Oxidative Stability During Storage of Structured Lipids Produced from Fish Oil and Caprylic Acid

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ABSTRACT: Structured lipids produced by enzymatic or chemical methods for different applications have been receiving considerable attention. The oxidative stability of a randomized structured lipid (RFO), produced by chemical interesterification from fish oil (FO) and tricaprylin, and a specific structured lipid (SFO), produced by enzymatic interesterification from the same oil and caprylic acid, was compared with the stability of FO. Oils were stored at 2°C for 11 wk followed by storage at 20°C for 6 wk. In addition, the antioxidative effect of adding the metal chelators EDTA or citric acid to SFO was investigated. FO contained the largest amount of PUFA and RFO the lowest. However, SFO had a higher PV initially and during storage at 2°C, whereas the PV of FO was highest during storage at 20°C. The level of volatile oxidation products was highest in SFO during the entire storage period, and off-flavors were more pronounced in SFO. The lower oxidative stability of SFO was probably related to the initially lower quality (regarding oxidation products), which is apparently a result of the long production procedure required. Addition of metal chelators did not reduce the oxidation of the SFO.

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Technological developments in oil modification methods and an increased interest in products beneficial to the health have led to the production of structured lipids (SL). SL are lipids that are modified to have a desired FA composition. The most well known are TAG that are designed to have specific FA placed in randomized order on the glycerol backbone (randomized structured lipids, RSL) or to have specific FA located in specific positions (specific structured lipids, SSL). SSL with a long-chain PUFA in the *sn*-2 position and medium-chain FA in the *sn*-1 and *sn*-3 positions may be particularly beneficial to individuals with an increased energy requirement, since these SSL provide quick energy from the medium-chain FA and increased absorption of the essential long-chain PUFA in the *sn*-2 position (1–3). SSL can, for example, be produced to contain the beneficial n-3 FA from fish oil (FO). However, FO is susceptible to lipid oxidation due to its native polyunsaturation, and this process leads to flavor deterioration of the lipids. Because SL may be incorporated into food products, for the resulting products to be of an acceptable sensory quality, it is important that the SL have an acceptable sensory quality and an oxidative stability that is comparable to the corresponding traditional lipids.

Transition metal ions are able to catalyze the initiation of oxidation of unsaturated FA and the decomposition of lipid hydroperoxides (4,5). This reaction may be inhibited by metal chelators such as citric acid and EDTA. Citric acid is widely used as a metal chelator in vegetable oils, where it has been shown to work effectively in concentrations of around 50 ppm and in synergy with the tocopherols that occur naturally in the vegetable oils (4).

The aim of the present study was to investigate the oxidative stability of three different oils: an RSL (RFO) and an SSL (SFO), both based on FO, and the original FO. RFO was purified by batch deodorization and SFO by short-path deodorization, whereas FO was deodorized by the supplier and used without any further purification. In addition, we investigated the antioxidative effect of adding the metal chelators EDTA or citric acid to the SFO. A concentration of 75 mg/kg EDTA was used, since it has been found to work in FO-enriched mayonnaise (6), and an equimolar amount of citric acid (42 mg/kg) was tested.

The oils were stored for up to 11 wk at 2°C and subsequently for 6 wk at 20°C in the dark. Samples were analyzed for PV and volatiles, and during the first 11 wk, they were also subjected to sensory analyses.

EXPERIMENTAL PROCEDURES

Materials. Refined and deodorized FO was obtained from Pronova Biocare (Lysaker, Norway). FFA composition and FA content are shown in Tables 1 and 2, respectively. Tricaprylin (purity >90%) was obtained from Fluka Chemie AG (Buchs, Switzerland). FFA content was 0.06%. Caprylic acid (8:0, purity >95%) was from Grünau Illertissen GmbH (Illertissen, Germany). Lipozyme RM IM, an *sn*-1,3-specific lipase obtained from *Rhizomucor miehei*, was donated by Novozymes A/S (Bagsværd, Denmark). EDTA disodium calcium salt was obtained from Sigma-Aldrich (Steinheim, Germany), and citric acid was from Bie & Berntsen (Højbjerg, Denmark).

Production of the RSL (RFO). The SL, with a randomized structure based on tricaprylin and FO, was produced by chemical interesterification, a conventional method with sodium

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methoxide as the catalyst. The reaction was conducted in a 45-L batch reactor. Twenty kilograms blended oil mixture (FO and tricaprylin, 75:25 w/w) was loaded into the reactor, and stirring was regulated to 230 rpm. The oil mixture was dried at 90°C and 100 mbar vacuum before reducing the temperature to 60°C and adding 0.1 wt% sodium methoxide (20 g) while stirring. After 30 min the reaction was stopped by addition of 3 L aqueous citric acid solution (4%). The oil was washed three or four times with the same volume of citric acid solution until the pH was below 7 and dried again under vacuum for 30 min at 90°C before cooling to room temperature. FFA and volatiles were removed in a 50-L batch deodorizer at 190°C and 2–3 mbar vacuum for 2.5 h.

Production of the SSL (SFO). Enzymatic interesterification by lipase-catalyzed acidolysis using Lipozyme RM IM (*R. miehei* lipase immobilized on a macroporous resin) in a pilot packed-bed reactor was used to produce the lipid with a specific structure based on FO and caprylic acid (substrate mole ratio 1:6 of oil/acyl donor, 1:1 in weight ratio). The production conditions were chosen based on our previous study (7). Short-path distillation was used to remove FFA (8).

Storage experiment. Five oil batches were produced [FO, RFO, SFO, SFOE (SFO with EDTA), and SFOc (SFO with citric acid)]. The metal chelator (75 mg/kg EDTA or 42 mg/kg citric acid) was added to 2 kg of the SFO and suspended by ultrasound. Oil samples were stored in brown screw-capped glass bottles in the dark at 2°C for up to 11 wk. At each sampling time, one bottle was used for sensory analysis and another for the determination of FFA, PV, and volatile oxidation products. Samples were taken at the start of the storage experiment and after 2, 4 (only chemical), 6, 8, and 11 wk. After 11 wk, the remaining bottles were transferred to room temperature (20°C, dark), and the bottles for chemical analysis were taken weekly from weeks 12 to 17. Sensory analysis was not performed in weeks 12 to 17. The bottles for chemical analysis were stored at −30°C and thawed before analysis. Replicates were withdrawn from the same bottle.

