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Characterization of oxidative stability of fish oil and plant oil enriched skimmed milk

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Abstract

Oxidative deterioration of skimmed milk emulsions supplemented with 1.0 wt% fish oil and blends of fish- and plant oils was investigated. In order to select oil blends with high oxidative stability, fish oil was blended with various proportions (5, 10, 30, 50 %) of either crude camelina or oat oil, and oxidative stability assessed by the Schaal oven weight gain test and by measuring oxidative stability index by the Rancimat method. Results showed that increased proportions of plant oil in the blends enhanced protection against oxidation. Oat oil resulted in distinct better protection than camelina oil. When pure fish oil and blends containing 10% plant oil were incorporated into skimmed milk emulsions the oxidative stability was severely deteriorated. However, fish oil blended with oat oil conferred the lowest PV and lower amounts of volatile compounds during the storage period of 14 days at 4 °C. In contrast, skimmed milk supplemented with fish-oat oil blend gave the highest scores for off-flavors in the sensory evaluation. Fluorescence spectroscopy were able to measure increasing lipid oxidation in fish oil enriched milk stored in the dark at 4 °C for 21 days. This study showed that oxidative stability of PUFA differs according to their form; bulk or emulsion. Several methods, including sensory analysis, should be combined to illustrate the complete picture of lipid oxidation in emulsions.

1. Introduction

Marine *n*-3 polyunsaturated fatty acids (PUFAs) have received increased attention during the last decade due to potential health benefits in human nutrition [1, 2]. Fish oil is the main dietary source of the long-chain (LC) *n*-3 PUFAs, especially eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6). However, intake of marine foods is below the recommended level in many countries [3, 4]. Enrichment of LC *n*-3 PUFA to commonly consumed foods is a way of increasing consumption of these fatty acids in the diet. Due to the high degree of unsaturation of EPA and DHA, triglycerides rich in these fatty acids are prone to oxidation. Lipid oxidation can adversely affect the nutritional value, shelf-life and sensory quality of foods. Oxidative deterioration of lipids results in the formation of primary oxidation products, lipid hydroperoxides, which are tasteless and odorless. When these primary oxidation products are decomposed they form mixtures of volatile and non-volatile secondary oxidation products. The volatiles are responsible for various unpleasant flavor compounds, while the non-volatiles remain odorless [5]. In addition to the primary and secondary products, tertiary oxidation products in the form of fluorescent chromophores can be formed by interactions of secondary oxidation products with proteins, phospholipids, and nucleic acids, showing characteristic fluorescence spectra [5]. Fluorescence spectroscopy, measured directly on intact samples, is a rapid and sensitive technique with regard to determination of lipid oxidation formed by secondary oxidation products and amino acids [6-8].

In order to get a complete picture of the oxidation process, the degree of oxidation should be measured by more than one method, including methods detecting both the primary, secondary and tertiary oxidation products [5].

In complex systems such as lipid containing emulsions, a series of factors can affect the initiation and propagation of oxidation [4, 9, 10]. Physical and chemical properties of the added ingredients are among these factors [11]. Such ingredients may include marine oils, antioxidants, water, emulsifiers, proteins and so forth. Different strategies such as addition of antioxidants have been applied to retard oxidative deterioration in lipid emulsions [10, 12], among which the natural antioxidants have been of special interest [13, 14]. Vegetable oils contain naturally occurring antioxidant compounds, where the most abundant ones are tocopherols [15-17]. Tocopherols function mainly by scavenging peroxy radicals, and thus interrupting propagation reactions [18].

Camelina *sativa* also known as false flax is an oilseed crop with high levels (30-40%) of α-linolenic acid (C18:3 *n*-3), making it vulnerable to oxidation. However, camelina oil has been found to be very resistant to oxidation and rancidity partly due to a high content of γ-tocopherol [19-22]. Blending fish oil with camelina oil has been shown to improve odor scores when produced as spreads [23].

