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ORIGINAL PAPER

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# Oxidative stability of mayonnaise and milk drink produced with structured lipids based on fish oil and caprylic acid

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**Abstract** The oxidative stabilities of traditional fish oil (FO), randomized lipids (RFO), or specific structured lipids (SFO) produced from fish oil were compared when incorporated into either milk drink or mayonnaise. Furthermore, the effect of adding the potential antioxidants EDTA (240 mg/kg) or lactoferrin (1000 mg/kg) to the milk drink based on SFO was investigated. The lipid type significantly affected the oxidative stability of both mayonnaises and milk drinks: The oxidative stability decreased in the order RFO>FO>SFO. The reduced oxidative stability in the SFO food emulsions could not be ascribed to a single factor, but was most likely influenced by the structure of the lipids and differences in the processes used to produce and purify the lipids. In milk drinks based on SFO, EDTA slightly reduced oxidation, while lactoferrin did not exert a distinct antioxidative effect.

**Keywords** Enzymatic interesterification · Chemical interesterification · Sensory assessment · EDTA · Lactoferrin

## Introduction

Structured lipids can be defined as triacylglycerols (TAG) restructured or modified to change the fatty acid composition and/or their positional distribution in the glycerol molecule by chemical or enzymatic processes. Through enzymatic interesterification it is possible to incorporate a desired fatty acid onto a specific position of the TAG, whereas chemical interesterification does not possess this regiospecificity due to the random nature of the reaction [1]. In the following, the products of these processes will be referred to as specific structured lipids (SSL) and randomized structured lipids (RSL), respectively. SSL can be produced with specific nutritional and/or functional properties. Interesterification using *sn*-1,3-specific lipase can produce SSL with medium-chain fatty acids in *sn*-1/3 positions and long-chain fatty acids in the *sn*-2 position. It has been suggested that this type of SSL, of so-called MLM-structure, may be useful in medicine, for instance for patients with malabsorption problems [1, 2, 3, 4].

The *n*-3 long chain polyunsaturated fatty acids (PUFA), which are abundant in marine lipid sources, have been reported to have several nutritional benefits. Their most well-documented effects include their ability to protect against cardiovascular diseases and their important role in the brain, retina and nervous tissue. Moreover, docosahexaenoic acid (DHA) also seems to play an important role in the neural and visual development of infants [5, 6, 7].

Numerous structured lipids have been described in the literature and potential food applications for them have been suggested. However, only a few research groups have studied how SSL behave in real foods. Data on the oxidative stability and sensory properties of such SSL-enriched foods are necessary in order to stimulate the interest of the health/functional food industry in SSL. Previously, we found that the oxidative stability of milk drink and mayonnaise enriched with SSL (produced from sunflower oil and caprylic acid) was reduced compared to milk drink and mayonnaise produced with randomized or traditional sunflower oil [8, 9]. In the present study we aimed to combine the nutritional benefits of MLM-type lipids with the benefits of *n*-3 fatty acids from fish oil. Therefore, we incorporated structured lipids based on fish oil into the same two food products as in our previous studies. The primary aim of this study was to compare the

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oxidative stability of mayonnaises/milk drinks containing randomized (RFO) or specific structured fish oil (SFO) with a mayonnaise/milk drink containing original fish oil (FO).

Previous results had shown that SFO would need antioxidants to protect the lipid against oxidation [10]. The efficacy of antioxidants in milk type emulsions depend on the oxidation and antioxidation mechanisms in the system. Metal ions (copper and iron) present in milk seem to be the most important catalyst of lipid oxidation in milk enriched with fish oil [11].

Therefore, a second objective of this study was to investigate if EDTA, a metal chelator, could improve the oxidative stability in milk drink with SFO. EDTA had been applied successfully in milk drink and mayonnaise containing SSL based on sunflower oil [8, 9]. We therefore wished to investigate whether EDTA would also be efficient in a milk drink containing more polyunsaturated fatty acids.

EDTA is a synthetic antioxidant and it is therefore desirable to replace this antioxidant with a naturally occurring metal chelating compound such as lactoferrin. Recently, it was reported that bovine lactoferrin, in concentrations ranging from 1–20 $\mu$M, was a strong metal chelating antioxidant in simple oil-in-water (o/w) emulsions [12]. In contrast, we did not observe any anti- or prooxidative effect of 10 $\mu$M lactoferrin in mayonnaise [8]. Therefore, a third objective of our study was to investigate if lactoferrin (1000 mg/kg ~12.5 $\mu$M) would have the same antioxidative properties in milk drink as EDTA.

## Materials and methods

### Materials

Refined fish oil was obtained from Pronova Biocare (Lysaker, Norway), and rapeseed oil from Århus Olie (Århus, Denmark). Tricaprylin (purity >90%) was obtained from Fluka Chemie AG (Buchs, Switzerland), and caprylic acid (purity >95%) from Grünau Illertissen GmbH (Illertissen, Germany). Egg yolk with 3% salt (NaCl) was from Danæg (Copenhagen, Denmark), potassium sorbate from Merck (Darmstadt, Germany), tarragon vinegar (7%) from Lagerberg (Hamburg, Germany), and EDTA (calcium disodium ethylene diamine tetraacetate) was from Sigma (Steinheim, Germany). Lactoferrin was donated by DMV International (Veghel, The Netherlands). Strawberry flavoring 11088 (nature-identical), Grindsted FF 5105 stabilizer (guar gum and sodium alginate), and Recodan emulsifier (mono- and diglycerides of fatty acids, carageenan, and guar gum) were donated by Danisco Ingredients (Brabrand, Denmark). Other chemicals and solvents were of analytical grade.

### Production of randomized and specific structured lipids

#### *Randomized lipid, RFO*

The structured lipid with a randomized structure based on tricaprylin and fish oil was produced by chemical interesterification and subjected to a batch deodorization, as described by Nielsen et al [10]. After deodorization, the oil was stored at −30 °C until use.