Determination of total FA composition and FA in the sn*-2 position*. The composition of FA in TAG was determined by GC after methylation using KOH (9). The FA composition in the *sn*-2 position was determined by Grignard degradation prior to methylation and GC analysis (10). Measurements were made in triplicate.

Determination of TAG species. Molecular species of TAG were separated by gradient reversed-phase HPLC (11) and identified by atmospheric pressure chemical ionization HPLC-MS (12). Measurements were made in duplicate.

Determination of induction time with an Oxidograph. The oxidative stability of the oils was determined by accelerated oxidation at 70°C in the presence of oxygen using an Oxidograph (MikroLab, Århus, Denmark). Oxidation was recorded as a drop in the oxygen pressure in the reaction flasks as a result of oxygen consumption. The induction time (i.e., the oxidative stability) was determined as the crossing point of the tangents to the curve. Measurements were made in duplicate.

Determination of tocopherols. The tocopherol content of the oils was measured according to AOCS Method Ce 8-89 (13) using a high-performance liquid chromatograph with a fluorescence detector and a Spherisorb column (S5W, 25 cm, 4.6 mm i.d.; Spherisorb, Norwalk, CT). After dilution of the oil sample with *n*-heptane, the tocopherol isomers (α-, β-, γ-, and δ-tocopherol) were determined simultaneously using an external standard. Analyses were made in duplicate.

Determination of FFA. FFA was determined according to AOCS Method Ca 5a-40 (13) using titration with NaOH and phenolphthalein as an indicator. The amount of FFA was calculated as the percentage of oleic acid. Analyses were made in duplicate.

Determination of primary oxidation products by PV. PV was determined according to the International Dairy Federation method described by Shantha and Decker (14). Absorption was measured on a Shimadzu UV-160 spectrophotometer, and analyses were performed in triplicate.

Determination of volatile secondary oxidation products. Oil (4 g) was weighed into a pear-shaped glass flask together with *n*-dodecane as an internal standard. Headspace volatiles from the oil were released by heating to 45°C and purging with nitrogen for 30 min while collecting them in Tenax tubes. Afterward, volatiles were separated and quantifed on a gas chromatograph with a capillary column (DB 1701, 30 m, 0.32 mm i.d., 1.0 μm film thickness; J&W Scientific, Folsom, CA) and an FID. The oven temperature program was as follows: 3 min isothermal at 35°C, increased at 3°C/min to 140°C, increased 5°C/min to 170°C, increased at 10°C/min to 240°C, and 8 min isothermal at 240°C. Compounds were identified by their retention times and confirmed by GC–MS and by spiking with external standards. Results from the analyses are given as peak area/g oil. Analyses were performed in triplicate.

Sensory assessment. Twelve assessors were selected for the panel. Prior to the experiment, all were trained with vegetable oil and FO in different oxidative states. During the training, the panel discussed which words to use to describe the oils, agreed on a scale for each single descriptor for quantification, and subsequently developed a profiling scheme to evaluate unknown samples. The scheme contained the following attributes: (i) aroma (smell): *fishy, rancid train oil, dry, metallic, green, acidic, synthetic, burned, nutty,* and *miscellaneous*; and (ii) flavor (taste): *fishy, rancid train oil, sticky, dry, metallic, green, acidic, bitter, synthetic, burned, nutty,* and *miscellaneous*. *Fishy* describes the smell and taste of fresh fish, *train oil* describes oxidized FO, and *rancid* describes the general smell and taste of oxidized oil (also vegetable). *Sticky* and *dry* describe the mouthfeel rather than the taste. Portions of 20 mL oil were poured into small, brown glass jars with caps and heated to 50°C before serving. Samples were blinded and served in randomized order to minimize possible carryover effects. Distilled water, heated to 50°C, as well as crisp bread was provided for oral rinsing at the beginning of sessions and between oil samples. Attributes were rated on separate 9-cm unstructured scales using a Psion mini-computer (Psion PLC, London, United Kingdom).

Data analysis. Data obtained from the storage experiment (PV, volatile secondary oxidation products, and sensory data) were analyzed by multivariate data analysis using The Unscrambler®, version 7.6, software program (CAMO, Oslo, Norway). A preliminary ANOVA partial least squares regression (APLSR) was performed on sensory results to project away differences in the sensory score levels of the assessors (15). The resulting so-called sensory residuals were used in the following data analysis. All data were analyzed by APLSR using the design variables as x-data and the measured variables as y-data. The design variables were for lipid type: FO, RFO (the RSL), and SFO (the SSL); for metal chelator type: NoM (no metal chelator), S-EDTA (addition of EDTA to SFO), and S-citr (addition of citric acid to SFO); for replicates: Rep1, Rep2, Rep3, and Average; and for interactions: FO*NoM, RFO*NoM, SFO*NoM, SFO*E (where E is EDTA addition), and SFO*c (where c is citric acid addition).

Cross-validation on treatments was used to validate the APLSR model. All variables were weighed by 1/SD. By using the jack-knifing feature in The Unscrambler software, it was possible to assess whether regression coefficients for the different design variables were significantly positive or negative ($P < 0.05$) for each of the measured variables.