Also addition of rapeseed oil to fish oil for the purpose of protecting fish oils in milk emulsions has been shown to be effective against oxidative flavor deterioration [24]. Grains, which are a staple dietary component for most of the world`s population, have largely been ignored as important contributors of dietary antioxidants. Oat is a cereal grain that differs from other cereals due to higher lipid and antioxidant content. Oat oil is rich in linoleic acid (C18:2 *n*-6) (31-44 %) [25] and contains a wide range of compounds with antioxidative properties, including tocopherols, tocotrienols, and phenolic compounds [26-28]. Natural antioxidants are best preserved in cold pressed oils. However, crude oils may have a color and distinct smell and taste that may be difficult to find acceptability among consumers.

Cow`s milk is a widely consumed oil-in-water emulsion, thus an interesting medium for addition of fish oil rich in LC *n*-3 PUFA. The content of naturally occurring protein material has been shown to be sufficient to emulsify fish oil added in small amounts [29], thus no extrinsic emulsifier is therefore needed.

The objective of the present study was to investigate the oxidative stability of skimmed milk enriched with fish oil and blends of fish oil and crude plant oils high in natural antioxidants, camelina and oat oil, respectively. Several methods were used for determination of primary, secondary and tertiary oxidation products, including peroxide value, volatile oxidation products, fluorescence spectroscopy and sensory evaluation.

2. Materials and Methods

2.1 Materials

Refined food grade fish oil (blend of cod liver oil and salmon oil) (FO) with added antioxidants (total amount less than 2 % w/w stated by the supplier) was provided by Borregaard Industries Ltd, division Denomega Pure Health, Norway. Crude cold pressed camelina oil (CO) was provided by Bioforsk Øst (Apelsvoll, Norway). After harvest, seeds were stored in a cold-storage chamber at 5 °C. Crude oil was obtained by using a pilot press for small samples (BT Bio Presse Type 50, BT biopresser aps, Dybvad, Denmark).

Oil fractions were frozen at - 40 °C immediately after pressing. Crude food grade Oat oil (OO), extracted with ethanol was obtained from CreaNutrition (Swedish Oat Fiber AB, Sweden). Skimmed milk powder was obtained from TINE BA (Oslo, Norway) with a fat content of < 1.0%

2.2 Characterization of Oils

Initial peroxide value of the three oils was measured by the AOCS Official method Cd8b-90 [30]. The fatty acid composition of FO was provided by the manufacturer, whereas the fatty acid compositions of CO and OO were provided by Nofima. The contents of fatty acids were measured as fatty acid methyl esters [31] using gas chromatography (GC) [32] with flame ionization detection (FID). Peaks were identified by means of external standards. The concentration of the individual fatty acids was expressed in % of total fatty acids. The tocopherol profile of the oils was analyzed by Eurofins Scientific (Moss, Norway), an accredited laboratory, and Nofima, by using normal phase high-performance liquid chromatography (HPLC) based on a method described by Panfili et al [33].

2.3 Determination of Oxidative Stability of Oils

Blends of FO with CO or OO, as well as pure oils, were tested for their oxidative stability by measuring the Oil Stability Index (OSI) according to AOCS Offical Method Cd 12b-92 [34]. The binary blend ratios were 50:50, 70:30, 90:10 and 95:5 for both FO:CO and FO:OO. Each binary ratio was prepared as a well-mixed batch; the minor oil component (i.e. plant oil) was weighed first, and the remaining was filled up with fish oil to obtain the desired ratio. Samples (5.00 ±0.04 g) were placed in glass tubes, sealed with a two hole rubber stopper equipped with aeration and effluent tubes, and installed into the Oxidative stability instrument (Omnion Inc., Rockland, MA, USA). The probe measuring the conductivity signal was connected to a computer which processed the data and generated OSI curves and OSI times automatically. All the samples were run at 70.0 ± 0.1°C, air pressure was set at 4.0 – 4.25 psi. Relatively low temperature of 70 °C compared to the temperature of 110 °C described in the AOCS Official Method was chosen due to the high susceptebility of FO to oxidation. The air pressure was reduced from the one prescribed in the AOCS Official Method (5.5 psi), due to an extensive foaming of OO under the flow of oxygen, as a prevention against contamination of the conductivity measurement tube containg deionized water and probe by the oily foam. The determinations were carried out in six replicates.

