respectively. Sophorolipid was used at 0.1% (by weight) as an emulsifier. The emulsions were initially homogenized by using a Silverson homogenizer (model L4RT, Silverson Machines, U.K.) at 5000 rpm for 2 min and then passed through a microfluidize processor (M-110Y,

**Table 1. Total and sn-2 Positional Fatty Acid Profiles (Percent) of Fish Oil (FO), Soybean Oil (SO), Physically Blended Lipid (PBL), and Structured Lipid (SL)[a]**

| | FO | | SO | | PBL[b] | | SL[c] | |
|---|---|---|---|---|---|---|---|---|
| fatty acid[d] | total | sn-2 | total | sn-2 | total | sn-2 | total | sn-2 |
| C14:0 | 1.24 ± 0.03 | 1.33 ± 0.01 | ND[e] | ND | 0.29 ± 0 | 0.2 ± 0.01 | 0.28 ± 0 | 0.32 ± 0.02 |
| C16:0 | 5.43 ± 0.05 | 5.15 ± 0.09 | 11.49 ± 0.03 | 0.75 ± 0.02 | 10.46 ± 0.01 | 1.38 ± 0.14 | 10.42 ± 0 | 9.61 ± 0.10 |
| C16:1 | 1.48 ± 0.02 | 1.47 ± 0.06 | 0.08 ± 0 | 0.1 ± 0.03 | 0.34 ± 0 | 0.27 ± 0.01 | 0.34 ± 0 | 0.33 ± 0.02 |
| C18:0 | 1.35 ± 0.00 | 1.66 ± 0.11 | 4.54 ± 0.02 | 0.42 ± 0.07 | 3.81 ± 0 | 0.62 ± 0.03 | 3.82 ± 0.01 | 3.9 ± 0.00 |
| C18:1 | 17.82 ± 0.01 | 26.23 ± 0.36 | 21.59 ± 0.06 | 24.13 ± 0.44 | 20.59 ± 0.1 | 23.18 ± 0.2 | 20.78 ± 0.01 | 21.85 ± 0.69 |
| (n-7)C18:1 | 2.08 ± 0.01 | 1.37 ± 0.12 | 2.45 ± 0 | 0.78 ± 0.49 | 2.31 ± 0.07 | 1.23 ± 0.14 | 2.29 ± 0.1 | 2.01 ± 0.06 |
| C18:2 | 7.19 ± 0.08 | 15.29 ± 0.22 | 52.38 ± 0.01 | 68.5 ± 0.05 | 44.23 ± 0.1 | 62.47 ± 0.18 | 44.54 ± 0.05 | 49.58 ± 0.44 |
| C20:0 | 0.78 ± 0.00 | ND | ND | ND | 0.46 ± 0 | ND | 0.45 ± 0.01 | ND |
| (n-6)C18:3 | 0.84 ± 0.01 | ND | 0.49 ± 0 | 0.43 ± 0.02 | 0.42 ± 0 | ND | 0.42 ± 0.03 | ND |
| C20:1 | 0.60 ± 0.04 | ND | 0.23 ± 0 | 0 ± 0 | 0.31 ± 0 | ND | 0.32 ± 0.04 | ND |
| (n-3)C18:3 | 2.32 ± 0.07 | 4.58 ± 0.25 | 6.28 ± 0.05 | 4.88 ± 0.04 | 5.03 ± 0.02 | 5.3 ± 0.04 | 5.09 ± 0 | 5.16 ± 0.07 |
| C20:2 | 0.33 ± 0.03 | ND | ND | ND | 0.09 ± 0 | ND | 0.09 ± 0 | ND |
| C22:0 | 0.84 ± 0.01 | ND | 0.47 ± 0 | ND | 0.5 ± 0 | ND | 0.49 ± 0 | ND |
| (n-6)C20:3 | 2.28 ± 0.00 | 2.33 ± 0.00 | ND | ND | 0.38 ± 0 | 0.27 ± 0.04 | 0.38 ± 0 | 0.39 ± 0.02 |
| (n-9)C22:1 | 0.95 ± 0.04 | 1.12 ± 0.10 | ND | ND | 0.15 ± 0 | ND | 0.14 ± 0.01 | ND |
| C22:2 | 0.53 ± 0.02 | ND | ND | ND | 0.11 ± 0 | ND | 0.1 ± 0.02 | ND |
| C24:0 | 0.40 ± 0.00 | ND | ND | ND | 0.19 ± 0.01 | ND | 0.18 ± 0 | ND |
| C24:1 | 1.66 ± 0.01 | 1.52 ± 0.00 | ND | ND | 0.24 ± 0.03 | 0.19 ± 0.03 | 0.23 ± 0 | 0.26 ± 0.00 |
| EPA | 3.40 ± 0.00 | 2.23 ± 0.20 | ND | ND | 0.58 ± 0.01 | 0.26 ± 0.02 | 0.54 ± 0 | 0.46 ± 0.14 |
| DPA | 12.4 ± 0.00 | 8.35 ± 0.21 | ND | ND | 2.09 ± 0.07 | 1.13 ± 0.11 | 2.02 ± 0.01 | 1.68 ± 0.05 |
| DHA | 26.86 ± 0.06 | 22.33 ± 0.89 | ND | ND | 4.54 ± 0.01 | 3.14 ± 0.21 | 4.53 ± 0 | 3.94 ± 0.76 |
| unknown | 9.22 ± 0.15 | 5.06 ± 0.31 | | | 2.57 ± 0.08 | 0.34 ± 0.03 | 2.53 ± 0.11 | 0.48 ± 0.09 |
| total n-3[f] | 44.99 ± 0.08 | 37.49 ± 1.56 | 6.28 ± 0.05 | 4.88 ± 0.04 | 12.23 ± 0.10 | 9.83 ± 0.38 | 12.18 ± 0.01 | 11.23 ± 1.02 |