RESULTS

Analytical data on oils. The analytical data for FO, RFO, and SFO at the start of the experiment are shown in Tables 1 and 2. At the start of the experiment, FFA was low in all oils and increased in the order FO, RFO, SFO, whereas PV was lowest in RFO, followed by FO, and highest in SFO (Table 1). Furthermore, the oxidative stability, as measured by the induction time, was highest in FO (276 min), whereas it was 40% lower in RFO and 68% lower in SFO (Table 1). The addition of EDTA to the SSL did not alter the induction time, whereas citric acid increased the induction time slightly (data not shown). The α-tocopherol content was highest in FO and lowest in SFO. The contents of γ- and δ-tocopherol were similar in FO and RFO, whereas they were lower in SFO. The results in Table 1 demonstrate that even at the start of the study, oxidation parameters were different in the three oils.

RFO and SFO contained approximately the same amount of 8:0 (33 and 38%; see Table 2), indicating that approximately the same amounts of 8:0 were incorporated into the TAG by the chemical and enzymatic interesterification processes. As a result of the incorporation of 8:0, the contents of almost all other FA were lower in SFO and RFO; PUFA, in particular, were lower in RFO than in FO. The total content of PUFA was reduced from 39% in the original FO to 24 and 33% in RFO and SFO, respectively. The amount of PUFA in the *sn*-2 position was similar in FO (37%) and SFO (36%) and lower in RFO (22%). On the contrary, 37% 8:0 was found in the *sn*-2 position of RFO, whereas only 3.1% was present in the *sn*-2 position of SFO. Approximately 88% of the TAG in RFO and SFO had at least one medium-chain FA incorporated; 3–4% of the TAG contained only long-chain FA, whereas the remaining TAG were not identified. Thus, from Table 2 and the abovementioned results, it was obvious that a large part of the structured TAG in the SFO had the desired TAG structure, MLM (with M being the medium-chain FA 8:0 and L a long-chain FA). In contrast, only a small portion of the RFO had the desired structure, as RFO had a high content of M in the *sn*-2 position. Furthermore, the lipids in FO were solely of the LLL type, since only long-chain FA were present in this oil (Table 2).

FFA during storage. The level of FFA was constant during storage and relatively low especially in FO, followed by RFO and SFO, irrespective of the metal chelator added. FFA levels were on average 0.03, 0.07, and 0.09% in the oil types, respectively.

APLSR analysis. Data from the storage experiment (PV, volatile secondary oxidation products, and sensory results) were analyzed by APLSR. Four principal components were validated. Together, these explained 73 and 78% of the variation in the x- and y-data, respectively. Principal components 1 and 2 (PC1 and PC2) explained 37 and 60% of the variation in the x- and y-data, respectively (Figs. 1A and 1B). PC3 showed a difference between SFOE and SFOc, and PC4 showed a difference between RFO and the other design variables. However, the measured variables were not very well explained; therefore, the plot of PC3/PC4 is not shown.

Correlation loadings plot. The loadings plot of PC1 and PC2 was difficult to interpret because of the many variables. Therefore, the plot was split into two: one with PV and the sensory variables (Fig. 1A) and one with the GC variables (Fig. 1B). The design variables are shown in both plots. The replicates are not shown, since they were located in the center and therefore did not explain any variation.

Design variables. The design variables for lipid types FO

TABLE 1
Oxidation-Related Data for Oil Batches at the Start of the Experiment[a]

	FFA (%)	PV (meq/kg)	Induction time (min)	α-Tocopherol (μg/g)	γ-Tocopherol (μg/g)	δ-Tocopherol (μg/g)
FO	0.03 ± 0.00	2.14 ± 0.03	276 ± 4	106.8 ± 1.5	29.0 ± 0.8	10.6 ± 0.2
RFO	0.06 ± 0.01	0.89 ± 0.05	164 ± 4	41.6 ± 1.7	31.0 ± 0.4	12.6 ± 0.5
SFO	0.08 ± 0.01	2.63 ± 0.05	88 ± 4	4.5 ± 2.5	4.1 ± 0.2	ND

[a]Values are mean ± SD (n = 2–3). FFA and PV were taken from week 0 results. Induction time was determined at 70°C on an Oxidograph (MikroLab, Århus, Denmark). FO, fish oil; RFO, randomized structured lipid based on FO and tricaprylin; SFO, specific structured lipid based on FO and caprylic acid; ND, not detected.

TABLE 2
FA Composition in TAG and in the *sn*-2 position for FO, RFO, and SFO[a]

	FA profile of TAG			FA profile of *sn*-2		
FA	FO	RFO	SFO	FO	RFO	SFO
8:0	ND	37.5	33.2	ND	37.2	3.1
14:0	9.9	6.3	6.6	17.5	6.8	15.3
16:0	20.0	12.8	12.6	27.3	13.5	27.3
16:1	9.7	6.2	5.5	11.2	6.5	10.6
18:0	3.2	2.0	1.3	0.7	2.1	0.7
18:1n-9	13.0	8.4	5.6	4.3	8.5	4.5
18:1n-7	3.1	2.0	1.3	0.9	1.9	1.0
18:2n-6	1.3	0.8	0.6	0.8	0.8	0.8
20:1n-9	1.1	0.7	0.5	0.3	0.6	0.3
18:4n-3	3.7	2.3	3.5	3.4	2.4	3.2
20:5n-3	18.8	11.3	14.1	10.8	10.7	10.9
22:5n-3	1.8	1.1	1.6	3.1	1.0	3.5
22:6n-3	10.9	6.5	11.5	17.6	6.0	16.3
Sum PUFA	38.8	23.5	32.8	37.1	22.2	36.0

[a]Values are given as mol%. Only the FA present at levels of >1.0% for at least one of the lipids are listed. For abbreviations, see Table 1.

and RFO were located to the left in the third and second quadrant, respectively (Figs. 1A and 1B). SFO was located to the far right very close to the PC1 axis. The design variables S-EDTA and S-citr, describing the addition of metal chelators EDTA and citric acid, also were to be found on the right side of the diagram on the PC1 axis. The design variable describing no metal chelator addition (NoM) was located between the two correlation rings to the left on the PC1 axis. Thus, PC1 described the difference between FO and RFO and the SSL with and without metal chelator (SFO), but also a smaller difference between SFO with and without metal chelator. PC2 described the difference between FO and RFO. All interactions (FO*NoM, RFO*NoM, SFO*NoM, SFO*E, and SFO*c) were located on the same vertical position as the corresponding design variable but close to the PC2 axis except SFO*NoM, which was located just right of the PC2 axis. Therefore, the interactions did not supply any additional information.