#### Specific structured lipid, SFO

The structured lipid with a specific structure based on fish oil and caprylic acid was produced by enzymatic interesterification [13]. Short path distillation was used to remove free fatty acids [14].

### Production of mayonnaise and milk drinks

#### *Mayonnaise*

Mayonnaises were produced in 1 kg batches (codes: Ma-FO, Ma-RFO, or Ma-SFO), composed of 16% lipid (FO, RFO, or SFO), 64% rapeseed oil, 10.4% water, 4.0% tarragon vinegar, 4.0% egg yolk, 0.3% NaCl, 1.0% sugar, 0.1% potassium sorbate, and 0.2% Grindsted FF5105. All percentages are w/w. Mayonnaises were produced as previously described [8].

#### *Milk drink*

Five different milk drinks based on skimmed milk (codes: M-FO, M-RFO, M-SFO, M-SFOE, M-SFOL) were produced. The compositions of the milk drinks were 0.5% lipid (FO, RFO, or SFO), 4.5% rapeseed oil, 5.0% sugar, 0.2% Recodan emulsifier, and 0.13% strawberry flavoring. Antioxidants (240 mg/kg EDTA (E) or 1000 mg/kg lactoferrin (L)) were added to two batches with SFO. The amount of skimmed milk was adjusted so that the total amount of ingredients was 4500.0 g in all experiments. Milk drinks were produced as described earlier [9]. During the mixing process and the HTST heat treatment process the milk drinks were heated to 70 °C and 140 °C, respectively.

### Storage experiments

#### *Mayonnaise*

Mayonnaises were stored in 50 mL capped, brown glass jars in the dark at 20 °C for 0, 2, 4, 6, 8 and 10 weeks. All glass jars contained the same amount of mayonnaise and they had the same headspace volume above the sample. After the desired storage period, the glass jars were frozen at −30 °C until peroxide values (PV) and secondary volatile oxidation products were determined. Mayonnaises were thawed and centrifuged at 25,400 *g* for 10 min to separate out the oil phase for the determination of PV.

#### *Milk drinks*

Milk drinks were stored in the dark at 2 °C for up to 10 weeks. Separate bottles were used for sensory assessments (500 mL bottles) and chemical analyses (PV and volatiles, 250 mL bottles), which were performed after 0, 2, 4, 6, 8, and 10 weeks. All bottles for chemical analyses contained the same amount of milk drink and the same headspace volume above the sample. The same was the case for the sensory bottles, but the headspace volume above the sample in the sensory bottles was not the same as the one in the bottles for chemical analysis due to different bottle sizes. Samples for chemical analyses were frozen at −30 °C and thawed before analysis. Fat was extracted from the milk drinks according to the method by Bligh and Dyer [15], and the extract was used for PV analyses.

### Analytical methods

#### *Analysis of fatty acid composition and TAG structure*

The composition of fatty acids in TAG was determined by gas chromatography of methyl esters. The fatty acid composition in the *sn*-2-position was determined by Grignard degradation prior to

methylation and GC analysis [16]. Molecular species of TAG were separated by gradient RP-HPLC [17] and identified by APCI HPLC-MS [18]. Analyses were performed in duplicate (fatty acids and TAG-species) or triplicate (*sn*-2).

*Determination of induction time by Oxidograph*

The oxidative stability of the oils was determined by accelerated oxidation at 70 °C in the presence of oxygen, using the 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 (the oxidative stability) was determined as the crossing point of the tangents to the curve. Measurements were made in duplicate.

*Analysis of oxidation parameters in emulsions*

Peroxide values were determined using the ferro-thiocyanate method described by Shanta and Decker [19]. Identification and determination of volatile secondary oxidation products were performed by dynamic headspace GC-FID/-MS as described earlier [8, 9]. Results from the analyses are given as concentration ($\mu$g/kg) or as peak area/g mayonnaise or milk drink. Analyses were performed in duplicate (PV) or triplicate (volatiles).

*Sensory assessment (milk drink only)*

The procedure used was that outlined by Timm-Heinrich et al [9]. Twelve assessors were selected for the panel and trained prior to the experiment. The panel agreed on the following attributes for profiling the milk drinks:

*Aroma* (smell):
fishy, strawberry, sweet, exotic fruit, milk, vanilla, rancid, dry, and miscellaneous.
*Flavor* (taste, including retronasal perception):
fishy, strawberry, sweet, milk, vanilla, rancid, acidic, burned, bitter, and miscellaneous.
*Texture*:
mealy, thick, slimy, oily, dry, and miscellaneous.

Milk drinks were served at 5 °C in order to mimic realistic consumer behavior. Blind samples were served in random order to minimize carry-over effects. The panel rated all attributes for each sample on separate 9 cm unstructured scales using a PSION mini computer (PSION plc, London, UK).

Data analysis

*Mayonnaise*

Data obtained from the storage experiment, as well as data from the rheological measurements, were analyzed together by multivariate data analysis using The Unscrambler v 7.6 software (CAMO, Oslo, Norway). The data were analyzed by ANOVA principal least squares regression (APLSR) using the design variables as X-data and the measured variables as Y-data. The design variables were, for lipid type: FO (fish oil), RFO (randomized lipid), and SFO (specific structured lipid), and for replicates: Rep1, Rep2, Rep3, and Mean. Full cross-validation was used to validate the APLSR model. All variables were weighed by 1/standard deviation. By using the jack-knifing facility 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.