[a]All data are mean values ± standard deviation of duplicate measurements. [b]Physically blended lipid was a mixture of fish oil/soybean oil = 1:3 (w/w). [c]Structured lipid was synthesized by fish oil/soybean oil = 1:3 (w/w) with Lipozyme RM IM 10% for 6 h at 55 °C. [d]The name of each fatty acid is shown as follows: myristic acid (14:0); palmitic acid (16:0); pamitoleic acid (16:1); stearic acid (18:0); oleic acid [18:1(n-9)c]; vaccenic acid [18:1(n-7)]; linoleic acid [18:2(n-6)]; arachidic acid (20:0); α-linolenic acid [18:3(n-3)]; gadoleic acid (20:1); γ-linolenic acid [18:3(n-6)]; eicosadienoic acid (20:2); behenic acid (22:0); eicosatrienoic acid (20:3); docosadienoic acid (22:2); lignoceric acid (24:0); nervonic acid (24:1); EPA [20:5(n-3)]; DPA [22:5(n-3)]; DHA [22:6(n-3)]. [e]ND, not detected. [f]Total n-3 = (n-3)C18:3 + EPA + DPA + DHA.

Microfluidics, Newton, MA) with two passes at 3000 psi for the final state of emulsion. Each SL- and PBL-emulsion was mixed with PFSPE in three different concentrations (200, 500, and 1000 ppm). Catechin, a natural antioxidant, was used as a positive control at 200 ppm. Each emulsion was placed in a vial with a cap and stored in a dark oven at 35 °C for 16 days. For evaluation of oxidative stabilities, peroxide value (POV for measuring hydroperoxide) and 2-thiobabituric acid-reactive substances (TBARS value for substances such as malonaldehyde) were measured every 2 days during the first 8 days and every 4 days during the later 8 days, resulting in measurements on days 2, 4, 6, 8, 12, and 16.

Lipid peroxides were measured according to the method of Mei et al.[24] with a little modification. Thiocyanate/$Fe^{2+}$ solution was made by mixing 1 mL of 3.94 M ammonium thiocyanate with 1 mL of $Fe^{2+}$ solution, which was obtained from the supernatant of a mixture of 3 mL of 0.144 M $BaCl_2$ in 0.4 M HCl and 3 mL of freshly prepared 0.144 M $FeSO_4$. Emulsion sample (20 μL) was mixed with 3 mL of methanol/1-butanol (2:1, v/v) and 30 μL of freshly prepared thiocyanate/$Fe^{2+}$ solution. Twenty minutes later, the absorbance was measured at 510 nm. Lipid peroxides were quantified from a standard curve using $H_2O_2$.

TBARS content was also measured according to the method of Mei et al.[25] TBA solution was prepared by dissolving 0.1125 g of TBA and 4.5 g of trichloroacetic acid into 25.421 mL of 0.25 N HCl. Emulsion sample (200 μL) was then mixed with 100 μL of 2% BHT methanol solution, and then 2 mL of TBA solution and 0.7 mL distilled water were added. The mixtures were heated in a boiling water bath for 30 min. After cooling and centrifuging at 2000*g* for 15 min, the absorbance was measured at 532 nm. TBARS values were quantified by standard curves prepared using 1,1,3,3-tetraethoxypropane.

**Statistical Analyses.** Analysis of variance (ANOVA) was performed by using SPSS 16.0 (SPSS Inc., Chicago, IL). A difference was considered to be statistically significant when $p < 0.05$. All data were measured in duplicate.