Primary oxidation products. (i) Storage at 2°C. All PV from storage at 2°C (PV-0–PV-11) were located to the right close to SFO, S-EDTA, and S-citr (Fig. 1A). This finding indicated that PV were highest in the SFO and lowest in FO and RFO during storage at 2°C. The raw data (Table 3) confirmed that PV were lowest in RFO, followed by FO and then SFO (with or without metal chelators). In FO and RFO, PV developed slowly during the 11 wk. The initial PV level was higher in all the SFO than in FO and RFO. From week 4, PV was lowest in SFO without metal chelators compared to SFO with metal chelator. In contrast to FO and RFO, all three SFO reached the highest PV level (SFOE) or a plateau (SFO and SFOc) between weeks 2 and 8, after which PV decreased again. Because PV was low after 11 wk of storage, the temperature was increased to 20°C, since oils are often stored at this temperature.

(ii) Storage at 20°C. All PV variables from storage at 20°C (PV-12–PV-17) were located on the lowest part of the PC2 axis close to FO in the vertical direction (Fig. 1A). This location indicated that PV were higher in the FO than in the other samples. This interpretation of the model was indeed confirmed by the raw data obtained after the oils had been transferred to room temperature. A significant increase in PV for FO was observed, along with a smaller increase in PV for the three SFO. In contrast, PV in the RFO decreased from 1.3 to 0.9 meq/kg after transfer to room temperature, but increased again to 1.9 meq/kg in week 14. Thus, the highest PV were observed in FO, followed by SFO (with or without metal chelator), and the lowest PV were found in RFO. PV in the SFO batches were relatively constant; these values varied between 2.2 and 3.5 meq/kg and tended to decrease at the end of the storage period. PV were generally higher in SFOc than in SFO, whereas the effect of EDTA was more moderate.

Volatile secondary oxidation products. A wide range of volatile compounds was identified and quantified by dynamic headspace GC–MS and GC with FID. In our study, alkanes and alkenes were found at high levels, primarily in the SFO during storage (data not shown). However, since these types of compounds do not contribute to the sensory characteristics of the oils, they were not considered further (4). Nineteen other volatiles were considered to be relevant sensory oxidation products (Table 4). The correlation loadings plot containing all 19 volatile compounds gives an overview of the location of the compounds relative to the design variables (Fig. 1B). The plot revealed a large cluster of compounds located at the right side of the PC1 axis close to SFO, whereas fewer compounds were located near FO and very few near RFO. Thus, these findings indicated that most of the volatiles were found at the highest levels in the three SFO, at lower levels in FO, and at the lowest level in RFO. By examining the development of the volatiles during storage, it was possible to categorize them in six groups having different patterns of development. During storage, with a shift of temperature the pattern changed for some compounds; therefore, it is important to note that the volatiles may belong to one group during storage at 2°C and to another group during storage at 20°C.

