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ORIGINAL PAPER

Characterization and Oxidative Stability of Structured Lipids: Infant Milk Fat Analog

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Abstract Structured lipids (SLs) for infant milk formulation were produced by enzymatic interesterification of tripalmitin with vegetable oil blends and fish oil. The SLs were characterized by fatty acid content and structure, melting profiles, oxidative stability index, free fatty acid (FFA) concentration, and tocopherol content. Oxidative stability was studied using accelerated methods by quantifying FFA, peroxides (peroxide value) and aldehydes (p-anisidine value) production. Total oxidation (TOTOX value) was calculated as 2 × (peroxide value) + (p-anisidine value). The structured lipids after purification by distillation and addition of antioxidants had melting profiles, oxidative stability index, and initial FFA concentration that were similar to that of the starting oil blends, while the fatty acid composition and structure of the SLs were similar to that of human milk fat. Oxidative stability of the SLs was improved with tocopherol addition as antioxidants and was comparable to that of the vegetable oils and oil blends.

Keywords Infant milk fat analog · Oil blends · Oxidative stability · Oxidative stability index · Palmitic acid · Structured lipids

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Introduction

Developing infant milk fat similar to human milk fat (HMF) is of great interest and a challenge to food processors. In most vegetable oils used for infant milk fat production, the sn-1,3 positions of the TAGs are occupied mainly by saturated fatty acids, while in human milk these positions contain mainly unsaturated fatty acids [1]. The location of saturated fatty acids especially palmitic acid at the sn-2 position of triacylglycerols (TAGs) increases the efficiency of absorption of fatty acids from the lumen and also decreases calcium loss in infants [2]. Unlike vegetable oils, HMF contains about 40–60% palmitic acid at the sn-2 position of the TAGs [3].

Structured lipids (SLs) containing similar fatty acid structure as HMF can be produced by interesterification reactions using an sn-1,3-specific lipase that gives high selectivity and mimics the natural pathways of metabolic processes [4]. Preliminary studies in our laboratory have revealed that enzymatic interesterification of tripalmitin with a vegetable oil blend containing coconut, safflower and soybean oils, using lipozyme RM IM as a biocatalyst, can be successful in the production of SL that mimic the fatty acid composition and structure of HMF. Safflower and soybean oils are two sources of polyunsaturated fatty acids that together with coconut oil, produces an acceptable combination of fat that can be employed in infant milk fat formulation [5]. Furthermore, HMF contains small amounts of long chain polyunsaturated fatty acids (LCPUFAs), and the fortification of infant milk with these LCPUFAs by addition of fish oil is becoming increasingly popular for additional health benefits [6].

However, the successful production of SLs for infant milk formulation can be impeded by their high susceptibility to oxidative deterioration [7–9]. SLs or infant milks

produced with lipids containing unsaturated fatty acids can deteriorate during storage and produce off-flavors and odors characteristic of oxidation [8, 10]. Furthermore, the production process for SLs increases free fatty acids (FFA) concentration, which is also responsible for off-flavor development [10]. A high concentration of FFA in infant milk induces a rancid and bitter taste that is unacceptable. Also, unsaturated fatty acids in infant milk fat can be oxidized into hydroperoxides (primary oxidation products), which can then be rapidly decomposed to secondary oxidation products such as alkanes, alkenes, aldehydes, and ketones [10]. Oxidative deterioration and high FFA concentration can alter the nutritional quality of SLs [9] and render infant milks containing these lipids potentially toxic and unacceptable to consumers [10]. Oxidation stability of structured lipids intended for infant milk formulation, provides insight on the acceptability of these milks for consumption. This paper therefore aims to characterize and study the oxidative stability of SLs intended as infant milk fat analogs.

Materials and Methods

Materials

Coconut, safflower and soybean oils were purchased from local retail outlets (Athens, GA, USA). Menhaden fish oil, tripalmitin and mixed tocopherols were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Benzene, ethanol, methanol, glacial–acetic acid, hexane, chloroform, iso-octane and other organic solvents and chemicals were purchased from the J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) or Fisher Scientific (Norcross, GA, USA). Lipid standards including heptadecanoic acid, methyl esters of caprylic, capric, lauric, myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, arachidonic, behenic, eicosapentaenoic, and docosahexaenoic acids were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Lipozyme RM IM, an immobilized sn-1,3-specific lipase from *Rhizomucor miehei*, was obtained from Novozymes A/S (Bagsvaerd, Denmark). Apha (α-) tocopherol and tocotrienol standards were obtained from Fluka (Sigma Aldrich, St. Louis, MO, USA) and beta (β-), gamma (γ-), and delta (δ-) tocopherol standards were obtained from the Sigma Chemical Co.