The same blends as for the OSI test were used for the Schaal oven weight gain test. Oil samples (5.00 ± 0.01 g) were weighed into open glass Petri-dishes (inner diameter 7.0 cm, height 1.2 cm) and placed into a laboratory drying oven (TS 8136, Termaks AS, Bergen, Norway) at 70 ± 1°C in the dark with no air circulation. The dishes were taken out of the oven for weighing every 8 hours during the first 21 days and then twice or once a day, cooled to ambient temperature, reweighed and returned to the oven. Weight changes were recorded for up to 53 days. The time required to reach a 0.5% weight gain was calculated and taken as an index of stability. Each sample was analyzed in triplicate.

2.4 Preparation of emulsions

Skimmed milk powder (100g/l) was mixed with water (20°C) using an Ultra Turrax Super Dispax SD 45/2 (IKA-Werke GmbH & Co. KG, Staufen, Germany). The resulting milk (3 L) was pasteurized by heating to 72°C within 3 min, holding for 15 s and then cooled to room temperature. Three different batches of emulsions were then prepared as described in Table 1; FO:CO and FO:OO were mixed together in ratio 90:10 and the oil blend (1 wt %) were then added to the skimmed milk. For pure FO, 1 wt % was added to the skimmed milk. The samples were subsequently homogenized (18 MPa) in a two-valve Rannie homogenizer (Model LAB 4580/71, Copenhagen, Denmark) under cooling conditions (7-9 °C). The pH of the emulsions was 6.7. Samples were stored in closed Pyrex bottles (50 mL for PV and HS-CG/MS analysis, 1 L for sensory analysis and 250 mL for fluorescence spectroscopy) at 4°C in the dark. Samples for PV and volatile analysis were taken at day 0, 5, 8 and 14, immediately flushed with nitrogen (quantity 99.9, AGA AS, Oslo, Norway), and stored at -25°C. Samples were thawed immediately before analysis. Sensory evaluation of the emulsions was carried out after 0, 5 and 8 days of storage, whereas fluorescence spectrometry was performed at day 0, 5, 8, 14 and 21.

2.5 Analysis of primary oxidation products

Lipids were extracted from the emulsions by chloroform:methanol (1:1 w/w) [35], using a reduced amount of solvent [36]. PV were measured directly in the oil extracted from the milk emulsion by colorimetric ferric-thiocyanate method [37]. The samples were analyzed in duplicate.

2.6 Analysis of volatile secondary oxidation products

Volatiles were trapped on Tenax tubes (Perkin Elmer, Norwalk, CN, USA) by purging 4 g milk emulsions with N_2 (150 ml/min) for 30 min at 45 °C. 4-methyl-1-pentanol in rapeseed oil was used as internal standard. An automatic thermal desorber (ATD-400, Perkin Elmer, Norwalk, CN) was used to desorb (200 ° C) the volatiles, and subsequently they were cryofocused on a Tenax GR cold trap. Separation of the volatile compounds was achieved by gas chromatography (HP 5890 IIA, Hewlett Packard, Palo Alto, CA, USA) as described by Timm-Heinrich et. al. (2003). The volatiles were analyzed by mass spectrometry (HP 5972 mass-selective detector) and identified by MS library searches (Wiley138K, John Wiley and Sons, Hewlett-Packard) and by authentic external standards. The individual compounds were quantified through calibration curves. The formation of nine volatiles 1-penten-3-one, 1-penten-3-ol, (E)-2-pentenal, 2-penten-1-ol, hexanal, (E)-2-hexenal, 2,4-heptadienal, (E,E)-2,4-heptadienal and (E,Z)-2,6-nonadienal, responsible for off-flavors [38-40], was followed during 14 days of storage at 4°C. The samples were analyzed in triplicate.