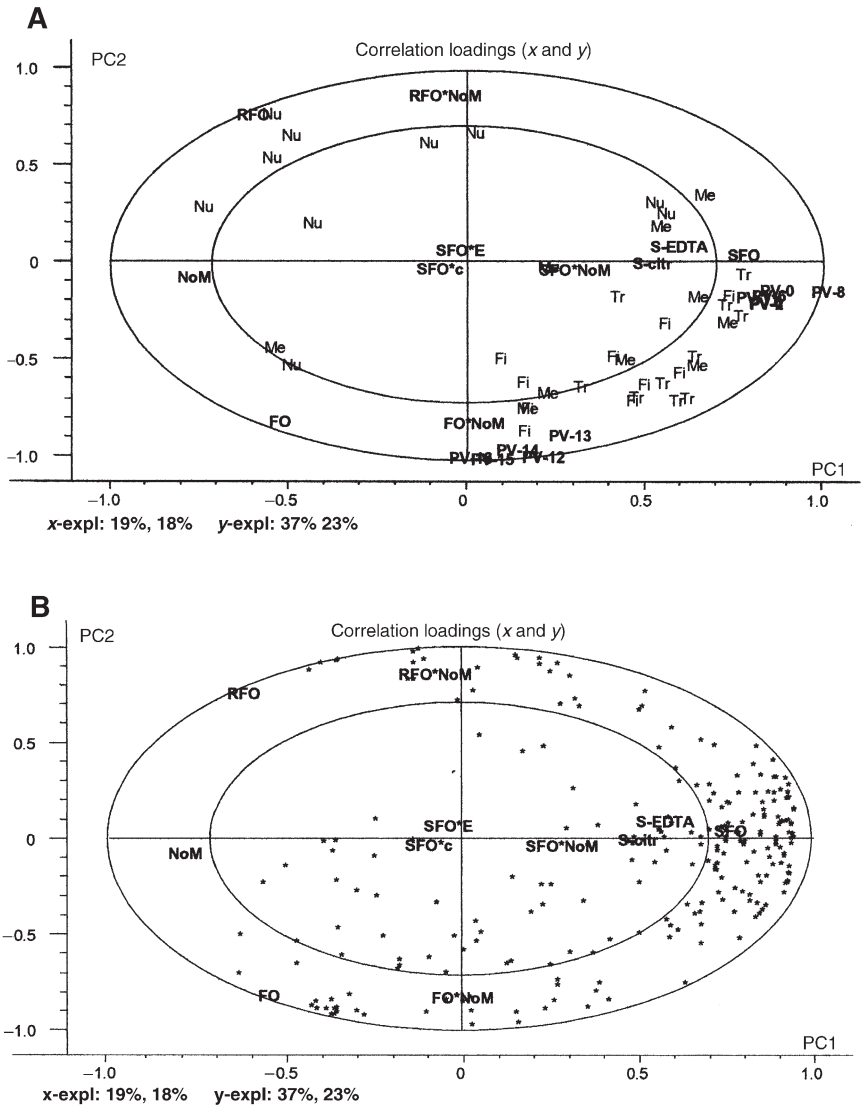

FIG. 1. Correlation loadings plot of ANOVA partial least squares regression analysis on design variables as x-data and measured variables as y-data. The location of the samples and variables corresponds to the first letter in the sample/variable name. FO, fish oil; RFO, randomized structured lipid based on FO and tricaprylin; SFO, structured lipid based on FO and caprylic acid; S-EDTA, addition of EDTA to SFO; S-citr addition of citric acid to SFO; NoM, no metal chelator added. (A) PV and sensory data. PV numbers indicate the week of storage: 0–11 at 2°C and 12–17 at 20°C. Nu, nutty aroma and flavor; Tr, train oil; Fi, fishy; and Me, metallic. (B) Secondary volatile oxidation products. An asterisk (*) indicates a volatile compound in that week. The inner ellipse indicates 50% explained variance, and the outer ellipse indicates 100% explained variance. PC1 and PC2 refer to principal components 1 and 2.

The groups shown in Table 4 are as follows. In group A, volatiles were present in the highest amount in SFO with or without metal chelator. Twelve of the 19 compounds belonged to this group and included all types of volatiles (aldehydes, ketones, and alcohols). In group B, the levels seemed to increase equally in all oils. One compound (*c*-4-heptenal) during storage at 2°C and two additional compounds (1-penten-3-ol, *t*-2-heptenal) at 20°C constituted this group. The compounds in group C were found in lower levels in RFO compared with the other lipids. This group included *t,t*-2,4-hexadienal, *t,t*-2,4-heptadienal (2°C), and *t*-2-octenal (20°C). In group D, 1-octen-3-ol was found at highest level in FO. This was also observed for hexanal during storage at 2°C and for *t,t*-2,4-hexadienal and decanal during storage at 20°C. In group E, on the contrary, lower levels of *c*-3-hexenal and octanal were observed in FO compared with the other oils during storage, and at 20°C the levels of *c,t*-2,4-heptadienal and nonanal were also low in FO. In addition, the levels of *c*-3-hexenal and nonanal were found to be highest in RFO. Figures 2A–2E show the development during storage of one compound belonging to each of the groups, A to E.

Significant regression coefficients. To interpret the effect of

TABLE 3
PV During Storage of Oil Samples[a]

Week	Temp. (°C)	FO	RFO	SFO	SFOE	SFOc
0	2	0.38 ± 0.02	0.13 ± 0.17	1.01 ± 0.02	1.01 ± 0.07	1.04 ± 0.01
2	2	0.86 ± 0.06	0.24 ± 0.01	1.85 ± 0.03	2.16 ± 0.02	1.81 ± 0.06
4	2	1.08 ± 0.03	0.44 ± 0.03	1.91 ± 0.01	2.24 ± 0.06	2.77 ± 0.50
6	2	1.26 ± 0.02	0.70 ± 0.01	1.94 ± 0.13	2.59 ± 0.04	2.88 ± 0.50
8	2	1.67 ± 0.06	1.05 ± 0.04	1.89 ± 0.13	3.36 ± 0.11	2.89 ± 0.02
11	2	1.58 ± 0.06	1.32 ± 0.08	1.46 ± 0.01	1.82 ± 0.03	2.36 ± 0.05
12	20	2.14 ± 0.03	0.89 ± 0.05	2.63 ± 0.06	2.38 ± 0.03	2.45 ± 0.07
13	20	4.32 ± 0.05	1.79 ± 0.04	2.93 ± 0.04	2.99 ± 0.05	3.36 ± 0.07
14	20	3.33 ± 0.04	1.87 ± 0.10	2.28 ± 0.11	2.95 ± 0.05	2.69 ± 0.08
15	20	4.24 ± 0.09	1.84 ± 0.06	2.52 ± 0.14	2.92 ± 0.06	3.26 ± 0.10
16	20	3.80 ± 0.13	1.41 ± 0.03	2.55 ± 0.14	2.19 ± 0.12	2.65 ± 0.05
17	20	4.20 ± 0.10	1.42 ± 0.03	2.41 ± 0.02	2.30 ± 0.03	2.74 ± 0.04

[a]Values were calculated as meq peroxides/kg and given as mean ± SD (n = 3). SFOE, SFO containing 75 mg EDTA/kg; SFOc, SFO containing 42 mg citric acid/kg; for other abbreviations see Table 1.

metal chelator and lipid type on the formation of volatiles in further detail, the regression coefficients obtained from the APLSR model for the various design variables were studied in relation to the different compounds after 8, 11, 16, and 16 wk (Table 4). The regression coefficients were mainly negative during storage at both 2 and 20°C for FO, RFO, and NoM and were mainly positive for SFO, S-EDTA, and S-citr. These data confirm that SFO had higher levels of volatiles than RFO and FO and that EDTA or citric acid did not reduce volatile formation. Instead, EDTA and citric acid seemed to increase volatile formation.