## RESULTS AND DISCUSSION

**Fatty Acid Composition.** Table 1 shows the total and positional fatty acid profiles of FO, SO, PBL, and SL. FO contained a high amount (44.99%) of n-3 fatty acids, in which major fatty acids were long-chain polyunsaturated fatty acids (LPUFA) such as docosahexaenoic acid (DHA, 26.86%), docosapentaenoic acid (DPA, 12.40%), eicosapentaenoic acid (EPA, 3.40%), and α-linolenic acid (C18:3n-3, 2.32%). The fatty acid profile at the sn-2 position showed a similar composition, which contained 22.33% DHA, 8.35% DPA, 2.23% EPA, and 4.58% C18:3n-3. Meanwhile, SO contained large amounts of linoleic acid (C18:2, 52.38%) and oleic acid (C18:1, 21.59%). C18:3n-3 (6.28%) was the only n-3 fatty acid in SO. Both C18:2 and C18:1 were major fatty acids of PBL and SL, whereas the contents of n-3 LPUFA (DHA, DPA, EPA, and C18:3n-3) were similar. However, as expected, the fatty acid contents at the sn-2 position in PBL and SL were different. After interesterification reaction, major changes of the fatty acid content occurred for C16:0 (1.38% of PBL and 9.61% of SL) and C18:2 (62.47% of PBL and 49.58% of SL). By comparison of SL to PBL, the contents of DHA, DPA, and EPA at the sn-2 position were not significantly changed ($p > 0.05$).

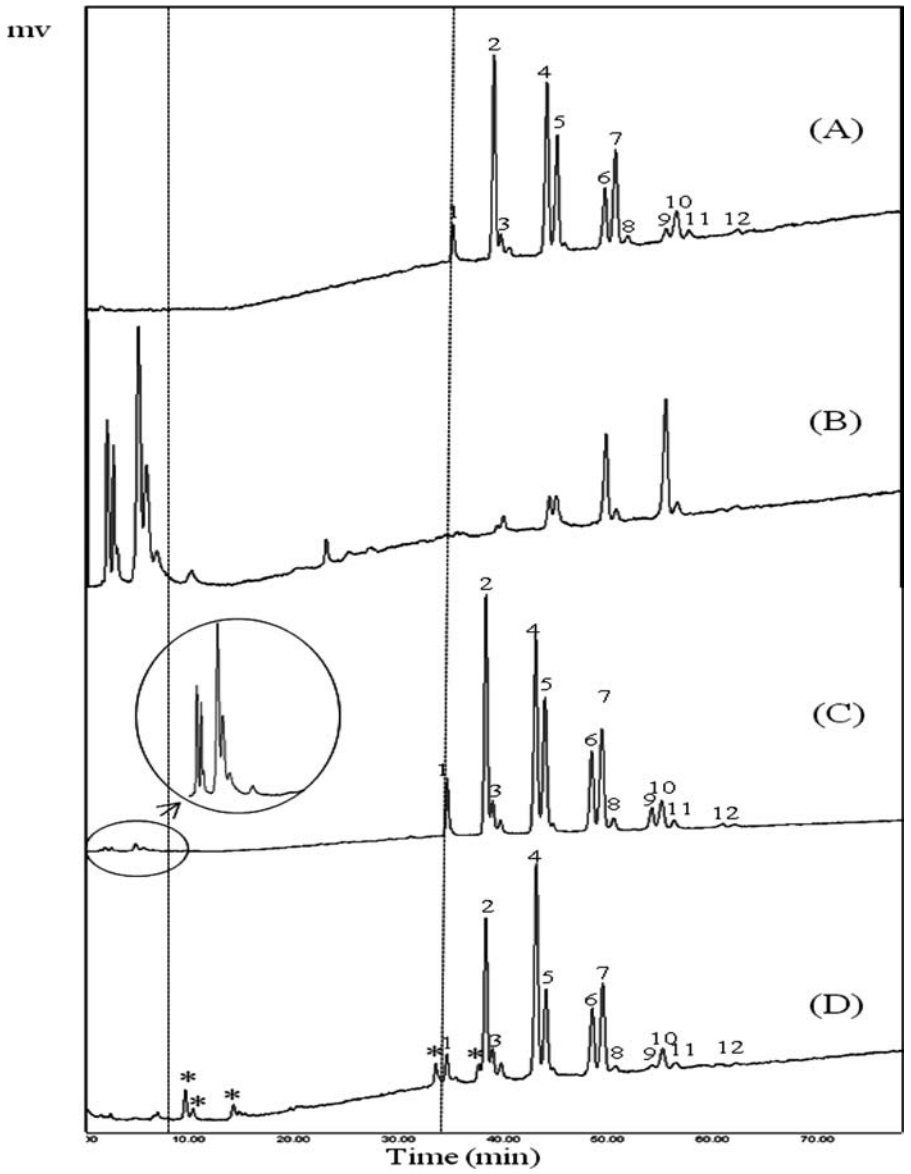


**Figure 1.** TAG profiles from the HPLC of (A) soybean oil, (B) fish oil, (C) physically blended lipid (fish oil/soybean oil = 1:3, w/w), and (D) structured lipid (fish oil/soybean oil = 1:3, w/w, RM IM 10%, w/w, for 6 h at 55 °C). Peaks: 1, LLLn; 2, LLL; 3,OLLn; 4, LLO; 5, PLL/OOLn; 6, LOO; 7, LLS/PLO; 8, PLP; 9, OOO; 10, SLO; 11, POO; 12, SOO/PPP. *, newly synthesized peaks.