*Milk drinks*

Sensory data were imported into the Unscrambler software in order to perform a preliminary ANOVA partial least squares regression (APLSR) [20] to determine and project away differences in the sensory score levels of the assessors [21]. The resulting so-called sensory residuals were used for further data analysis. Data obtained from the storage experiment (PV, volatiles and sensory residuals) were analyzed by multivariate data analysis using the Unscrambler software as described above. The design variables were, for lipid type: FO (fish oil), RFO (randomized lipid), and SFO (specific structured lipid); for antioxidant type: NoA (no antioxidant), EDTA, and LAC (lactoferrin); and for replicates: Rep1, Rep2, Rep3, and Mean. Furthermore, interactions (FO*NoA, RFO*NoA, SFO*NoA, SFO*E, and SFO*L) were included as design variables.

## Results

### Chemical data for the lipids employed

Analytical data for the lipids employed to produce mayonnaise and milk drinks (Table 1) showed that PV were lowest in RFO, followed by FO, and highest in SFO. Furthermore, the oxidative stability as measured by the induction time was lowest for SFO, whereas it was doubled for RFO, and three times as high for FO. Finally, the tocopherol content was ten times lower in SFO compared with RFO, while FO had the highest content. The results in Table 1 demonstrate that the processes employed to produce and purify randomized lipids and structured lipids affected the oxidative status as discussed by Nielsen et al [10].

RFO had a lower total content of PUFA (23%) than SFO (33%) and FO (39%) (Table 1). Therefore, RFO and (partially) also SFO could be expected to be less prone to oxidation than FO. Both SFO and RFO contained 33–38% C8:0, indicating that approximately the same amounts of C8:0 were incorporated into the TAG by both the chemical and enzymatic interesterification processes. The proportions of PUFA in the *sn*-2 position were the same

**Table 1** Oxidation-related data for lipids used for mayonnaise and milk drink production (mean value ±SD, $n$=2–3)

| | PV[a] (meq/kg) | Induction time[b] (min) | $\alpha$-tocopherol ($\mu$g/g) | $\gamma$-tocopherol ($\mu$g/g) | $\delta$-tocopherol ($\mu$g/g) | SFA[c] sum (%) | MUFA[d] sum (%) | PUFA sum (%) |
|---|---|---|---|---|---|---|---|---|
| FO[e] | 2.14 | 276 | 106.8±1.5 | 29.0±0.8 | 10.6±0.2 | 33.1 | 26.9 | 38.8 |
| RFO[f] | 0.89 | 164 | 41.6±1.7 | 31.0±0.4 | 12.6±0.5 | 58.6 | 17.3 | 23.5 |
| SFO[g] | 2.63 | 88 | 4.5±2.5 | 4.1±0.2 | n.d. | 53.7 | 12.9 | 32.8 |

[a] The standard deviation (SD) was <0.05 meq/kg; [b] determined at 90 °C on the Oxidograph, the SD was ±4 min; [c] saturated fatty acids; [d] monounsaturated fatty acids; [e] fish oil; [f] randomized structured lipid; [g] specific structured lipid; n.d.=not detected. For further details please refer to Nielsen et al [10]

**Table 2** Peroxide values during storage (meq/kg, mean value $n$=2)

| | Mayonnaise stored at 20 °C | | | Milk drink stored at 2 °C | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ma-FO | Ma-RFO | Ma-SFO | M-FO | M-RFO | M-SFO | M-SFOE | M-SFOL |
| Week 0 | 0.38 (+) | 0.23 (–) | 0.36 (+) | 0.53 | 0.63 | 0.52 (–) | 1.10 (+) | 0.58 (–) |
| Week 2 | 1.99 | 0.65 (–) | 3.88 (+) | 1.31 | 0.55 (–) | 1.56 (+) | 0.76 (–) | 0.67 (–) |
| Week 4 | 5.60 (+) | 4.21 | 3.85 (–) | 5.72 (+) | 3.19 (–) | 4.10 | 3.06 (–) | 3.55 |
| Week 6 | 5.45 (–) | 5.43 (–) | 14.44 (+) | 6.09 (+) | 3.56 (–) | 4.21 | 3.66 | 4.05 (+) |
| Week 8 | 8.57 (+) | 6.02 | 3.93 (–) | 7.68 (+) | 4.98 (–) | 6.37 | 5.82 | 5.76 |
| Week 10 | 14.03 (+) | 4.77 (–) | 5.03 (–) | 8.39 (+) | 3.07 (–) | 4.02 | 2.00 | 2.30 |

Ma=Mayonnaise, M=Milk, FO=Original fish oil, RFO=Randomised fish oil, SFO=Specific structured fish oil, E=EDTA, L=Lactoferrin. The relative SD of the PV determinations was <10% for values >1.0 meq/kg. (+) Indicates a significant positive regression coefficient, (–) indicates a significant negative regression coefficient ($p$<0.05) obtained from APLSR models

for FO (37%) and SFO (36%), and almost twice as much as for RFO (22%) [10]. Finally, approximately 28% of the TAG in SFO, and a maximum 27% of the TAG in the RFO had the TAG structure MLM, where M is the medium-chain fatty acid C8:0 and L is a long-chain fatty acid [10].

## Effect of oil type and antioxidants on lipid oxidation in mayonnaise and milk drinks

Our previous data indicated that the oil type may effect the oxidative stability of mayonnaise and milk drink [8, 9]. To evaluate the effect of oil type and antioxidant addition on lipid oxidation in mayonnaise and milk drink in the present study, data obtained from the storage experiments were analyzed by a so-called APLSR analysis. In this analysis, oil type (FO, RFO, and SFO) and antioxidants were used as design variables. The advantage of this type of analysis is that it provides a graphical overview of the correlations between the measured variables (such as PV, secondary oxidation products, and sensory data) and between the measured variables and the design variables. This is of particular advantage in experiments with many measured variables, as in the present experiment. Moreover, by studying the regression coefficients obtained from the APLSR analysis, it is possible to determine the effect of the design variables on the measured variables. This part of the APLSR analysis can be compared to a traditional ANOVA. We discuss the effect of oil type and antioxidants on the different measured variables in mayonnaise and milk drink below, based on both the raw data and the regression coefficients obtained from the APLSR models. Finally, the correlation loading plots from the APLRS models are shown to give an overview of the correlations between the different variables.