Sensory data. Eleven descriptors were developed to describe the aroma (smell) and 13 descriptors to describe the flavor (taste) of the oils. Of these 24 descriptors, 5 descriptors describing smell and 7 describing taste were considered the most important in a preliminary statistical evaluation. The 3 descriptors most characteristic of oxidized fish oil (*fishy, train oil*, and *metallic*) and one pleasant descriptor are shown in Figure 1A. In the correlation loadings plot, the pleasant descriptor *nutty* was located to the left, close to the design variables FO and RFO. On the contrary, the unpleasant aroma and flavor descriptors *fishy, train oil,* and *metallic* were located to the right, closer

TABLE 4
Significant Regression Coefficients[a] for Secondary Volatile Oxidation Products After 8 and 11 wk (storage at 2°C) and 16 and 17 wk (storage at 20°C)

	Storage at 2°C							Storage at 20°C						
	Gr	FO	RFO	SFO	NoM	S-EDTA	S citr	Gr	FO	RFO	SFO	NoM	S-EDTA	S-citr
t-2-Butenal	A	–	– –	+ +	– –	+ +		A	–	– –	+ +	– –	+ +	+
1-Penten-3-one	A	– –	– –	+ +	– –	+ +	+ +	A		– –	+ +	– –	+	+
1-Penten-3-ol	A	– –	–	+ +	– –	+ +	+ +	B	–		+ +	– –	+	+
t-2-Pentenal	A	–	–	+ +	– –	+ +	+	A	–		+ +	– –	+	+
Hexanal	D	++	– –					A	–	– –	+ +	– –	+ +	
c-3-Hexenal	E	– –		+ +	– –	+ +		E	– –	+ +				
t -2-Hexenal	A	–	– –	+ +	– –	+ +	+ +	A	–	–	+ +	– –	+ +	+
Heptanal	A	–	– –	+ +	– –	+ +	+ +	A	– –	–	+ +	– –	+ +	+
c-4-Heptenal	B	– –	+					B	–	+	+	–	+	
t,t-2,4-Hexadienal	C		– –	+ +	– –	+ +	+	C,D	+	– –			+	
t-2-Heptenal	A		– –	+ +	– –	+ +	+	B	+	– –				
1-Octen-3-ol	D	+ +	–			+		D	+		–	+		+
Octanal	E	– –	+			+ +	+	E	– –	+ +			+	+
c,t -2,4-Heptadienal	A	– –	–	+ +	– –	+ +	+	E	–		+	–	+	
5-Ethyl-2-furanone	A	–	– –	+ +	– –	+ +	+	A		- –	+ +	– –	+	+
t,t-2,4-Heptadienal	C		– –	+ +	– –	+	+	A		–	+ +	– –	+	+
t-2-Octenal	A		– –		–		+	C,D			+	–	+	
Nonanal	A	–	–	+ +	– –	+ +	+	E	– –	+ +			+	
Decanal	A		– –	+ +	–		+	C,D	+	– –				

[a]A plus sign indicates a (+) significant positive regression coefficient; a minus sign indicates a (–) significant negative regression coefficient ($P < 0.05$). Two plus (+ +) or minus signs (– –) indicate that the regression coefficients were significantly positive or negative in both week 8 and week 11 for storage at 2°C, or in both week 16 and week 17 for storage at 20°C. A blank cell indicates that the design variable was not significant. Letters in groups (Gr) refer to the following: A, SFO was highest the first 4–6 wk, and thereafter SFO with EDTA (S-EDTA) or citric acid (S-citr) was highest; B, SFO with and without metal chelator (NoM) was highest; C, all increased; D, RFO was lowest; E, FO was highest; F, FO was lowest, and in some RFO was highest. For the interpretation of design variables, please refer to Figure 1; for other abbreviations, see Table 1.

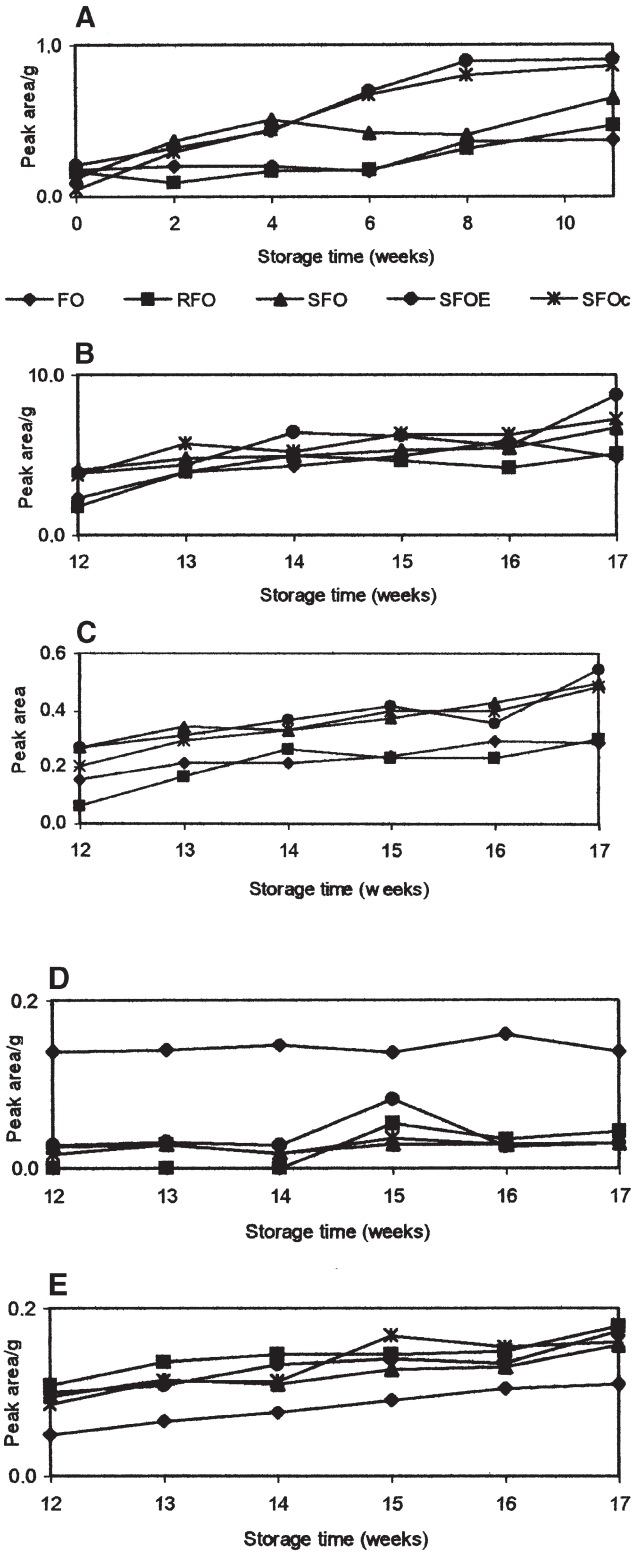

FIG. 2. Formation of secondary oxidation products in oil batches during storage at 2°C (0–11 wk) and 20°C (12–17 wk). (A) 1-Penten-3-one (group A); (B) 1-penten-3-ol (group B); (C) *t,t*-2,4-heptadienal (group C); (D) 1-octen-3-ol (group D); (E) octanal (group E). SFOE, SFO containing 75 mg EDTA/kg; SFOC, SFO containing 42 mg citric acid/kg; for other abbreviations see Figure 1.