**TAG Profile.** The HPLC separations of TAG species in SO, FO, PBL, and SL are shown in Figure 1. In addition, in Figure 1A, the identification of peaks in SO is also shown, in which LLL (peak 2), LLO (peak 4), PLL/OOLn (peak 5), LOO (peak 6), and LLS/PLO (peak 7) were the major TAGs, comprising 84.9% of total peak area. For FO, the TAG species containing high unsaturated fatty acids (i.e., DHA, DPA, and EPA) were eluted within the first 10 min (Figure 1B). When compared to PBL (Figure 1C), SL (Figure 1D) showed more than five new peaks in the chromatogram; the areas of peaks 2 (LLL) and 9 (OOO) decreased, whereas peak 4 (LLO) was increased in SL. Furthermore, the amounts of TAG, DAG, MAG, and FFA in PBL and SL were also compared by HPLC-ELSD (Figure 2). The area percentages of TAG, DAG, and MAG in PBL were 88.1, 8.3, and 3.6%, respectively, whereas the area percentages of TAG, DAG, MAG, and FFA in SL were 74.5, 20.5, 3.3, and 1.8%, respectively. The results implied that SL contained more DAG as byproduct than PBL due to the interesterification.

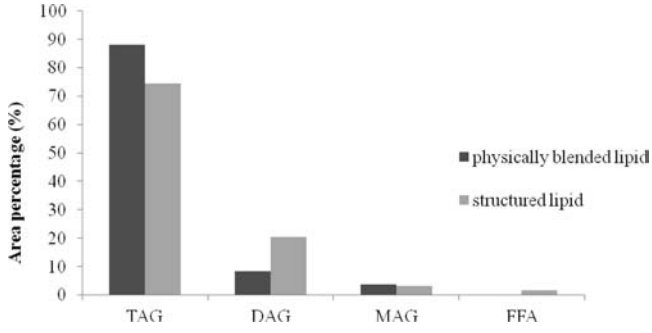


**Figure 2.** Area percentages of TAG, DAG, MAG, and FFA in physically blended lipid (PBL) and structured lipid (SL).

**Total Anthocyanins and HPLC Characterization.** The content of total anthocyanins in PSFPE was 2.42 mg/g extract (data not shown). The HPLC chromatographic profile of anthocyanins that were extracted from PFSP (PFSPE) is


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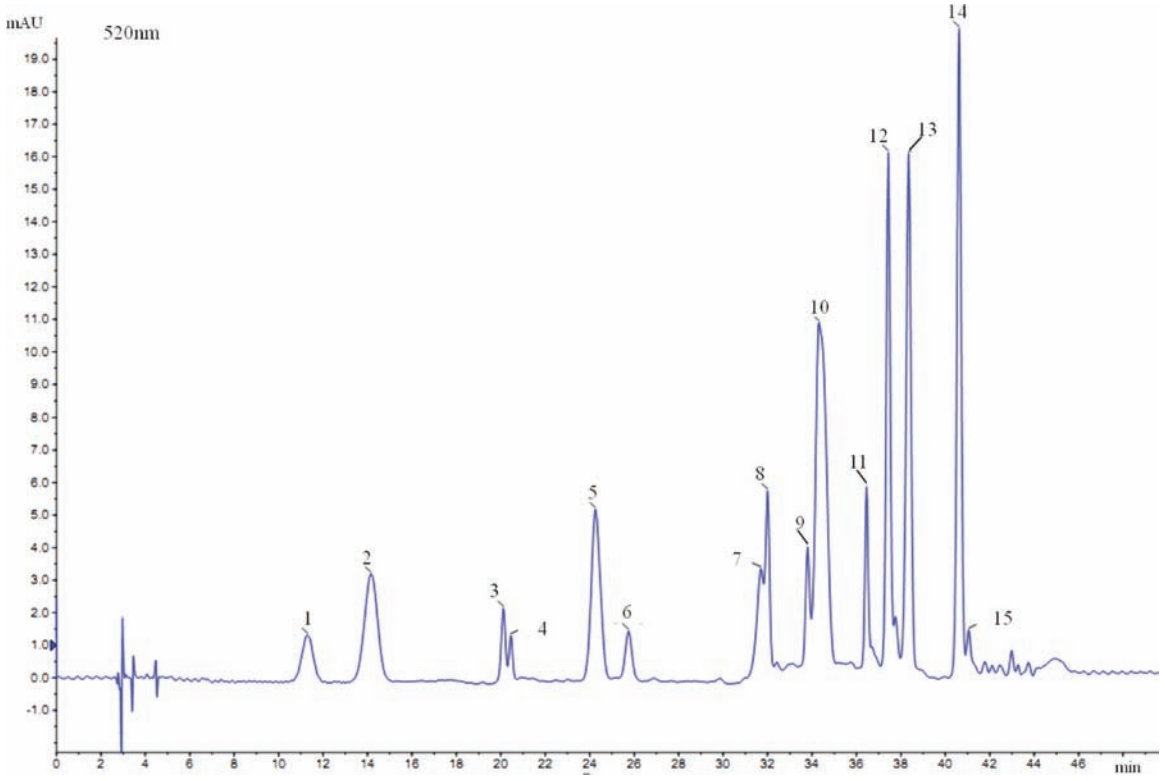


**Figure 3.** HPLC chromatographic profile at 520 nm of anthocyanins from purple-fleshed sweet potato extract. Peak numbers correspond to the peaks in Table 2.