2.7 Sensory evaluation

The emulsions were evaluated by a trained sensory panel (12 panellists) at Nofima (Ås, Norway) using Quality Descriptive Analysis ISO 6564:19865(E) and ISO 13299:2003(E). The panelists have been selected and trained according to recommendations in ISO 8586-1:1993(E). Prior to the assessments, the panel went through a training session to agree on attributes for the enriched emulsions and for the variation in attribute intensity. The descriptors used for odor and flavor assessment were fishy, metallic, stearin/paraffin and paint. The coded samples (50 ml) were served in blind trials at 0, 5 and 8 days of storage and randomized according to sample, assessor and replicate. The panelists evaluated the samples in duplicate, during two sessions. Emulsions were evaluated on a continuous intensity scale ranging from 1 to 9, where 9 is the maximum intensity. The sensory laboratory has been designed according to guidelines in ISO 8589: 1988(E) with separate booths. Data were collected on Eye Question, v. 3.8.6 (Logic 8, Nederland).

2.8 Fluorescence Spectroscopy

Fluorescence emission spectra were measured directly on FO milk emulsions. The samples (12 ml) were filled into sample cuvettes that exposed a flat, circular surface with a diameter of 5 cm.

Samples were illuminated by 382 nm excitation light, and fluorescence emission spectra were measured in the range 410-640 nm according to [41]. Excitation at 382 has been shown to give good results regarding measurements of tertiary oxidation products [41, 42]. The excitation light was generated by a 300W Xenon light source (Oriel 6258, Oriel Corporation, Stratford, CT) and passed through a 10 nm bandwidth interference filter (Orel 59920). The light was directed onto the samples at an angle of 45°. Spectra were collected by a spectrograph (Acton SP-150, Acton Research Corp., Acton, MA) connected to a sensitive charge coupled device (CCDcamera; Princeton TEA/CCD-512-TKBM1, Princeton Instruments Inc., Trenton, NJ). A cut-off filter at 400 nm (Melles Griot 03FCG049, Melles Griot Inc., Irvine, CA) was positioned in front of the spectrograph slit to suppress excitation light reflected from the sample. Exposure time was 0.5 s for all the measurements. Samples were measured at 4°C. Four spectra were collected for each sample, giving four replicates. Spectrograph and detector were controlled by the software Win Spec Ver. 1.4.3.4 (Princeton Instruments Inc.)

2.9 Statistical analysis

Data were evaluated by one-way analysis of variance and Tukey's test using Minitab Statistical software (Addison-Wesley, Reading, MS, USA). Differences were considered to be significant at $p < 0.05$. Sensory descriptions and fluorescence emission intensity was plotted against wavelength in R ver. 2.14.1, which is a free software environment maintained by the R Development Core Team (http://www.r-project.org/). Smoothing of curves was performed using the method local polynomial regression fitting (LOESS) in the R software.

3. Results and Discussion

3.1 Properties of oils

FO, CO and OO used in the present work were characterized in terms of fatty acid profile, initial PV and tocopherol profile (Table 2). Levels of fatty acids and tocopherols in CO and OO were in good agreement with previous reports [19-21, 27, 28, 43-45]. FO and CO had the highest γ-tocopherol levels (1310 and 784 ppm respectively), whereas OO had the highest levels of α-tocopherol (90 ppm). FO had the highest total content of tocopherols, but the predominant part is added tocopherol, whereas CO and OO were crude oils with only naturally occurring tocopherols. The oils may also contain other antioxidants not analyzed in this study. In particular, tocotrienols in OO and phenolic compounds in CO and OO may contribute to protection against oxidation [20]. In general, the tocotrienols have a stronger antioxidant effect on lipid oxidation than tocopherols [46]. Tocopherols and tocotrienols act by donating their phenolic hydrogens to lipid free radicals, and have donating power in the order α > β > γ > δ [47]. Relative antioxidant activity of tocopherols depends on factors such as the lipid composition, temperature, physical state (bulk or emulsion) and the tocopherol concentration [48].