Preparation of Oil Blends

Two different oil blends were prepared by mixing a 2.5:1.1:0.8, v/v/v blend of coconut, safflower, and soybean oils, respectively (B1), and by mixing a 2.1:1.1:0.8:0.4, v/v/v/v blend of coconut, safflower, soybean and fish oils, respectively (BFO). Coconut oil was melted to a liquid at 40 ± 2 °C before blending to ensure uniform distribution of the oil mixture.

Synthesis of Structured Lipids

Structured lipids (SLs) were synthesized in a batch-wise packed-bed bioreactor by enzymatic interesterification of a 1:3 molar ratio of tripalmitin to B1 and 1:3 molar ratio of tripalmitin to BFO to give S1 (SL synthesized from B1) and SFO (SL synthesized from BFO), respectively. Table 1 indicates oil blend ratios and their components. Lipozyme RM IM (10% weight of total reactants) was used as a biocatalyst in the reaction. The bioreactor was set-up according to the process described by Xu et al. [11]. The reaction was carried out at a reaction temperature of 55 °C and an incubation time of 14.4 h.

Purification of Synthesized Structured Lipids

The synthesized SLs were purified by removal of FFA as described by Akoh and Moussata [8]. A UIC KDL-4 short-path distillation system (UIC Inc., Joliet, IL, USA) equipped with a 0.04 m^2 heated evaporated surface and a 500-mL 2-neck receiver, was used for the purification process.

Table 1 Fatty acid (mol % /100 g) composition of the oil blends, purified structured lipids, and human milk fat

Fatty acids	HM*	B1	BFO	S1	SFO
C8:0	–	7.9 ± 0.1[a]	4.1 ± 0.9[a]	1.5 ± 0.1[c]	1.2 ± 0.2[c]
C10:0	2[d]	7.4 ± 0.1[a]	4.5 ± 0.1[b]	2.9 ± 0.3[c]	2.4 ± 0.3[cd]
C12:0	7[e]	26.6 ± 0.7[a]	20.7 ± 1.0[c]	23.6 ± 1.1[b]	17.1 ± 1.2[d]
C14:0	8[c]	7.5 ± 0.1[cd]	10.0 ± 0.3[a]	7.2 ± 0.2[d]	9.0 ± 0.3[b]
C16:0	23[b]	5.2 ± 0.2[d]	9.3 ± 0.3[c]	24.6 ± 1.3[ab]	26.0 ± 1.4[a]
C16:1n-7	3[a]	ND	0.8 ± 0.4[b]	ND	0.3 ± 0.0[b]
C18:0	7[a]	5.1 ± 0.1[b]	5.7 ± 0.6[b]	3.2 ± 0.4[c]	3.0 ± 0.1[c]
C18:1n-9	38[a]	32.1 ± 0.3[c]	34.6 ± 0.2[b]	29.6 ± 1.4[d]	30.7 ± 0.7[cd]
C18:2n-6	9[a]	7.2 ± 0.2[b]	7.3 ± 0.7[b]	6.4 ± 0.2[b]	7.1 ± 0.0[b]
C18:3n-3	1[c]	0.9 ± 0.0[c]	1.6 ± 0.4[a]	1.1 ± 0.0[bc]	1.5 ± 0.0[ab]
C20:5n-3	0.2[c]	ND	1.3 ± 0.2[a]	ND	1.2 ± 0.0[b]
C22:6n-3	0.4[a]	ND	0.3 ± 0.0[b]	ND	0.2 ± 0.0[c]

* Source: [3, 6]. *HM* Human milk fat, *B1* oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils, *BFO* oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils, *S1* structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h, *SFO* structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h, *ND* not detected. Data with the same letters within rows are not significantly different ($p < 0.05$)

The heating oil temperature was 185 °C, while the water temperature was 20 °C. Free fatty acids were removed under vacuum (<1 mmHg) from the SLs at a flow rate of 100 mL/h. The purified SLs (S1 and SFO) were both divided into two equal portions, and vitamin E (mixed tocopherols) was added to one portion at 1 mg/4.4 g (227 ppm) to give S1V and SFOV, respectively. The second portion was left as it was and served as a control. All samples were then flushed with nitrogen and stored at −80 °C until needed for analysis.