**Table 2. Mass Spectrometric Data and Identification of Anthocyanin Compounds in Purple-Fleshed Sweet Potato Extract**

| peak[a] | retention time (min) | molecular ion $[M^+]$ ($m/z$) | aglycon ($m/z$) | other fragment ion ($m/z$) | proposed anthocyanin | references[b] | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 11.31 | 773 | 287 | ND[c] | cyanidin 3-sophoroside-5-glucoside | T1 | C1 | Ti1 |
| 2 | 14.51 | 787 | 301 | 625 | peonidin 3-sophoroside-5-glucoside | T2 | C2 | Ti4 |
| 3 | 20.12 | 893 | ND | 449 | cyanidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside | T3 | C3 | Ti5 |
| 4 | 20.45 | 935 | ND | 773 | cyanidin 3-(6′-caffeoylsophoroside)-5-glucoside | T4 | C4 | Ti6 |
| 5 | 24.26 | 907 | 301 | 463 | peonidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside | T5b | C5 | Ti7 |
| 6 | 25.75 | 949 | ND | 787 | cyanidin 3-(6′-feruloylsophoroside)-5-glucoside | T6 | C7 | Ti10 |
| 7 | 31.70 | 935 | ND | ND | unknown | | | |
| 8 | 32.00 | 935 | 287 | 449 | cyanidin 3-caffeoylsophoroside-5-glucoside | T7 | | Ti17 |
| 9 | 34.06 | 1097 | ND | ND | cyanidin 3-(6′,6′-dicaffeoylsophoroside)-5-glucoside | T10 | C9 | Ti16 |
| 10 | 34.32 | 949 | 301 | 787 | peonidin 3-caffeoylsophoroside-5-glucoside | T11d | C14a | Ti21 |
| 11 | 36.59 | 1081 | 287 | 919 | cyanidin 3-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside | T14 | C10 | Ti19 |
| 12 | 37.16 | 1111 | 301 | 463 | peonidin 3-(6′, 6″-dicaffeoylsophoroside)-5-glucoside | T13 | C13 | |
| 13 | 38.28 | 1069 | ND | ND | peonidin 3-(6′, 6″-caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside | T16 | C14b | Ti20 |
| 14 | 40.52 | 1125 | 301 | 963 | peonidin-3-(6′,6′-caffeoylferuloylsophoroside)-5-glucoside | T17 | C15 | |
| 15 | 41.05 | 1143 | ND | ND | unknown | | | |

[a]Peak numbers refer to Figure 3. [b]References: ref 13 for compounds T1−T17; ref 14 for C1−15; ref 12 for Ti1−Ti21. [c]ND, not detected.

presented in Figure 3. According to the HPLC condition in this study, 15 peaks were separated and 13 peaks were identified.

Indentification of the anthocyanin compounds was based on the data from HPLC-DAD/MS with several references.[12−14,26,27] In their results, most anthocyanins were peonidin or cyanidin 3-sophoroside-5-glucosides and their acylated derivatives. In Table 2, peak 1 contained the molecular ion $[M + H]^+$ at 773 and a fragment with $m/z$ at 287, which symbolized cyanidin aglycon. Peak 1 was therefore tentatively identified as cyanidin 3-sophoroside-5-glucoside. For peak 2, the molecular ion $[M + H]^+$ at 787, the peonidin aglycon with $m/z$ 301, and the fragment ion pattern of $m/z$ 625 due to the elimination of one molecule of glucose $[M - 162]^+$ indicated that peak 2 was peonidin 3-sophoroside-5-glucoside. Peak 3 had the molecular ion $[M + H]^+$ at 893 and fragment $m/z$ 449 $[M - 2 \times 162 - 120]^+$ for the loss of a sophorose molecule and


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*p*-hydroxybenzoic acid 120 [*p*-hydroxybenzoic acid − $H_2O$], pointing to a tentative identification as cyanidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside. Such three anthocyanins were first identified in the roots of PFSP by Truong,[13] and they were also found in leaves of Japanese purple sweet potato[26] and a cell line of Ayamurasaki cultivar purple sweet potato. [12]

Peak 4 had the molecular ion $[M + H]^+$ at 935 with a fragment ion of *m/z* 773 $[M - 162]^+$, suggesting the loss of glucose. There was no aglycon *m/z* in the chromatogram; considering the preceding studies on MS data,[13] it was tentatively identified as cyanidin 3-(6′-caffeoylsophoroside)-5-glucoside. Peak 6 (*m/z* $[M + H]^+$ at 949, 787 $[M - 162]$), peak 7 (*m/z* $[M + H]^+$ at 935), peak 13 (*m/z* $[M + H]^+$ at 1069), and peak 15 (*m/z* $[M + H]^+$ at 1143) showed similar results to peak 4. Peak 6 was tentatively identified as cyanidin 3-(6′-feruloylsophoroside)-5-glucoside, whereas peaks 7 and 15 cannot be identified due to the limited information. Peak 13 was one of the major anthocyanin compounds in PFSP, compared to previous studies.[13,26] According to the similar peak eluting order and the peak area, peak 13 was tentatively identified as peonidin 3-(6′,6″-caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside.