TABLE 5
Significant Regression Coefficients for Sensory Descriptors After 8 and 11 Wk of Storage at 2°C[a]

Aroma/Flavor	FO	RFO	SFO	NoM	S-EDTA	S-citr
A *Fishy*	+					
A *Rancid*	+				+	−
A *Train oil*	+					+
A *Metallic*	+		−	+		
A *Nutty*			+		+	
F *Fishy*	−	−	+			+ +
F *Rancid*	−	−	+	−	+	+
F *Train oil*		−	+	−		
F *Metallic*	−	−	+			
F *Bitter*	−	−	+	−		+ +
F *Synthetic*	−	+			+	+
F *Nutty*	− −	+ +		+		

[a]A plus sign (+) indicates a significant positive regression coefficient; (–) significant negative regression coefficient ($P < 0.05$). A blank cell indicates that the design variable was not significant or that results from weeks 8 and 11 were contradictory. For the interpretation of design variables, see Figure 1. A, aroma; F, flavor; for other abbreviations see Tables 1 and 4.

to the SFO design variables in the first and especially the fourth quadrant. *Rancid* was located in the middle of the plot, indicating that it was not well explained by the model (not shown). This observation corresponds to the raw data showing higher levels of the unpleasant descriptors in the SFO and a lower level of *nutty* flavor compared with FO and RFO (data not shown). The *train oil* flavor developed slower in RFO than in the other samples, whereas the level in FO was comparable to that of SFO during most of the storage period at 2°C (Fig. 3).

Significant regression coefficients for sensory data. Table 5 shows the regression coefficients obtained from the APLSR model for weeks 8 and 11 for the 12 sensory descriptors that were selected. As the most important, the FO lipid type correlated significantly negatively with 5 of the unpleasant flavor descriptors and the pleasant *nutty* flavor, and positively with the 4 unpleasant aroma descriptors. RFO correlated mainly negatively with the unpleasant flavor descriptors. On the contrary, the SFO lipid type correlated positively with the majority of the unpleasant flavor descriptors. No metal chelator addition correlated positively with the *metallic* aroma and *nutty* flavor and negatively with 3 of the unpleasant flavor descriptors.

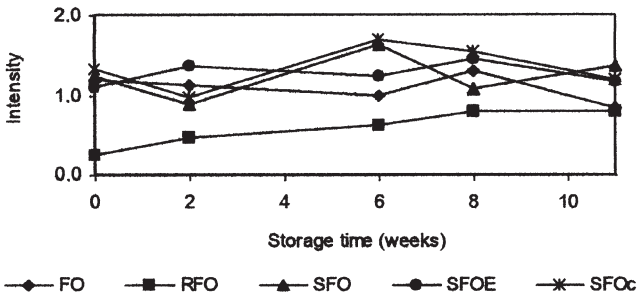

FIG. 3. Development of the *train oil* aroma in oil batches during storage at 2°C (assessors $n = 9–12$). For abbreviations, see Figures 1 and 2.

Addition of EDTA to SFO resulted in a positive correlation with the *rancid* aroma and flavor, whereas addition of citric acid was significantly positively correlated to the *fishy*, *rancid,* and *bitter* flavors. The latter off-flavor may arise from the citric acid itself. Addition of both EDTA and citric acid resulted in a synthetic flavor. These findings confirmed that the SFO developed more unpleasant off-flavors than FO and RFO and that addition of EDTA or citric acid did not prevent the formation of off-flavor in SFO. On the contrary, EDTA and citric acid seemed to promote *rancid* and *fishy* off-flavor formation in SFO.

DISCUSSION

The present study was performed to compare the oxidative stability and sensory properties of traditional FO, RFO, and SFO (the latter two produced to contain caprylic acid). In addition, the antioxidative effect of adding EDTA or citric acid to the SFO was investigated. An important issue was to use the oils as they were produced since further processing, such as an extra purification step, may be unrealistic for commercial production.

The results obtained demonstrated that the lipid type significantly affected the oxidative stability and the sensory properties of the oils. The accelerated oxidation test (using an Oxidograph) showed that the oxidative stability of SFO, measured as the induction time, was one-half and one-third of the induction time for RFO and FO, respectively. The content of FFA was slightly higher in SFO compared with FO and RFO. However, there was no increase in FFA content during storage, and differences between the lipids were very small since the values, in general, were very low. Thus, FFA were not released from TAG by hydrolysis in any of the lipids.

The PV of all oils increased during storage at 2°C. PV were higher in the SFO than in FO and RFO from week 0 to week 8. From week 8 to week 11, PV in the SFO decreased, whereas PV continued to increase in the FO and RFO. After the temperature was raised to 20°C, PV in the FO increased faster than at 2°C, whereas this was not the case for the other oils. These observations indicate that oxidation of the SSL (SFO) was initiated and had reached a maximal rate of peroxide formation at an earlier point in time than that of FO and RFO. FO seemed to be more resistant to oxidation than SFO, since its PV increased much slower than the PV of the SFO, at least at 2°C. At 20°C, the PV of the FO increased faster than in the SFO, but this phenomenon could be due to a faster decomposition of peroxides in SFO than in FO. RFO seemed even more resistant to oxidation than FO, as PV increased slower in RFO than in FO, both at 2°C and at 20°C.