Characterization of Structured Lipids

Fatty Acid Analysis

The fatty acid composition of the oil blends and SLs were determined. A 0.1-g amount from each of B1, BFO, SI and SFO was pipetted into a screw-cap reaction tube. Then 20 µL of heptadecanoic acid (0.1 mg/mL) was added to each tube as an internal standard. Thereafter, 3 mL of 6% HCl in methanol was added and the mixture was methylated by incubating at 75 °C for 2 h in a pre-heated oven. Upon methylation, the fatty acid methyl esters (FAME) were twice extracted with 2 mL hexane and 1 mL 0.1 M KCl, and centrifuged at 1,000 rpm ($400\times g$) for 3 min. The upper (hexane) layer was decanted and combined for each sample, then passed through anhydrous sodium sulfate columns. The excess solvent was evaporated under nitrogen until about 1 mL aliquot was obtained.

Gas Chromatographic (GC) Analysis of FAME

Fatty acid profiles were quantified using an Agilent 6890N gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). Helium was the carrier gas and the gas flow rate was 1.7 mL/min. The oven temperature was initially held at 80 °C for 3 min and then programmed to 215 °C for 10 min at a rate of 10 °C/min, then held isothermally for 20 min. The column used was a fused silica Heliflex capillary column (Alltech-AT-225: 30 mm × 0.25 mm × 0.25 µm film thickness; Deerfield, IL, USA). The different amounts of FAME (mol%) were analyzed and integrated by an integrator (model G2070AA, Agilent Technologies, Palo Alto, CA, USA) with reference to C17:0 as an internal standard.

Sn-2 Positional Fatty Acid Analysis by Pancreatic Lipase

Fatty acids at the sn-2 position were analyzed according to the method described by Sahin et al. [4].

Oxidative Stability Index

The oxidative stability index (OSI) of the vegetable oils, fish oil, oil blends, SLs with added tocopherols (SIV and SFOV), and SLs without added tocopherols (SI and SFO) were determined at 110 °C with an Oil Stability Instrument (Omnion, Rockland, MA, USA), according to the AOCS Cd 12b-92 method [12].

Free Fatty Acids Value

The FFA value was determined by the AOCS Ca 5a-40 method [12]. Titration was done with KOH, using phenolphthalein as an indicator. The FFA was calculated as % oleic acid.

Melting Profiles Determination

The melting profiles of the samples were determined by differential scanning calorimetry according to the AOCS Cj 1-94 method [12].

Tocopherol Determination

Tocopherol composition of the samples was determined according to the HPLC method described by Lee et al. [13], using a Shimadzu SRI203 HPLC system (Kyoto, Japan), equipped with a LC-6A pump, RF-10A spectrofluorometric detector. A normal phase column LiChrosorb Si 60 (5 µm 25.0 × 0.4 mm i.d.; Alltech Assoc., Inc., Deerfield, IL, USA) coupled with a precolumn (30–40 µm) packed with Perisorb A, was used for the separation. The mobile phase consisted of hexane and 2-propanol (99.7:03, v/v). The excitation and emission wavelengths of the detector were set at 290 and 330 nm, respectively. Five standard isomers (α, β, γ, δ-tocopherol and α-tocotrienol) were used for the quantification of vitamin E in all samples. Total tocopherol was calculated as the sum of the overall proportion of individual tocopherol isomers.

Oxidation Experiments

Five-gram amounts of the vegetable oils, fish oil, oil blends, SLs and SLs with added tocopherols were weighed individually into soda-glass tubes (25 mm × 200 mm) and oxidized uncovered for 72 h at 60 °C in the dark using a shaking water bath (New Brunswick Scientific Co., Edison, NJ, USA) as described by Akoh and Moussata [8]. The oils

were sampled at 0, 24, 48, and 72 h of oxidation for the FFA value, peroxide value (PV), and p-anisidine value (P-AV). The PV was determined by the acetic acid–isooctane method of AOCS Cd 8b-90 [12]. The P-AV was determined by the spectrophotometric method of AOCS Cd 18–90 [12]. The TOTOX (total oxidation) value was calculated as 2 × (PV) + (P-AV) as described by Shahidi and Wanasundra [14].

Statistical Analysis

All experimental data were produced in triplicate, analyzed by analysis of variance, and mean differences between treatments (oils, oil blends, non-structured and structured lipids) using the general linear model program of the SAS package [15]. All results are presented as means of the replicates.