Peak 5 (*m/z* $[M + H]^+$ at 907, peonidin aglycon with *m/z* at 301, 463 [M − sophorose-*p*-hydroxybenzoic acid]) and peak 8 (*m/z* $[M + H]^+$ at 935, cyanidin aglycon with *m/z* at 287, 449 [M − sophorose − ferulic acid]) were identified as peonidin 3-*p*-hydroxybenzoylsophoroside-5-glucoside and cyanidin 3-caffeoylsophoroside-5-glucoside, respectively. Peak 11 was a cyanidin compound (*m/z* 287), and the fragment ion of *m/z* 919 $[M - 162]$ indicated the elimination of glucose. Compared with the previous studies, this compound was tentatively identified as cyanidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside.

Peak 10 (*m/z* $[M + H]^+$ at 949, peonidin aglycon with *m/z* at 301, 787 $[M - 162]$), peak 12 (*m/z* $[M + H]^+$ at 1111, peonidin aglycon with *m/z* at 301, 463 [M − sophorose − caffeic acid − caffeic acid]), and peak 14 (*m/z* $[M + H]^+$ at 1125, peonidin aglycon with *m/z* at 301, 963 $[M - 162]$) were also the major anthocyanin compounds of PFSP. Peaks 10 and 12 were identified as peonidin 3-caffeoylsophoroside-5-glucoside and peonidin-dicaffeoylsophoroside-5-glucoside, respectively. Such two compounds were previously found in Japanese purple sweet potato (*I. batatas* L.).[14] Meanwhile, peak 14 was identified as peonidin-caffeoyl-feruloylsophoroside-5-glucoside, which did not appear as a major anthocyanin compound in PFSP roots in previous studies. It was reported that the peonidin/cyanidin ratio in purple-fleshed sweet potato affects the color of the roots.[28] This indicated that a higher ratio of peonidin to cyanidin showed a greater degree of redness, whereas the sweet potatoes with a large amount of cyanidin showed a greater degree of blueness. The PFSP in this study showed red-purple color, indicative of a higher peonidin/cyanidin ratio.

**Antioxidative Activity of PFSPE in PBL and SL-Based O/W Emulsion.** Hydroperoxides were generally accepted as the first oxidation product of lipid. Therefore, POV was measured to observe the initial rates of oxidation in PBL- and SL-based emulsions with different concentrations of PFSPE. As shown in Figure 4, compared with POV of control (blank), POVs were obviously reduced by PFSPE in both PBL- and SL-based emulsions in which higher PFSPE concentration generally led to lower POV. For PBL-based emulsion (Figure 4A), antioxidants (PFSPE and catechin) significantly ($p < 0.05$) decreased the POVs. There was no significant difference ($p > 0.05$) between POVs of emulsions with 500 and 1000 ppm PFSPE during 16 days of oxidation. Such concentrations of PFSPE, however, showed higher POVs than 200 ppm catechin. For SL-based emulsion (Figure 4B), different concentrations of PFSPE showed somewhat different results. The lowest concentration of PFSPE (200 ppm) led to the highest level of POV (15.70 mequiv/L emulsion), and there was no significant difference with that of control (16.25 mequiv/L emulsion) on the last day of oxidation. However, the highest level of PFSPE (1000 ppm) showed a low level of POV during 16 days with no significant difference ($p > 0.05$) to POV of 200 ppm catechin.