## Primary oxidation products

### *Mayonnaise*

PV were generally lowest in mayonnaise Ma-RFO. In contrast, PV were highest in mayonnaise Ma-FO after 4, 8, and 10 weeks, and in Ma-SFO after 2 and 6 weeks (Table 2). The regression coefficients (RC), obtained from the APLSR analysis on volatiles and PV, were significantly positive for Ma-FO in weeks 4, 8, and 10 and for Ma-SFO in weeks 2 and 6, and significantly negative for Ma-RFO after 0, 2, 6, and 10 weeks. A significant positive RC indicates that the design variable, Ma-FO say, had a significant positive effect on the measured variable, while it has a negative effect if the RC is negative. Therefore, the RC confirmed the conclusion from the raw data that PV generally were significantly lower in Ma-RFO, followed by Ma-SFO and then Ma-FO. The pattern in the development of PV varied between the different mayonnaises. PV in mayonnaise Ma-FO increased gradually from 0.4 meq/kg in week 0 to 14.0 meq/kg in week 10. PV in mayonnaise Ma-RFO increased from 0.2 to 6.0 meq/kg in week 8 and then decreased to 4.8 meq/kg in week 10. In contrast, in Ma-SFO PV increased rapidly from 0.4 to 14.4 meq/kg in week 6 and then decreased to 4–5 meq/kg in week 8 and 10. These findings indicate a more rapid formation of lipid hydroperoxides in mayonnaise Ma-SFO, followed by a rapid decomposition to secondary oxidation products.

### *Milk drinks*

M-FO had significantly higher PV in weeks 4 to 10 than the other milk drinks (Table 2), followed by M-SFO and M-RFO. The RC were significantly positive for M-FO in weeks 4 to 10 and significantly negative for M-RF0 in weeks 2 to 10, while there was no consistent pattern for the milk drinks based on SFO. Both milk drinks with added antioxidant had about the same PV, which was lower than the PV in M-SFO. After 10 weeks, the maximum value (8.4 meq/kg) was measured in M-FO, while PV peaked in week 8 in the other four batches: 5.0, 6.4, 5.8, and 5.8 meq/kg in M-RFO, M-SFO, M-SFOE, and M-SFOL, respectively. Therefore, in both experiments, the lowest PV was generally observed in samples with RFO.

**Table 3** Significant regression coefficients of volatile compounds in mayonnaise and milk drinks after 8 and 10 weeks of storage

| Compound | Abbreviation | Mayonnaise | | | Milk drink | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | FO | RFO | SFO | FO | RFO | SFO | NoA | EDTA | LAC |
| 2-Propanone | Ket3 | n.d. | n.d. | n.d. | | − − | | | | |
| 2-Butanone | Ket4 | n.d. | n.d. | n.d. | | − − | + | − | | |
| 3-Methylbutanal | MAld4 | − − | + | ++ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Pentanal | Ald5 | ++ | | − − | − | − − | ++ | − − | | ++ |
| 2-Pentylfuran | Fur5 | − | | ++ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2-Hexanone | Ket6 | ++ | ++ | − − | ++ | − − | | − | ++ | |
| Hexanal | Ald6 | − − | − − | ++ | | − − | ++ | − − | ++ | |
| 2-*E*-Hexenal | Mo6 | + | − − | ++ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2-Heptanone | Ket7 | − | | + | ++ | | − − | ++ | | − − |
| Heptanal | Ald7 | | | | | − | + | − | + | |
| 2-*E*-Heptenal | Mo7 | | − − | ++ | | − | + | − | − − | ++ |
| 2,4-*E*,*Z*-Heptadienal | Di7 | | − − | ++ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2,4-*E*,*E*-Heptadienal | Di7 | | − − | ++ | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Octanal | Ald8 | − | | + | − − | | ++ | − − | ++ | + |
| 2-*E*-Octenal | Mo8 | + | − − | + | − | | | − | − | + |
| Nonanal | Ald9 | n.d. | n.d. | n.d. | + | − | | | − | |
| 2-*E*-Nonenal | Mo9 | | | | ++ | − | | | − | + |
| Decanal | Ald10 | n.d. | n.d. | n.d. | − | | | | | |
| 2-*E*-Decenal | Mo10 | n.d. | n.d. | n.d. | | − | | | − | + |
| 2,4-*E*,*Z*-Decadienal | Di10 | | − − | + | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2,4-*E*,*E*-Decadienal | Di10 | | | | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d.=Not detected. + Indicates a significant positive regression coefficient, − indicates a significant negative regression coefficient ($p<0.05$). ++ or − − Indicates significance of results in both weeks. A blank cell means that the design variable was not significant in any of the weeks. For interpretation of design variables and abbreviations please refer to Fig. 3