Results and Discussion

Characterization of Structured Lipids

Fatty Acid Composition

The fatty acid composition of the oil blends and their corresponding SLs are given in Table 1. The enzymatic interesterification reaction increased the C16:0 content of the SLs. There was a 78 and 64% increase in this fatty acid content for S1 and SFO, respectively. S1 showed a decrease in C8:0 and C10:0 content, in comparison to the oil blends, while the opposite trend occurred for C16:0 with about 6% greater decrease in S1 than SFO (Table 1). These decreases in medium chain saturated fatty acid content in the SLs are attributed to the increased amount of C16:0 incorporated into the TAGs of the oils (Table 1). The SLs have a fatty acid profile nearer to that of the human milk fatty acid composition in comparison to the vegetable oil blends.

BFO contained LCPUFAs as a result of its fish oil content, which is also responsible for its significant EPA and DHA content compared to B1. Likewise, the LCPUFA content of SFO is attributable to the fish oil content of its starting oil blend (Table 1). Fatty acid composition of BFO and SFO indicate that the enzymatic interesterification process had minimal effect on the EPA and DHA concentration. About 94 and 92% of these LCPUFAs were retained after the interesterification reaction with sn-1,3 specific lipase from *R. miehei*. These results are similar to those reported by Jennings and Akoh [7], Akoh and Moussata [8], and Sahin et al. [6].

The sn-2 Positional Fatty Acid Composition

The sn-2 positional fatty acid composition of the SLs and oil blends are given in Table 2. The incorporation of C16:0 at the sn-2 position increased significantly from the oil blends to the SLs due to the interesterification reaction. Both SLs (S1 and SFO) had about a 40–46% C16:0 incorporation suggesting that most of the C16:0 contained in these SLs are located at the sn-2 position. This is unlike the results obtained for the unreacted oil blends (Table 2). Similar results were obtained by Nielsen et al. [10], where a good portion of the C16:0 was incorporated at the sn-2 position of the human milk fat substitutes produced by enzymatic reactions.

There was a decrease in the C12:0 at the sn-2 position of the SLs in comparison to their unreacted counterparts. Trace amounts of EPA and DHA were also incorporated at the sn-2 position of SFO, which is due to the fish oil content of its starting material (Table 2). These results are similar to that reported by Sahin et al. [6], where less than 1% total EPA and DHA were incorporated at the sn-2 position after esterification reactions using lipozyme RM IM.

The SLs had increased amounts of C18:0 located at the sn-2 position, unlike the oil blends. This suggests that a good portion of this fatty acid migrated to the sn-2 position during the enzymatic interesterification reaction (Table 2). The amount of C18:0 at the sn-2 position of the SLs were closer to that of HMF, in comparison to the oil blends, which show trace amounts of C18:0 at the sn-2 position. The sn-2 fatty acid profiles of the SLs more closely resemble HMF, in comparison to the oil blends.

Table 2 The sn-2 fatty acid profile (mol% /100 g) of the oil blends, purified structured lipids and human milk fat

Fatty acids	HM*	B1	BFO	S1	SFO
C8:0	–	0.6 ± 0.1[c]	0.8 ± 0.0[c]	4.1 ± 0.2[b]	5.5 ± 0.3[a]
C10:0	0.2[c]	2.6 ± 0.3[b]	2.4 ± 0.1[b]	3.6 ± 0.2[a]	3.7 ± 0.2[a]
C12:0	2.1[d]	67.5 ± 1.7[a]	63.9 ± 1.5[b]	16.3 ± 0.4[c]	17.1 ± 0.6[c]
C14:0	7.3[b]	7.1 ± 0.6[b]	7.0 ± 0.8[c]	9.5 ± 0.5[a]	5.1 ± 0.5[c]
C16:0	40–60[a]	0.7 ± 0.1[b]	0.7 ± 0.3[b]	40.8 ± 0.8[a]	46.9 ± 1.5[a]
C16:1n-7	4.7	ND	ND	ND	Tr
C18:0	3.3[a]	Tr	Tr	2.6 ± 0.1[b]	1.0 ± 0.2[c]
C18:1n-9	12.7[b]	13.2 ± 0.6[b]	16.0 ± 1.2[a]	14.3 ± 0.5[ab]	8.7 ± 0.4[c]
C18:2n-6	7.3[c]	7.8 ± 0.3[b]	9.4 ± 0.8[a]	8.6 ± 0.2[ab]	6.7 ± 0.3[c]
C18:3n-3	0.6[a]	0.5 ± 0.1[ab]	0.3 ± 0.0[c]	0.3 ± 0.0[c]	0.4 ± 0.1[bc]
C20:5n-3	Tr	ND	0.1 ± 0.0	ND	Tr
C22:6n-3	Tr	ND	0.1 ± 0.0	ND	Tr