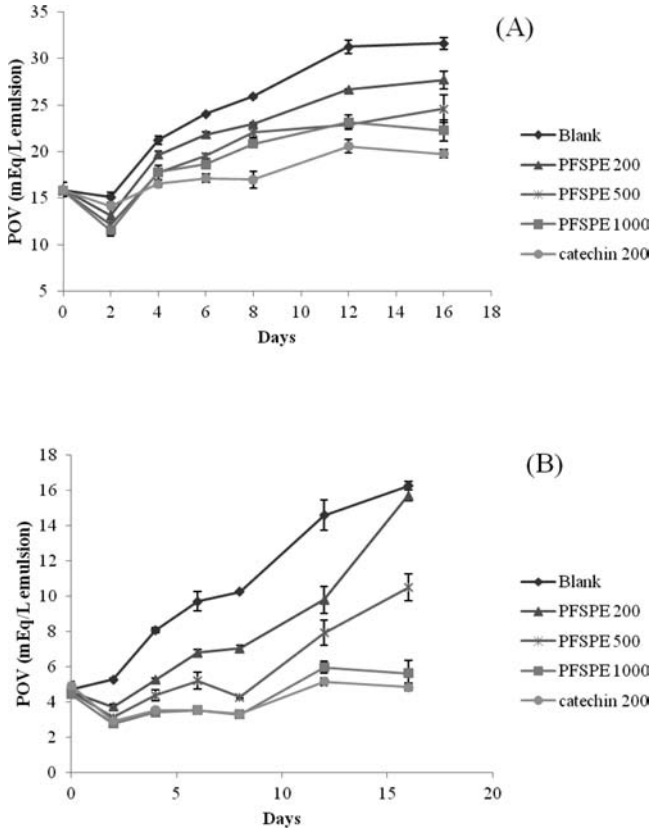


**Figure 4.** Changes in POV with time in physically blended lipid-based emulsion (A) and structured lipid-based emulsion (B). PFSPE 200, PSPE 500, and PSPE 1000 designate the emulsions with purple-fleshed sweet potato extract in the concentrations of 200, 500, and 1000 ppm, respectively. Catechin 200 designates the emulsion with 200 ppm catechin. Data shown are the average of duplicate samples. Error bars on the chart represent standard deviation.

TBARS are secondary oxidation products formed from the degradation of oxidized polyunsaturated fatty acids. Oxidation products of PBL- and SL-based emulsions in the presence of different concentrations of PFSPE were observed (Figure 5). The presence of PFSPE and catechin reduced the TBARS of both PBL- and SL-based emulsions when compared with TBARS of control (blank). For PBL-based emulsions (Figure 5A), concentrations of PFSPE showed no significant effect ($p > 0.05$) on TBARS, and all three levels of PFSPE showed higher TBARS than 200 ppm catechin from the 2nd to the 16th day. For SL-based emulsions (Figure 5B), however, TBARS were not significantly different ($p > 0.05$) among 500 and 1000 ppm

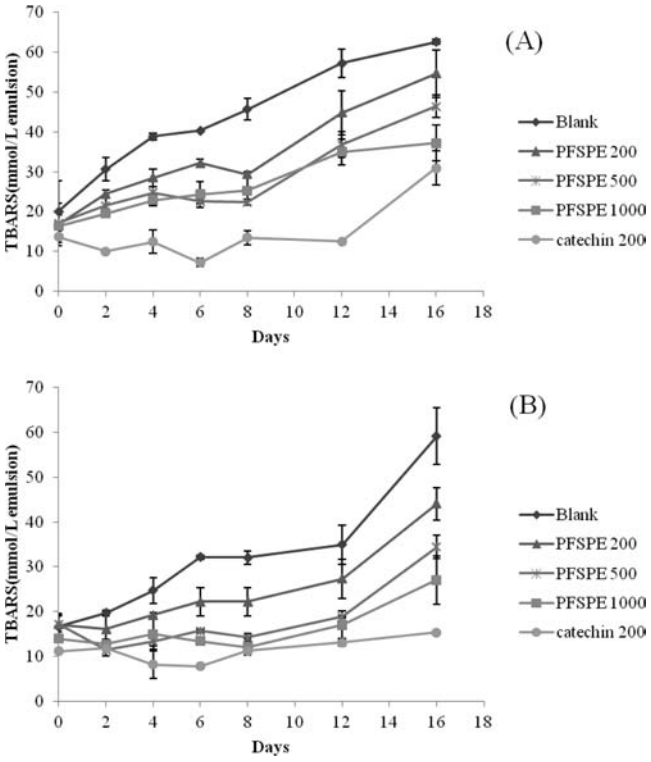


**Figure 5.** Changes in TBARS with time in physically blended lipid-based emulsion (A) and structured lipid-based emulsion (B). PFSPE 200, PSPE 500, and PSPE 1000 designate the emulsion with purple-fleshed sweet potato extract in the concentrations of 200, 500, and 1000 ppm, respectively. Catechin 200 designates the emulsion with 200 ppm catechin. Data shown are the average of duplicate samples. Error bars on the chart represent standard deviation.

PFSPE and 200 ppm catechin up to 12 days. In addition, 200 ppm PFSPE showed lower TBARS than control, but higher than the others (500 and 1000 ppm PFSPE; 200 ppm catechin) during 16 days of oxidation. When TBARS were compared on the 16th day, SL-based emulsions with antioxidants (catechin 200 ppm, 15.36 mmol/L emulsion; PFSPE 1000 ppm, 27.05 mmol/L emulsion; PFSPE 500 ppm, 34.43 mmol/L emulsion; PFSPE 200 ppm, 44.07 mmol/L emulsion) showed significantly lower ($p < 0.05$) TBARS value than the corresponding PBL-based emulsions (catechin 200 ppm, 30.94 mmol/L emulsion; PFSPE 1000 ppm, 37.20 mmol/L emulsion; PFSPE 500 ppm, 46.42 mmol/L emulsion; PFSPE 200 ppm, 54.62 mmol/L emulsion), respectively, whereas the control (blank) from PBL- and SL-based emulsions showed no significant differences from one another. The result indicated that PFSPE and catechin showed more effective antioxidative capacity in SL-based emulsion than in PBL emlusion.