**Table 4** Concentration ($\mu$g/kg) of volatile compounds in mayonnaise and milk drinks in week 8 (mean value $n$=3)

| Compound | Mayonnaise | | | Milk drink | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ma-FO | Ma-RFO | Ma-SFO | M-FO | M-RFO | M-SFO | M-SFOE | M-SFOL |
| Pentanal | 174.5 | 108.4 | 97.7 | 14.9 | 9.5 | 17.4 | 21.5 | 26.4 |
| 2-Hexanone | n.q. | n.q. | n.q. | 3.8 | 1.0 | 1.4 | 4.0 | 2.5 |
| Hexanal | 18.7 | 6.0 | 176.1 | 28.7 | 22.9 | 33.8 | 53.6 | 37.0 |
| 2-*E*-Hexenal | 121.2 | 79.9 | 125.0 | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2-*E*-Heptanone | n.q. | n.q. | n.q. | 4.0 | 3.5 | 3.3 | 3.3 | 3.1 |
| Heptanal | 47.9 | 43.0 | 55.4 | 17.5 | 14.8 | 15.2 | 21.3 | 19.6 |
| 2-*E*-Heptenal | 90.0 | 74.2 | 103.2 | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2,4-*E*,*Z*-Heptadienal | n.q. | n.q. | n.q. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2,4-*E*,*E*-Heptadienal | 206.0 | 135.3 | 255.5 | n.d. | n.d. | n.d. | n.d. | n.d. |
| Octanal | n.q. | n.q. | n.q. | 8.3 | 11.5 | 11.9 | 19.6 | 18.6 |
| 2-*E*-Octenal | n.q. | n.q. | n.q. | 3.9 | 4.9 | 4.9 | 3.9 | 8.4 |
| 2-*E*-Nonenal | n.q. | n.q. | n.q. | 10.3 | 2.8 | 9.6 | 2.8 | 12.5 |
| Decanal | n.d. | n.d. | n.d. | 4.7 | 18.5 | 13.0 | 25.3 | 31.6 |
| 2-*E*-Decenal | n.d. | n.d. | n.d. | 15.1 | 8.6 | 22.3 | 7.5 | 28.7 |

The relative SD of volatile concentrations was <20%. n.q.=Not quantified, n.d.=not detected. For interpretation of code names please refer to Table 2

## Secondary volatile oxidation products

### *Mayonnaise*

In total 16 volatiles were identified by GC-MS and quantified by dynamic headspace GC-FID (Table 3). To interpret the effect of lipid types on the formation of volatiles, the RC for the various design variables were studied in relation to the different compounds of the last two samplings (Table 3). After 8 and 10 weeks of storage, the main effect of the lipid type SFO was positively correlated to 11 out of 16 volatiles, while FO and RFO were positively correlated to only four and two volatiles, respectively. RFO was negatively correlated to seven volatiles while FO was negatively correlated to only five volatiles. Therefore, the RC indicated that the oxidative stability increased in the order Ma-SFO<Ma-FO<Ma-RFO. In accordance with this interpretation, the raw data showed that Ma-SFO generally had the highest level of all volatiles throughout the storage period except for pentanal, 2-hexanone, and 2-*E*-nonenal (data not shown). Pentanal and 2-hexanone were found in higher levels in Ma-FO, while the patterns were less clear for 2-*E*-nonenal. Table 4 shows the concentrations in week 8 for those

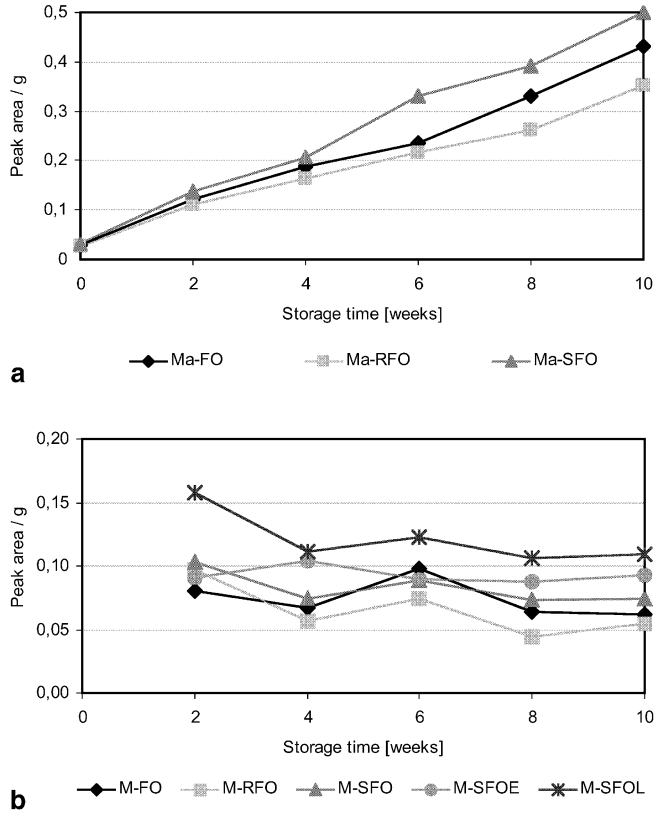


**Fig. 1** Volatile formation during the storage of mayonnaise (20 °C) and milk drinks (2 °C) ($n$=3). **a** 2-*E*-heptenal in mayonnaise; **b** pentanal in milk drink. For interpretation of code names please refer to Table 2

volatiles that were quantified in μg/kg by calibration curves. Firstly, this table also shows the same order of oxidation stability as mentioned above. Secondly, 2,4-*E*,*E*-heptadienal was found in much higher concentrations than the other compounds in all samples (135–255 μg/kg). The second highest concentrations were generally observed for hexanal and 2-*E*-hexenal. Hexanal was only found in high concentrations in Ma-SFO (176 μg/kg) though. Figure 1a, which depicts the formation of 2-*E*-heptenal, is an example of the increased formation of volatiles in Ma-SFO followed by Ma-FO and Ma-RFO.