* Source: [3, 6]. *HM* Human milk fat, (B1, BFO, S1, SFO: see Table 1 for explanation of abbreviations), *ND* not detected, *Tr* trace. Data with same letters within rows are not significantly different ($p < 0.05$)

Oxidative Stability Index

The OSI of the vegetable oils, fish oil, oil blends, SLs with added tocopherols and their controls are given in Table 3. Coconut oil had the highest oxidative stability in comparison to the other oils. This is due to the high content of saturated fatty acids in coconut oil, leading to a high resistance to oxidation. On the other hand, fish oil had the lowest OSI value (0.2 h at 110 °C), and therefore the lowest resistance to oxidative deterioration. The low OSI value of fish oil is attributed to its high-unsaturated fatty acids content, especially the LCPUFAs. Although safflower and soybean oils, which contain high amounts of monounsaturated fatty acids (MUFAs) had lower OSI values than coconut oil, they also had better oxidative stability than fish oil (Table 3).

The enzymatic interesterification reaction appeared to increase the susceptibility of the SLs to oxidation, as the OSI values of S1 and SFO decreased significantly from that of B1 and BFO (Table 3). Factors such as loss of tocopherols and phospholipids [8, 16] during the short-path distillation process might be associated with the low stability of S1 and SFO samples. However, the addition of tocopherols as an antioxidant to the SLs positively affected their oxidative stability. The OSI values of SIV and SFOV were significantly higher than those of S1and SFO (Table 3). Although antioxidant addition increased the oxidative stability of the SLs (Table 3), the presence of LCPUFAs also had an impact on the OSI values of these SLs, irrespective of their tocopherol content. The results in Table 3 show that the OSI values of SIV and SFOV at 110 °C are more comparable to those of B1 and BFO, unlike the values of S1 and SFO at the same OSI temperature.

Table 3 Physico-chemical characteristic of the vegetable oils, fish oil, oil blends and purified structured lipids

Samples	Oxidative stability index at 110 °C (h)	Free fatty acid (%)	Melting profile (0 °C)
Coconut oil	79.1 ± 0.8^{a}	0.1 ± 0.0^{d}	−35 to 27
Safflower oil	3.5 ± 0.3^{cd}	0.1 ± 0.0^{d}	−35 to 27
Soybean oil	3.2 ± 0.3^{cd}	0.1 ± 0.0^{d}	−34 to 26
Fish oil	0.2 ± 0.0^{e}	0.3 ± 0.0^{b}	−31 to −6
B1	10.8 ± 1.0^{b}	0.2 ± 0.0^{c}	−27 to 22
BFO	4.4 ± 0.5^{c}	0.2 ± 0.0^{c}	−28 to 22
S1	0.9 ± 0.1^{e}	0.3 ± 0.0^{b}	−27 to 38
SFO	0.9 ± 0.2^{e}	0.4 ± 0.0^{a}	−25 to 38
S1V	10.4 ± 0.2^{b}	0.3 ± 0.0^{b}	−26 to 38
SFOV	2.7 ± 0.5^{d}	0.3 ± 0.0^{b}	−25 to 38

B1 Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils; (B1, BFO, S1, SFO: see Table 1 for explanation of abbreviations), *S1V* S1 with added tocopherols (227 ppm); *SFOV* SFO with added tocopherols (227 ppm)

Data with same letters within columns are not significantly different ($p < 0.05$)

Free Fatty Acid Content

The FFA content of the vegetable oils, fish oil and oil blends are given in Table 3. Although coconut oil had the lowest FFA content, the vegetable oils and oil blends all had FFA concentrations <1%, (Table 3). The FFA concentrations in the SLs were comparable to those of the starting materials, indicating that the short-path distillation process was successful in removing most of the FFAs generated during the enzymatic interesterification reaction. Akoh and Moussata [8] and Nielsen et al. [10] reported similar results of FFA removal using short-path distillation.