In accordance with the proposition above, that peroxides decomposed faster in SFO than in RFO and FO, volatiles were formed to a higher extent in the SFO compared with the other oils. Unlike the peroxides, which have a neutral aroma and flavor, the majority of their breakdown products (secondary oxidation products) are volatile and therefore give rise to a change in the sensory characteristics of the oils. A large number of relevant aroma volatiles were found at the highest levels (peak area) in SFO with metal chelator added during storage at 2°C and in all three SFO during storage at 20°C. These were 1-penten-3-one (*rancid*, *green* flavor; Ref. 16), 1-penten-3-ol (*sweet;* Ref. 16), 2-hexenal (*sour*, *green*; Refs. 16,17), and heptanal (16). These compounds have previously been found in oxidized FO and FO-enriched mayonnaise (16,17). Other volatiles found at high levels in SFO were hexanal, pentanal, and nonanal (*green*, *compost*). In addition, 2,4-hexadienal (*green*, *burnt*; Ref. 16) and 2,4-heptadienal (*fishy, painty*; Refs. 16,17) were found at high levels in SFO. The latter compounds have low flavor threshold values (16). The only compounds found at the highest level in FO at 2°C were hexanal and 1-octen-3-ol. However, after increasing the temperature, the significant flavor compound hexadienal also correlated with the FO design variable (Table 4). Only heptenal and octanal (*green*; Ref. 16) were found to correlate with the RFO design variable when stored at the low temperature, and at increased temperature hexenal and nonanal also correlated with this design variable. Altogether, both the loadings plot (Fig. 1B) and the regression coefficients (Table 4) demonstrated that the SFO design variable correlated with by far the most volatiles, and these observations indicated that the SFO oxidized faster than the RFO and the traditional FO.

The sensory evaluation of the oils demonstrated an obvious difference between the FO and RFO on one hand and the more *fishy/rancid* SFO on the other after storage at 2°C. The high levels of volatiles in the SFO are assumed to be responsible for the dominant *fishy, train oil*, and *rancid* flavors as well as the *bitter* flavor. The sensory profile after storage at 20°C can be assumed to be similar to the one observed after storage at 2°C, as indicated by the similarity between the regression coefficients for volatile data obtained after storage at 2 and 20°C. In summary, the sensory data supported the indications from the other data that SFO oxidized faster than FO and RFO. In contrast to this conclusion, the induction time showed that FO was less stable than FO and RFO. Therefore, one should be careful when drawing conclusions based on results barely obtained from measurements of induction time.

It is well known that the number of double bonds in a FA influences its oxidative stability. Moussata and Akoh (18) found that melon seed oil was less stable compared with an interesterified product in which linoleic acid was replaced by oleic acid. In our study, the content of PUFA was slightly higher for FO than for SFO and substantially higher than for RFO. Thus, the lower stability of the SFO compared with FO and RFO cannot be explained solely by their FA composition.

As previously described, the production conditions for RFO and SFO were different, since RFO was purified effectively by batch deodorization, whereas SFO was purified by the more gentle short-path distillation in order to avoid acyl migration of the FA on the TAG. Therefore the "history" of the oils (before the storage experiment) was not the same. It is most likely that the difference in oxidative stability was partly due to this different history of the oils. Akoh and Moussata (19) and Yankah and Akoh (20) found that SSL are more

susceptible to oxidation than the original oil containing a larger amount of unsaturated FA, probably due to the loss of antioxidants during production. We also observed a lower content of tocopherol, especially in SFO but also in RFO, compared with FO. Tocopherols were removed during the production of RFO and SFO; therefore, these fats may have reduced stability toward oxidation. Since oxidation is initiated by metals, addition of metal chelators could increase the stability of the oils. As mentioned, the effect of EDTA and citric acid was investigated in the present study. Citric acid has a low solubility in oil, yet it has been found to be effective in concentrations around 50 ppm. However, the addition of EDTA or citric acid in the concentration used in this study was not able to reduce the PV or the development of secondary volatile oxidation products. Rather, addition of the metal chelators seemed to slightly induce formation of certain volatile oxidation products. The lack of an antioxidative effect of EDTA and citric acid may be due to a precipitation of the metal chelators that would inhibit the binding of metals to the chelators. Alternatively, the concentration used may be too low. It has previously been suggested that EDTA may promote oxidation if the ratio between EDTA and Fe is below 1:1; this phenomenon could explain the observed pro-oxidative effect of EDTA. Further investigations using different concentrations of metal chelators alone and in combination with tocopherol would be relevant to determine how they affect oxidation in a different context.

In TAG, the position of the FA may also influence the oxidative stability. Wada and Koizumi (21) have proposed that unsaturated FA in the *sn*-1 and *sn*-3 positions, disregarding the chain length, are more susceptible to oxidation than the FA in the *sn*-2 position. Neff and El-Agaimy (22), on the other hand, found that TAG containing adjacent unsaturated FA oxidized faster than TAG containing unsaturated FA in the outer positions, probably due to interactions between the adjacent FA. Thus, SFO contained a large amount of MLL and FO contained only LLL (data not shown). Therefore, FO would, from an isolated point of view, be expected to have much lower stability than RFO and SFO. In summary, from the amount of unsaturated FA and the position of the unsaturated FA, it would be expected that FO would be more unstable than RFO and SFO.

The difference in the history of the oils resulted in a difference in the initial level of peroxides and volatiles as well as the content of tocopherol between the three lipid types. These differences were therefore most likely the primary cause of the difference in storage stability of the oils.

Our data show that SSL oxidized faster than randomized or traditional lipids. We also demonstrated that addition of EDTA (75 mg/kg) or citric acid (42 mg/kg) did not inhibit oxidation. On the contrary, they seemed to accelerate oxidation. Hence, further studies are needed to improve the sensory properties and oxidative stability of SSL.

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