The FO used in this study contained 10 and 12 % of the *n*-3 LC PUFAs EPA and DHA, respectively (Table 2). In CO and OO the majority of the PUFA consisted of the *n*-3 PUFA α-linolenic acid (ALA) and *n*-6 linoleic acid (LA) respectively. The high ALA content (37.9 %) in CO might be a nutritional advantage, but it can also be a driving factor for oxidation. PUFAs are susceptible to lipid oxidation, in the order DHA > EPA > ALA > LA. The order reflects the amount of reactive methylene groups available for peroxidation processes. Compared to FO and CO, the fatty acid profile of OO showed lower degree of unsaturation. In addition to triglycerides, the relatively high content of phospholipids (≥ 12 % w/w) in crude oat [49] may influence oxidative stability. Addition of CO and OO to FO may give small increases in the levels of LA and ALA in the blends, which can possibly affect the overall oxidative stability of the blends compared to pure oils [24, 50].

All the oils had low initial peroxide values. Peroxides, primary oxidation products in CO were higher by only 0.7 meq/kg than in OO, indicating that the two oils had similar levels of oxidation. The initial PV in the FO was very low (< 0.1 meq/kg). In fish oil enrich emulsion, a low initial peroxide value was shown to facilitate the control of oxidative deterioration [40]. The three pure oils showed no significant increase (p > 0.05) in the peroxide value during storage at 4 °C for 21 days (data not shown).

3.2 Results of stability tests of selected oil blends

The oxidative stability of oils and their blends was evaluated based on the measurement of the induction period (OSI) and the Schaal oven test at 70 °C (Table 3). These data were necessary for the selection of optimal blend ratios for the skimmed milk emulsions. The stability tests gave consistent results, indicating that addition of OO to the blends gave the best protection against oxidation (Table 3). Blends of FO+OO containing 5 and 10 % OO were roughly two-times as oxidative stable as blends of FO+CO with the same proportions of CO. Increasing the proportions of OO in the FO+OO blend to 30 and 50 %, increased the stability significantly. Increasing the proportions of CO in the FO+CO blends had only minor effects on the oxidative stability. The fatty acid composition and unsaturation indices of the oils used in this study suggest that FO would be the least stable, closely followed by CO, and then OO. Despite the high ALA content (~40 %) in CO, the induction period for CO was double as that observed for FO, which can be explained by differences in presence of minor compounds such as phenolic compounds and tocopherols as well as fatty acid profile [50].

The present results indicate that OO is very resistant to oxidation. However, the OSI test conditions did not allow determination of OSI values for pure OO, or blends with 30, and 50 % OO. This was due to extensive foaming of the oil in the glass tubes under air flow which inevitably contaminated the measurement probe. These values are therefore denoted as ND (not determined). Content of polar lipids and free fatty acids may have caused the foaming [51, 52]. Pure OO showed no increase in weight after more than 50 days in the oven at 70 °C, so the weight observation was terminated. Measurements on more polyunsaturated oils have shown to give best results by use of the weight gain method [53]. Addition of only 5 % OO gave increased protection, indicating that not only a dilution effect, but also the presence of tocopherols, tocotrienols, phenolics and other compounds in OO probably contributed to the dramatically prolonged induction period.