*Milk drinks*

Fourteen volatiles were identified by GC-MS and quantified by dynamic headspace GC-FID (Table 3). The RC after 8 and 10 weeks of storage (Table 3) showed, in agreement with the results from the mayonnaises, that the main effect of the lipid type RFO was significantly negative ($p$<0.05) for 10 out of 14 volatiles, while the main effect of the lipid type SFO was significantly positive for six volatiles. For the lipid type FO, four positive and four negative RC were determined. The main effect of lactoferrin addition (LAC) was significantly positive for six volatiles, while the effect of EDTA addition was less evident, with four significantly positive RC and five significantly negative RC, respectively. The no antioxidant design variable was negatively correlated to eight volatiles. These findings indicated that M-RFO was most stable, followed by M-FO and M-SFO. The interactions terms (data not shown) revealed that the order of oxidative stability (measured as correlation with formation of volatiles) for the three batches based on SFO was SFO*NoA>SFO*E>SFO*L, as the number of negative RC decreased (6 vs. 4 vs. 1) in this order, while the number of positive RC slightly increased. Pentanal is one of the volatiles that shows exactly this order of oxidative stability (Fig. 1b). However, the raw data showed that the levels for volatiles in general were low and that they did not increase significantly during storage (data not shown). In general, M-RFO had the lowest levels of volatiles, while the three batches based on SFO usually had the highest levels. Table 4, which shows the concentrations of those volatiles that were quantified by the use of calibration curves in week 8, supports this general trend. Moreover, it was evident that hexanal was found in higher concentrations than the other compounds, and that M-SFOE in particular had high concentrations of this compound (54 μg/kg). The other saturated aldehydes were also found in higher concentrations than the unsaturated aldehydes and ketones. Compared to the concentrations found in mayonnaise, the levels were much lower for milk drink and more stable throughout the storage period. Taken together, we obtained the same order of oxidative stability as for the mayonnaise samples: M-SFO<M-FO<M-RFO. Concerning antioxidant addition, the findings (especially the interactions) suggested that addition of EDTA did not improve the oxidative stability of M-SFO, while LAC addition resulted in even higher levels of volatiles.

Sensory data (milk drink only)

In total, 25 descriptors were used to describe aroma (smell), flavor (taste), and texture (mouthfeel) of the milk drinks. In a preliminary statistical evaluation, 13 of those were found to be most important. To clarify the effect of lipid type on the sensory perception, the RC were studied for the 13 descriptors (data not shown). The RC of the interaction terms in particular showed that RFO*NoA and SFO*E were positively correlated to strawberry and sweet taste, but negatively correlated to fishy aroma/flavor, and rancid and bitter tastes. In contrast, SFO*NoA was negatively correlated with the positive descriptors strawberry and sweet flavor, but positively with fishy aroma/flavor and bitter taste. The results for FO*NoA and SFO*L were less clear: both had negative RC for strawberry aroma/flavor and fishy smell, but a positive RC for rancid taste and rancid smell, respectively. These findings supported the conclusion that M-RFO was the milk drink with the best oxidative stability, while M-SFO had the lowest. In contrast to the conclusion based on the results of analysis of volatiles, sensory data indicated that

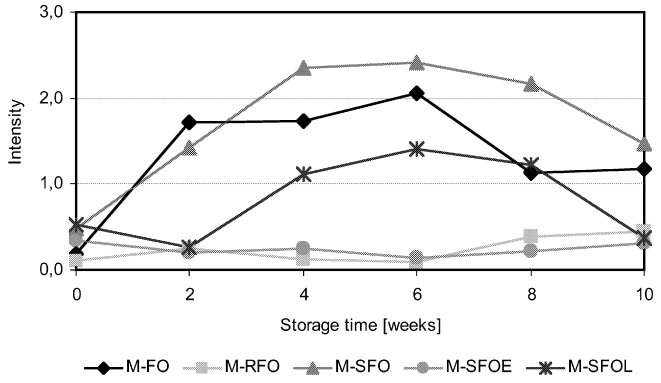


**Fig. 2** Development of fishy flavor perception during the storage of milk drinks at 2 °C (number of assessors varied between nine and twelve). For interpretation of code names please refer to Table 2

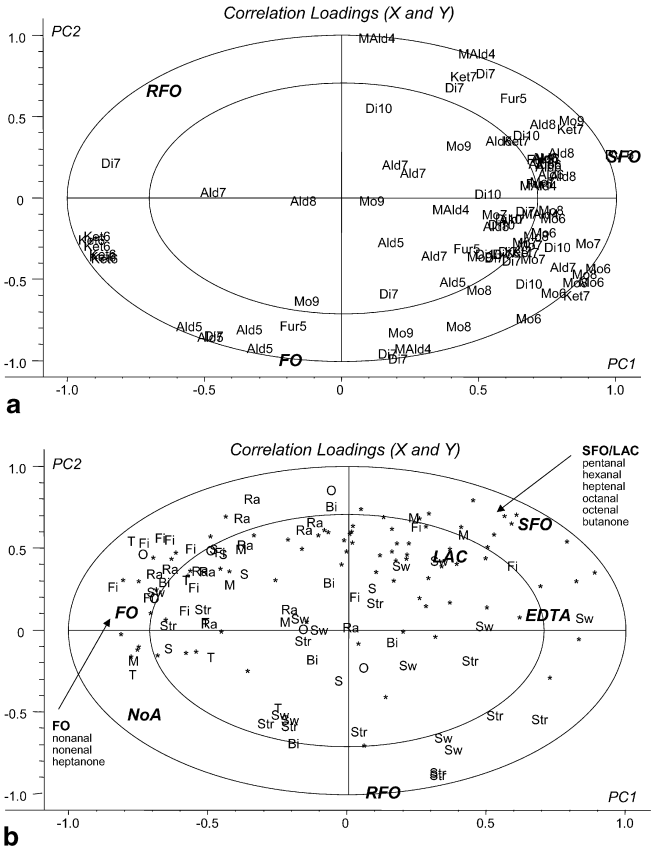


**Fig. 3** Correlation loading plots of APLSR analysis with design variables as X-data and measured variables as Y-data. FO=fish oil, RFO=randomized structured lipid, SFO=specific structured lipid, EDTA (E)=addition of EDTA, LAC (L)=addition of lactoferrin, NoA=no antioxidant addition. The inner ellipse indicates 50% explained variance and the outer ellipse 100% explained variance. The first letter in the variable name marks the real location of the variable in the loadings plot. Note that variables are found more than once as they represent weeks of storage. **a** Volatiles in mayonnaises. Two PC were validated; together they explained 43% and 74% of the variation in the X-data and Y-data, respectively. Ald=aldehydes, Mo=alkenals, Di=alkadienals, Ket=ketones, Fur=furans; the numbers at the end represent the carbon-chain length, as also indicated in Table 3. **b** Volatiles and sensory data for milk drinks. Four PC were validated; together they explained 73% of the variation in the X-data and 72% of the variation in the Y-data. Volatile compounds are indicated by *. Fi=fishy aroma/flavor, Str=strawberry aroma/flavor, Sw=sweet aroma/flavor, Ra=rancid aroma/flavor, Bi=bitter flavor, O=oily consistency, S=slimy consistency, T=thick consistency, M=mealy consistency