According to the HPLC-MS results, the main anthocyanin compounds in PFSPE were peonidin and cyanidin derivatives (Table 2). It is generally considered that the antioxidative capacity of phenolic compounds is usually enhanced when the number of hydroxyl groups in the B-ring increases. Therefore, the cyanidin with 3′,4′-di-OH would have higher radical scavenging activity than peonidin with only one OH group in the B-ring. Previous papers showed a general trend of decreasing antioxidative potency by glycosylation.[29,30] Therefore, catechin, which is free of glycosylation, showed higher antioxidative ability than the PFSPE in our study. In this study, the antioxidative activity of PFSPE increased with increasing concentration, where the concentration of 1000 ppm showed the highest antioxidative ability in both PBL- and SL-based O/W emulsions. A similar result was obtained by Viljanen et al.,[31] who found that anthocyanin inhibited lipid oxidation by 76%, inhibiting hexanal formation in purified rapeseed oil-based emulsion prepared with whey protein as an emulsifier.

It is generally considered that, apart from those generated by light, lipid oxidation in emulsion systems is usually induced by the radicals generated in the aqueous phase.[32] According to the previous papers, PFSPE possessed strong radical scavenging activity due to the appearance of anthocyanin compounds.[17,33] Such anthocyanins could capture the free radicals by donation of phenolic hydrogen atoms in the water phase to block the lipid radical reactions. This might be one of the dominant antioxidation mechanisms of PFSPE in emulsion systems. Meanwhile, we employed sophorolipids as an emulsifier for preparing the emulsions. Therefore, whether the sophoroside and glucoside groups in anthocyanin compounds could interact with the sophorolipids to act as a barrier in lipid oxidation needs further research.

It is notable that SL-based emulsions appeared to have better oxidative stability than PBL-based emulsions, whereas, in previous studies, SLs mostly showed less oxidation stability than the lipids before modification, partly due to the decrease of antioxidant content or the changes of fatty acid composition.[1,34] Therefore, we investigated the tocopherol contents in both PBL and SL (Table 3). As shown, only $\delta$-tocopherol showed a significant decrease after interesterification reaction. Thus, tocopherol content was assumed to have not much relevance to the different oxidation stability between PBL- and SL-based emulsions. The fatty acid compositions, in Table 1, of PBL and SL were not much different either. Therefore, it is assumed that the increased amounts of the reaction byproduct such as DAG would be one reason for the different oxidation stabilities of PBL- and SL-based emulsion systems. According to our current results, the SL-based emulsion with certain amounts of MAG and DAG showed smaller emulsion particle size and higher physical stability than the corresponding PBL-based emulsion (unpublished data). It seems that the higher physical stability of SL-based emulsion might lead to higher oxidative stability. Furthermore, reaction byproducts such as DAG and MAG could act as an emulsifier in the emulsion systems. It was reported that higher concentrations of emulsifiers led to lower oxidation rates in model O/W emulsions prepared with menhaden oil/caprylic acid SL.[35] In our study, the presence of higher amounts of DAG in the SL-based emulsion might result in tighter packing of surfactant molecules (including sophorolipids) at the oil−water interface than in the PBL-based emulsion. Therefore, such a tight membrane may act as an efficient barrier to the diffusion of

**Table 3. Tocopherol Contents (Milligrams per 100 g) of Physically Blended Lipid (PBL) and Structured Lipid (SL)[a]**

| | PBL | SL |
|---|---|---|
| $\alpha$-tocopherol | 2.98 ± 0.12 a | 2.76 ± 0.18 a |
| $\beta$-tocopherol | 0.26 ± 0.03 a | 0.23 ± 0.05 a |
| $\gamma$-tocopherol | 8.46 ± 0.19 a | 8.11 ± 0.18 a |
| $\delta$-tocopherol | 7.35 ± 0.13 a | 5.65 ± 0.13 b |
| total tocopherols | 19.05 ± 0.47 a | 16.75 ± 0.54 b |

[a]Values are the mean ± SD of duplicates. Means in rows with different letters (a, b) are significantly different ($p < 0.05$).


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lipid oxidation initiators into the oil droplets.[32] Further studies will be needed because the activity of antioxidants in emulsion systems depends on a complex multitude of factors including the colloidal properties of the substrate and the location and orientation of antioxidants as well as the conditions and stages of oxidation, etc. [32]

In conclusion, the PFSPE of 1000 ppm showed the highest antioxidative ability in both PBL-based emulsion (soybean oil/fish oil = 3:1, w/w) and SL-based emulsion. The strong radical scavenging activity might be one of the dominant antioxidation mechanisms of PFSPE in this emulsion system. Thus, the PFSPE can be a potential natural antioxidant for emulsion systems. Furthermore, the SL-based emulsion with PFSPE and catechin showed higher oxidative stability than PBL-based emulsion, leading to further studies.

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