Naturally, FO with its high unsaturation conferred the lowest OSI time (~ 57 h) and also reached 0.5 % weight gain at the earliest time point. The measured induction periods of FO using the two methods were relatively high compared with Rancimat measurements of anchovy, hake liver and sardine oils at the same temperature conditions [54]. The relatively high stability of FO can be attributed to the high total tocopherol content of 2660 ppm, and the presence of ascorbyl palmitate and other antioxidants (not shown), which may behave synergistically in reinforcing the antioxidant activity of tocopherols [55].

3.4 Oxidative Stability of Enriched Emulsions

Oil-in-water emulsions, enriched in LC n-3PUFA, were prepared with 1 wt % FO, and 90:10 FO:CO and FO:OO blends (Table 1). Stability tests indicated that a ratio of 95:5 was sufficient to increase oxidative stability in blends with OO, whereas ratios higher than 70:30 were needed for blends with CO. However, pre experiments showed that inclusion of higher levels of the plant oils (within the 1 wt%) than 90:10 resulted in a more characteristic taste and smell and also a poorer physical emulsion stability was observed.

3.4.1 Formation of primary oxidation products

Peroxide values are shown in Table 4. Peroxide values of the emulsions at day 0 were higher than initial PV of the oils, probably due to the fact that values obtained from the ferric thiocyanate method are generally higher than values obtained with the iodometric method [5]. In addition, it may also be presumed that the oxidation process initiated already during the processing of the emulsions. The FO emulsion increased more than 40-fold in peroxide value after 5 days of storage. On the contrary, the FO+OO emulsion showed no significant difference in PV during the 14 storage days. During further storage, the PV in FO enriched emulsion increased significantly. It have been suggested that low initial PV (< 0.1 meq/kg) is more critical for oxidation rates than polyunsaturated fatty acids or content of tocopherol in oils [24, 40]. In this study the low initial PV FO oxidized very rapidly when emulsified into skimmed milk despite a low initial PV. A significant increase in peroxide value was also shown in the FO+CO emulsion, with an initial value of 5.8 meq/kg to 42.7 meq/kg after 5 days of storage. This indicates that addition of CO to FO did not have a protective effect against oxidation in emulsion when considering the primary oxidation products. A decrease in PV was shown in FO+CO from day 5 to day 8, and from day 8 to day 14, which could indicate decomposition of hydroperoxides to secondary oxidation products. When relating the PV with results from the induction time measurements (Table 3), the same trend is observed concerning the oxidation rate of the oils and blends, which was found in the order; FO > FO+CO >FO+OO. Previous research have reported that milk emulsion with less than 0.01 % and also with 1 % milk fat content, enriched with 1.5 wt-% fish oil and 0.5 wt-% blend of fish oil and rapeseed oil respectively, resulted in relatively low peroxide values during storage at 2 °C for 14 days [24, 40]. The fish-rapeseed oil blend resulted in PV < 1 meq/kq, while skimmed milk enriched with cod liver oil (without antioxidants) gave peroxide values up to 5.3 meq/kq. Milk recombined from skimmed milk powder was used in the present study.

Since skimmed milk contain only <0.1 % fat, any interference with milk lipids in the measurements would be less significant compared to milk with higher fat content. Increased milk fat content may enhance oxidative stability since milk lipids are highly saturated. However, the similarity of milk batches was easier to achieve by the use of milk powder. Milk is a complex medium with several factors that can either inhibit or promote oxidation of the added oils. The present results indicated that interactions between the milk medium and the added PUFAs seemed to promote oxidation rather than inhibit it. Since heat is known to increase the oxidation rate of lipids, the temperature should in general be kept as low as possible during processing and storage [5]. Decomposition rate of hydroperoxides is high when exposed to high temperatures or high amounts of reactive transition metals. With this in mind, the emulsions in this study were homogenized at a low temperature to avoid the effect of temperature on the oxidation rate. Nevertheless, the emulsions reached high peroxide values during storage. However, it should be mentioned that other studies have shown that a high homogenization temperature and pressure increased oxidative stability in fish oil enriched milk emulsions prepared with skimmed milk instead of milk powder [29]. The emulsions were exposed to oxygen for a short period of time during the homogenization process, which is also a factor for the initiation of oxidation. The high levels of LC-PUFAs in FO and the high ALA levels in the CO are another factor that can accelerate oxidation, and may be a reason for PV values > 40 meq/kg after only 5 days storage. The significant increase in oxidation in the emulsions after just 5 days of storage at 4 °C is in contrast to the stability in bulk oils which further confirms that oxidative stability of PUFA is highly dependent on the food matrix, lipid composition and form [10].