addition of EDTA could improve the flavor stability of the milk drink M-SFO. These findings were confirmed by the raw data. Strawberry flavor was lowest in milk drink M-SFO in weeks 4, 6, and 10, followed by M-FO and M-SFOL, while the intensity was highest for milk drinks M-RFO and M-SFOE (data not shown). Fishy flavor intensity (Fig. 2) increased strongly in milk drink M-SFO, so that it was highest from week 4, followed by M-FO and M-SFOL, while the intensity did not increase in milk drinks M-RFO and M-SFOE throughout the storage period.

## Correlation loading plots from APLSR analysis

The correlation loading plots are shown in order to help visualize the correlations between the different mayonnaises or milk drinks and the corresponding analytical data, and to help visualize the effect of antioxidant addition on the milk drinks. Analytical variables and design variables located near each other are highly positively correlated, while variables located opposite each other are highly negatively correlated. Two reduced correlation loadings plots of principal component 1 versus principal component 2 (PC1 vs. PC2) are shown: one with volatiles from the mayonnaise experiment (Fig. 3a), and one with volatiles and sensory variables from the milk drink experiment (Fig. 3b).

### *Mayonnaise*

Figure 3a clearly illustrates that the oxidative stability was highest for Ma-RFO followed by Ma-FO and Ma-SFO, as most of the volatiles were located to the right near the SFO design variable (hexanal, octanal and 2-*E*-heptenal), while smaller clusters were observed near FO (pentanal) or between FO and RFO (2-hexanone). In contrast, almost no volatiles were located near RFO in the upper left corner. The locations of pentanal and 2-hexanone were in agreement with the positive RC for these two compounds for FO and/or RFO. These findings indicate that pentanal and 2-hexanone were the only compounds found in lower levels in Ma-SFO compared with the other two mayonnaises.

### *Milk drink*

In Fig. 3b, most volatiles (pentanal, hexanal and 2-*E*-heptenal) were found in the upper right part of the plot, close to SFO and LAC, but another cluster was found near FO to the very left (nonanal and 2-heptanone). Almost no

volatiles were located at the bottom close to RFO. Moreover, relatively few volatiles were located near EDTA. Regarding sensory variables, the milk drinks could be divided diagonally in the diagram into two groups: 1) RFO, EDTA and to a certain extent also NoA, correlated positively with the desired aroma descriptors strawberry and sweet, and 2) FO, LAC and SFO which had fishy and rancid off-flavors and an undesired mealy consistency. Taking volatiles and sensory data together, the plot illustrates that the oxidative stability of the milk drinks had the order M-SFO<M-FO<M-RFO. Concerning antioxidant addition, the findings suggested that M-SFOE was slightly better in taste than M-SFO or M-SFOL, even though the levels of volatiles were higher than in M-SFO.

## Discussion

In the present study, the oxidative stability of traditional fish oil, randomized lipids, or specific structured lipids (both produced from fish oil) were compared in terms of oxidative stability when incorporated into either mayonnaise or milk drink. Furthermore, the possible antioxidative effect of adding EDTA or lactoferrin to the milk drink with SFO was also investigated.

The results obtained demonstrated that the lipid type significantly affects the oxidative stability of the two food systems. In both experiments PV were lowest in the samples with RFO. However, in the mayonnaise experiment, PV increased more rapidly in the mayonnaise with SFO compared to FO, while the opposite was the case in the milk drink experiment. The results from the analysis of the secondary volatile oxidation products showed that the order of oxidative stability was the same in both products, namely SFO<FO<RFO. Sensory data on the milk drink confirmed this order of oxidative stability. Previous results obtained with mayonnaise and milk drink containing traditional sunflower oil, randomized sunflower oil, or specific structured sunflower oil showed that the emulsions with specific structured lipids were less stable than the emulsions with the other two lipid types [8, 9]. The present data therefore corroborates this conclusion and expands it to include specific structured lipids based on highly unsaturated lipids.

The reduced oxidative stability in the SL emulsions may be due to several different reasons. Firstly, PUFA contents in the three oils decreased in the order FO> SFO>RFO. The high oxidative stability of the RFO emulsions is in accordance with its lower PUFA content. In contrast, the lower oxidative stability of the SFO emulsions compared with to FO emulsions is not in accordance with their PUFA contents.

Secondly, the three lipids employed had been processed differently. The original fish oil was refined and deodorized by the oil producer and used without any further processing. In contrast, RFO was batch deodorized after the chemical interesterification, while the SFO went through a short path distillation process after enzymatic interesterification. The different process histories of the lipids affected their oxidative stability and tocopherol levels, as discussed by Nielsen et al [10], and as shown in Table 1. The effect of the process history of the pure lipids on the oxidative stability of the emulsions will be discussed in a moment.