Melting Profile

The melting profiles of the samples are given in Table 3. The vegetable oils have higher melting profiles than fish oil, which has a melting point range below 0 °C. Coconut oil has a melting profile similar to that of the vegetable oils, while safflower and soybean oils have melting points of −17 and −20 °C, respectively. The oil blends on the other hand showed different melting profiles relative to their individual oil content. BFO had a slightly lower and narrower melting point range than B1 (Table 3), which is due to its fish oil content. However, these oil blends appeared to have a narrower melting profile than the individual oils, which are attributable to higher content of medium-chain saturated fatty acids, with closer melting points. The SLs on the other hand had higher contents of long-chain saturated fatty acids (C14:0 and C16:0) in comparison to the individual oils, and higher contents of C16:0 in comparison to the oil blends. The SLs had higher melting profiles than the other oils (Table 3), attributable to the high degree of saturation of these oils relative to the individual oils and oil blends, and to the interesterification process. Since saturated fatty acids have higher melting points than unsaturated fatty acids, the incorporation of C16:0 in the SLs by interesterification therefore led to higher melting profiles in these oils. The melting point of the SLs is relevant to their physical state at a given temperature, which therefore affects their performance when used in the formulation of products like infant milks. Tocopherol addition had minimal effect on the melting profiles of the SLs, because the melting point range of S1 and SFO were comparable to those of SIV and SFOV (Table 3).

Tocopherol Content

The tocopherols contents of the samples are given in Table 4. Safflower and soybean oil had the highest total tocopherol values, with soybean oil having about 67% higher tocopherols than safflower oil. No detectable α-tocotrienol was found in both oils. Total tocopherol content of safflower oil consists mostly of α-tocopherol, while that of soybean oil consists of γ- and δ-tocopherols. Coconut oil had the highest content of α-tocotrienol. Fish oil had the lowest total tocopherol content (Table 4) consisting mainly of α-tocopherol and small amounts of δ-tocopherol. Jennings and Akoh [7] reported similar tocopherol results for unmodified menhaden fish oil. The oil blends contained significant amounts of all four types of tocopherols (α, β, γ, δ) and α-tocotrienol, with α- and γ-tocopherol making the highest contribution to the total. β-Tocopherol was low in all samples relative to the other tocopherol homologues (Table 3).

Purification of the SLs by short-path distillation had a negative impact on the tocopherol content (Table 4). There was a 54% loss in total tocopherol content from the oil blends during the short-path distillation processes (Table 4). Akoh and Moussata [8] reported similar tocopherol losses, where enzyme processing and exposure to light and heat may be responsible for the losses. According to Hamam and Shahidi [17], the formation of tocopheryl esters during the interesterification reaction might also be responsible for the loss of endogenous tocopherols present in the oils. There were higher losses of α-tocopherol in the samples relative to the other tocopherol types, which might be as a result of its higher heat lability and therefore greater susceptibility to destruction during the short-path distillation process [17]. The addition of mixed tocopherols (up to 227 ppm) as antioxidants to the SLs increased their total tocopherol content by about 42–50%, which is apparent in the total tocopherol content of S1V and SFOV, with respect to S1 and SFO. The lower content of γ- and δ-tocopherol in the enriched samples (SIV and SFOV) is due to the proportionate increase in other tocopherol isomers, with respect to total tocopherol content.

Oxidative Stability of Oils

Free Fatty Acid Value

The FFA values were low (<1%) for all samples throughout the 72 h accelerated oxidation period at 60 °C. There was no significant difference in the FFA content of safflower and soybean oils during the oxidation period, though coconut oil had the lowest value, and fish oil the highest (Data not shown). The high FFA value of fish oil is due to its higher concentration of polyunsaturated fatty acids, and therefore increased susceptibility to oxidation in comparison to the other oils. The SLs had higher FFA values than their starting materials, with SFO and SFOV having higher values than their counterparts (S1 and S1V) due to their fish oil content. However, the higher initial FFA values (>0.1%) of the SLs in comparison to the vegetable oils and oil blends may have been as a result of increased FFA production during the enzymatic interesterification process. The short-path distillation step resulted in a 0.3–0.4% residual FFA concentration in the SLs. Similar residual values were reported by Nielsen et al. [10], where about 0.3% FFA remained after the distillation process.