3.4.2 Formation of secondary volatile compounds

Nine volatile secondary oxidation products derived from degradation of *n*-3 and *n*-6 fatty acids [38-40] were selected as markers of oxidation during storage of the emulsions.

Except for 2-penten-1-ol and hexanal, the formation of the selected secondary volatile compounds increased already from the first storage days (Table 5). 2-penten-1-ol was below the detection limit in all the emulsions during the storage period. Hexanal was already present in high values in the emulsions from day 0. Significantly higher initial values were found in the emulsions containing the plant oils, OO (about 56×10^4 ng/g) and CO (about 20×10^4 ng/g) compared with the FO emulsion (about 12×10^4 ng/g). Hexanal is a common degradation product from the autoxidation of linoleic acid hydroperoxides, and have a very low threshold value for flavor and odor [5].

In oat, hexanal is one of the most abundant volatile compounds [56]. Both the OO and CO contain high levels of linoleic acid, 41.5 % and 15.6 % respectively (Table 2). During the storage period degradation of hexanal was detected in OO and CO emulsions, while hexanal increased in the FO emulsion.

Peroxide values showed a significant and a high increase in the FO and FO+CO emulsion from day 0 to day 5 (Table 4). As a result of the increase in hydroperoxides, a corresponding formation of secondary volatile compounds especially in FO and FO+CO was seen in this storage period (Table 5). The number of reactive methylene groups is higher, and the activation energy for abstracting proton from a methylene group in conjugation in FO and FO+CO is lower than for FO+OO with less degree of unsaturation in the fatty acid profile. At 8 and 14 days storage, the FO+OO emulsion showed significantly lower values of 1-penten-3-ol, 2,4-heptadienal and (E,E)-2,4-heptadienal than in FO and FO+CO emulsions (Table 5). (E,Z)-2,6-nonadienal was not detected in the FO+OO emulsion during the storage period, whereas a significant increase from 10.8 ng/g to 41.7 ng/g, during 5 to 14 days storage was found for (E,Z)-2,6-nonadienal in the FO emulsion. Development of the vinyl ketone 1-penten-3-one was higher in FO emulsion at day 8 and 14 compared to FO+CO and FO+OO emulsions. 1-penten-3-one, the diunsaturated aldehyde (E,E)-2,4-heptadienal and (E,Z)-2,6-nonadienal are compounds derived from degradation of n-3 PUFA, and have been characterized as very potent odorants, contributing to unpleasant rancid and fishy off-flavors in fish oil enriched milk and mayonnaise [38, 40, 57]. In general the FO emulsion developed higher levels of 1-penten-3-one, 1-penten-3-ol, (E)-2-pentenal, (E)-2-hexenal, 2,4-heptadienal, and (E,Z)-2,6-nonadienal during the storage period, closely followed by the FO+CO emulsion. A degradation of 1-penten-3-one, (E)-2-pentenal, 2,4-heptadienal and (E,E)-2,4-heptadienal was shown after 5 days of storage for the FO+CO emulsion, which indicate further oxidation or reactions with proteins to tertiary products. Overall the evaluation of volatile compounds showed the lowest values for the FO+OO emulsion for all compounds except for hexanal, followed by the FO+CO emulsion, as also observed when PV of the same emulsions were measured.