The reduced oxidative stability in the SFO food emulsions may partly be due to the higher initial content of lipid hydroperoxides in the SFO itself compared to the other two lipids (Table 1). In mayonnaise, however, PV and the concentration of volatiles were at the same level in Ma-FO, Ma-RFO and Ma-SFO in week 0, but PV and volatile concentrations increased much faster in Ma-SFO than in the other two mayonnaises. This observation showed that oxidation was indeed accelerated in Ma-SFO compared to the other two mayonnaises. The finding that PV were at different levels in the three bulk lipids, but at the same level in the three mayonnaises in week 0, could be explained by the fact that the fish lipids (FO, RFO, and SFO) were diluted by rapeseed oil (1:4) during the mayonnaise production. In milk drinks, PV were also at the same level in week 0, but PV increased faster in M-FO than in M-SFO. In contrast to the mayonnaise, volatile concentrations were not at the same level in week 0 in milk drinks, and concentrations did not increase much during storage. As the fish lipids were also diluted by rapeseed oil during milk drink production, these findings could indicate that volatile formation happened mainly during production of the milk drinks, probably during the heat treatment. Therefore, the order of volatile concentrations in milk drink during storage (M-RFO<M-FO<M-SFO) mainly reflected the differences between the samples that were already present in week 0. The slow formation of volatiles in milk drink compared with the mayonnaise could be explained by the fact that the milk drink only contained 0.5% SFO while mayonnaise contained 16%. Comparison of pentanal levels in the different mayonnaises and milk drinks showed that, in mayonnaise, pentanal formation was slower in the SFO sample, whereas the opposite was the case in the milk drink (Tables 3 and 4). Moreover, in mayonnaise, hexanal concentrations were much higher in the SFO sample than in the other samples, whereas in milk drink hexanal was only formed in slightly higher levels than in the samples with FO and RFO. This difference was probably due to different oxidation mechanisms in the two food systems caused by the different compositions of the food matrix.

The purification processes employed for the lipids significantly affected their tocopherol levels, which in turn may have affected the oxidative stability of the emulsions. However, the fish lipids were “diluted” by rapeseed oil, which contains much higher levels of tocopherol (~700 mg/kg). Therefore, the difference between total tocopherol levels in the emulsions were small, and therefore the different tocopherol levels in FO, RFO, and SFO were most likely of minor importance to the oxidative stability.

Apart from the factors described above, the oxidative stabilities of the emulsions may also have been influenced by the structure of the lipids. Therefore, previous studies

have indicated that the location of long chain PUFA in the TAG significantly influences its oxidative stability, but contradictory results are available in the literature [22, 23]. Due to the other factors mentioned above that may have influenced oxidative stability in the present study, our data do not allow us to make any conclusions about the extent the TAG structure influenced the oxidative stability in mayonnaise and milk drink. Further research using very pure structured lipids is necessary to elucidate this matter.

Interestingly, the induction times of the bulk lipids indicated that FO was more stable than RFO, followed by SFO, and these results were therefore not in accordance with the findings in the emulsions, where RFO was more stable than FO. These differences were most likely due to different oxidation mechanisms in emulsions compared with bulk oils. In mayonnaise and milk drink, metal ions are important oxidation catalysts, which may decompose preexisting lipid hydroperoxides to alkoxyl radicals, which may initiate and propagate oxidation [11, 24]. The FO lipid contained much higher levels of peroxides (2.1 meq/kg) than the RFO lipid (0.9 meq/kg). Our results therefore indicate that a high lipid PV has a greater effect on lipid oxidation in emulsions than on oxidation in lipids. This observation is also in accordance with our findings in fish oil enriched milk [11].

Interestingly, addition of EDTA to the milk drink did not reduce formation of the selected volatiles, but significantly reduced the formation of fishy off-flavors. In a previous study of milk drink based on specific structured sunflower oil, we observed that addition of EDTA in the same concentration was able to reduce both volatiles and off-flavor formation [9]. The contradictory results obtained in the present study may be due to the fact that volatile formation did not increase much during storage and concentrations were too low to determine any effect of antioxidant addition. The finding that volatile concentrations only increased slightly during storage at the low temperature used in the experiment (2 °C) could indicate that no oxidation took place in any of the samples. However, the intensity of the fishy off-flavor increased markedly in three of the milk drinks, including the M-SFO sample without EDTA, whereas this was not the case for the M-SFO sample with EDTA (Fig. 3b). Therefore, the sensory panel was able to perceive the antioxidative effect of EDTA in milk drink. These data suggest that EDTA prevented oxidation by chelating metal ions in the milk drink. Taken together, our data indicate that the sensory panel is more sensitive than our dynamic headspace GC method. However, due to practical reasons, the sample amount in the bottles used for sensory analyses was not the same as the sample amount in the bottles for the chemical analyses. Therefore, it cannot be ruled out that the oxygen content in the sensory bottles was higher than in the bottles for chemical analyses and that this resulted in a faster oxidation rate in the sensory bottles. This could explain why the sensory panel perceived the oxidative changes better than the dynamic headspace GC-FID method.

Addition of lactoferrin did not exert a clear effect on the oxidative stability of the milk drink, at least not in the concentration employed in the present study. Previous investigations have shown that lactoferrin (1–20 $\mu$M) was an effective antioxidant in simple buffered corn oil-in-water emulsions (10%) with soy lecithin as an emulsifier [12], and in a liquid infant formula [25]. The emulsions were not heated above 50 °C in these studies. In our study, the milk drink was heated both during the mixing/emulsification process (70 °C) and during the high temperature short-term treatment (140 °C for 5 s). In a study by Mata et al [26], it was shown that heating lactoferrin for a few seconds at temperatures between 72–135 °C decreased the iron binding ability of lactoferrin by about 25%, whereas heating at 85 °C for 20 min decreased its iron binding ability to 50%. The total heating time at 60–70 °C for our milk drink was approximately 5 min, which may have affected its metal chelating properties. In the present study we only investigated one concentration level of lactoferrin. More studies are required to determine whether lactoferrin can prevent lipid oxidation in milk drinks when added in other concentrations.

## Conclusions

In conclusion, the present study showed that the oxidative stability of mayonnaise/milk drink decreased in the order SFO<FO<RFO. Moreover, it was shown that EDTA could reduce off-flavor formation in milk drink containing SFO, while lactoferrin did not exert a distinct antioxidative effect. These results suggest that the oxidative stability of the specific structured lipids should be further improved to be comparable with traditional lipids.

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