Peroxide Value

The PV of the oils are presented in Fig. 1. Coconut oil had the lowest initial (<0.1 mequiv/1,000 g) and final

Table 4 Tocopherol content (mg/100 g) of the vegetable oils, fish oil, oil blends and purified structured lipids

Samples	Total tocopherol	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol
Coconut oil	2.4 ± 0.2[h]	0.19	0.0	0.2	ND	2.1
Safflower oil	32.4 ± 0.8[b]	30.4	0.5	1.2	0.3	ND
Soybean oil	97.4 ± 0.1[a]	6.6	0.6	60.3	29.9	ND
Fish oil	2.3 ± 0.1[h]	2.3	ND	ND	0.0	ND
B1	27.2 ± 1.7[d]	10.1	0.2	10.6	5.0	1.3
BFO	30.0 ± 0.3[c]	11.5	0.3	11.6	5.4	1.2
S1	12.5 ± 0.1[g]	2.2	0.0	6.2	4.1	ND
SF0	12.9 ± 0.1[g]	2.9	0.2	6.3	3.2	0.3
S1V	24.4 ± 0.9[e]	24.0	0.0	0.1	0.4	ND
SFOV	22.3 ± 0.3[f]	21.1	0.0	0.7	0.3	0.1

B1, BFO, S1, SFO, SIV, SFOV: see Tables 1 and 3 for explanation of abbreviations, *ND* not detected

Data with same letters within a column are not significantly different ($p < 0.05$)

(<0.2 mequiv/1,000 g) values (Fig. 1). Coconut oil also had the lowest increase in PV throughout the accelerated oxidation period, which is due to its lower unsaturated fatty acid content in contrast to the other oils. The initial PV of fish oil was about 50% higher than that of the other oils. High initial PV value signifies a high production of primary oxidation products, which leads to faster progression of oxidation. Fish oil had the highest increase in PV because of high LCPUFAs and low antioxidant contents (Fig. 1).

The SLs had a lower initial PV in comparison to the oil blends. This may be as a result of the enzyme reaction process, which increased the saturation of the lipids and also in part to the distillation process, which decreased the FFA, and other volatile contents of the SLs. Akoh and Moussata [8] reported the stabilization of SLs to oxidative deterioration by incorporation of saturated medium chain fatty acid (caprylic). SFO had higher PV than S1 (Fig. 1) throughout the accelerated oxidation period because of its higher LCPUFAs content in comparison to S1. Comparison of SFO with SFOV shows significantly lower values for the SL with added tocopherol. A similar result was obtained when S1 and S1V were compared, whereby S1V has a lower PV than S1 (Fig. 1).

The PV of S1V and SFOV in comparison to that of S1 and SFO shows the effect of tocopherol addition on the oxidative stability of the SLs. The PV were quite similar for the SLs with added tocopherols, irrespective of their LCPUFA content.

P-Anisidine Value

The p-anisidine assay is used to quantify the carbonyl compounds present in oils as a means to determine the past history of the oil [9, 14]. The vegetable oils had very low P-AV (Fig. 2), unlike fish oil, which had an initial P-AV greater than 1.0/g, and about 95% increase in this index after 72 h oxidation at 60 °C. The oil blends both showed a P-AV less than 0.2 after 72 h of oxidation (Fig. 2). The high P-AV of fish oils is attributed to a high rate of secondary oxidation products formation, unlike in the vegetable oils and oil blends.

Tocopherol addition as an antioxidant had a positive effect on the P-AV of the SLs throughout the accelerated oxidation period. Comparison of S1V and SFOV to B1, BFO, S1, and SFO shows that the tocopherol-fortified oils

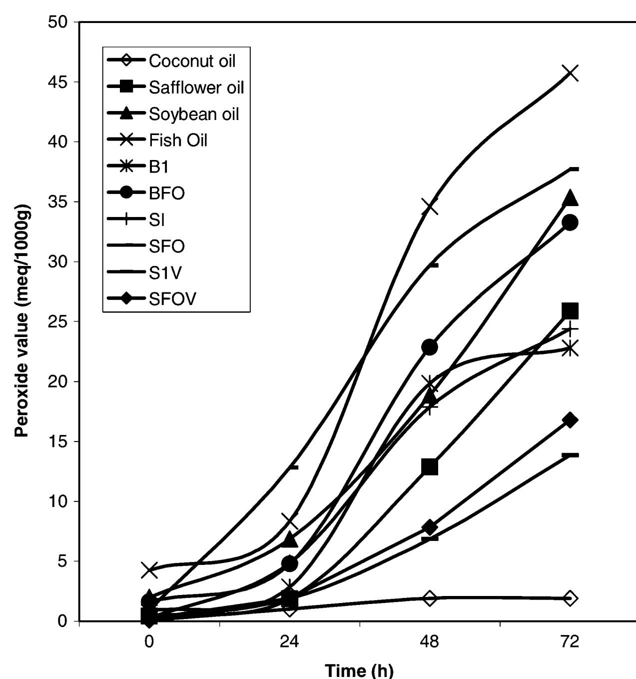

Fig. 1 Peroxide value of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h. *B1* Oil blend containing 2.5:1.1:0.8 (v/v/v) coconut, safflower, and soybean oils, *BFO* oil blend containing 2.1:1.1:0.8:0.4 (v/v/v/v) coconut, safflower, soybean, and fish oils, *S1* structured lipids made from 1:3 molar ratio of tripalmitin to B1 at 55 °C and 14.4 h; SFO: Structured lipids made from 1:3 molar ratio of tripalmitin to BFO at 55 °C and 14.4 h; S1V: S1 with added tocopherols (227 ppm); SFOV: SFO with added tocopherols (227 ppm)

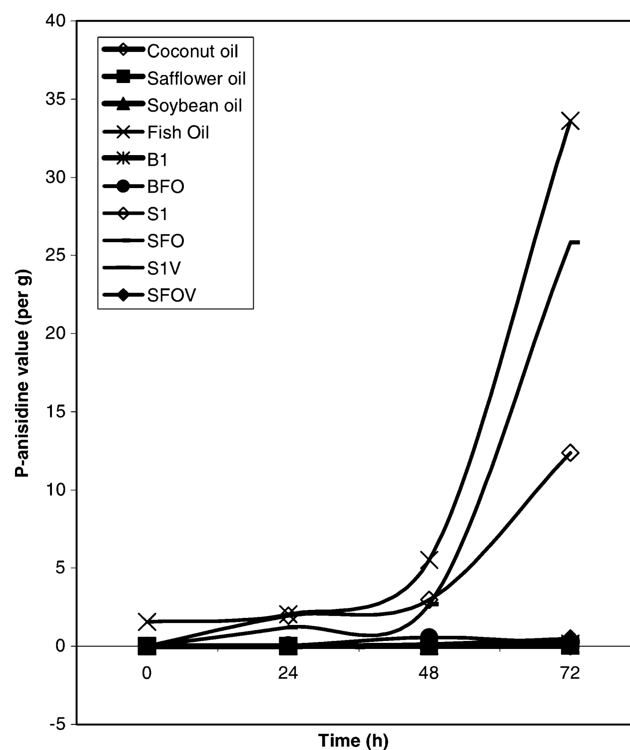

Fig. 2 The p-anisidine value of vegetable oils, fish oil, oil blends, purified structured lipids control and structured lipids with added tocopherols, oxidized at 60 °C for 72 h. (B1, BFO, S1, SFO, SIV, SFOV: see Fig. 1 for explanation of abbreviations)

had lower P-AV (Fig. 2). This is expected due to the low formation of hydroperoxides in these fortified SLs. Since the peroxides have to be present before subsequent breakdown to secondary oxidation products, it follows that low hydroperoxide formation increased the oxidative stability of these oils. The SLs with added tocopherols had P-AV that are comparable to their respective starting oil blends. S1 and SFO on the other hand, had P-AV at 24 h that are comparable to P-AV values of the oil blends at 72 h of accelerated oxidation (Fig. 2). Fish oil, S1 and SFO had higher P-AV than other samples, which suggests low oxidative stability of these oils with respect to the other oils.

TOTOX Value

The TOTOX value is a combination of the PV and P-AV normally used to determine the total oxidative stability of the oil. The SLs had higher TOTOX values than the oil blends, which is also expected due to the low antioxidant content of these oils that make them less stable to oxidation. S1V and SFOV on the other hand had lower TOTOX values than those of the oils blends, as a result of the combined effect of tocopherols and stabilizing action of high saturated fatty acids content of these samples. The SLs with added tocopherols had better total oxidative stability than their starting oils, and are therefore of better oil quality than the unfortified SLs.

Conclusion

Enzymatic interesterification increased the saturated fatty acid content and therefore the melting profile of the structured lipids. Purification of the reacted structured lipids by short-path distillation decreased the free fatty acid content as well as the total tocopherol content of the structured lipid. Addition of mixed tocopherol as an antioxidant improved the total oxidative stability of the structured lipids.

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