3.5 Sensory evaluation of enriched emulsions

The average sensory scores for the off-odors and off-flavors in milk emulsions stored for 8 days in the dark at 4°C are shown in Figure 1. Only small changes were detected in stearin/paraffin odors and flavors during the storage period, with FO+OO at day 8 having the highest score on 2.0 for odor and 2.3 for flavor (not shown).

Fish and paint odor and flavor increased from day 0 to day 8, in particular for the FO and FO+OO emulsions. . The highest scores were found for FO+OO emulsion at day 8. Paint odor and flavor showed significantly higher intensity in the FO+OO emulsion at day 8 (odor score 6.8 and flavor score 7.0), compared with the FO and FO+CO emulsions (odor score 4.5 and 1.7 respectively, flavor score 4.8 and 2.2). The intensity of fish and paint off-flavors in the FO+CO emulsions had a low intensity in the range 1.1- 2.2 during the entire storage period. Crude oils may have a strong characteristic product-related flavor [58]. In this study, crude CO had a very distinct odor and flavor even when mixed with FO and added to milk, which may have caused a masking effect of the off-flavors related to lipid oxidation in the FO+CO emulsion, resulting in low sensory scores for these attributes. This finding is in accordance with a recent study by Eidhin and Beirne (2010) showing that camelina oil had a masking effect on fish odors when blended with fish oil.

Results from the sensory evaluation contradicts with results from the analysis of secondary volatile compounds, where the FO+OO emulsion showed better oxidative stability compared with FO and FO+CO emulsions. Whether this can be explained by compounds not measured by HS GC/MS is unknown. This clearly demonstrates that both sensory analysis and instrumental methods are needed for a more complete evaluation when monitoring lipid oxidation in lipid enriched emulsions. One possible explanation for the high scores for off-flavors in the FO+OO emulsion can be related to the content of minor components including free fatty acids in oat [56]. The free fatty acids are formed during lipid extraction by hydrolysis of triglycerides either by lipases or by high temperature in the presence of water [59]. In emulsions, the polarity of free fatty acids and hydroperoxides can drive them to the surface of an emulsion droplet and interactions with aqueous-phase oxidation catalysts can occur [60].

3.6 Fluorescence Spectroscopy fish oil enriched emulsion

Figure 2 show the fluorescence emission spectra in the 420-480 nm region of FO emulsion during storage. Previous studies have shown formation of fluorescence oxidation products in this wavelength region, which increases with the degree of oxidation [61, 62]. According to Yamaki et. al [60] reactions between amino acids and lipid radicals, produces fluorescence emission spectra in the range 420-440 nm. Lipid oxidation products from turkey have been shown to give an emission peak in the 470 nm region [42].

The fluorescence intensity increased significantly ($p < 0.05$) in the FO milk emulsion during 14 days of storage at 4 °C in the dark in the 460 nm region. This result indicates increasing degree of lipid oxidation in the FO enriched emulsion during 14 days of storage, which is in agreement with the increasing oxidation products shown by PV, HS-GC/MS and sensory analysis of the FO emulsion.

Only the FO emulsion was chosen for investigation of the lipid oxidation process measured by fluorescence spectroscopy. The motivation was caused by the possibility of differences in fluorescence by addition of dissimilar oils which may cause challenges in comparison of spectra.

At day 21, naturally, microbiological spoilage had transpired in the emulsion due to the shelf-life of the skimmed milk. Therefore, no significant increase was shown at 460 nm after day 14. Day 21 was included in the fluorescence measurements to evaluate whether lipid oxidation continued to increase after 14 days of storage. The addition of FO to skimmed milk led to higher fluorescence emission intensity at day 0, compared with pure skimmed milk samples. No significant increase was shown for skimmed milk during storage time of 21 days (not shown). Degradation of the photosensitizer riboflavin (peak 530 nm) in the milk emulsions were not considered in this study since the samples were not stored under light exposure, also since the focus was on the development of lipid oxidation products.

Conclusion

This study demonstrated that blends of fish oil and oat oil achieved higher oxidative stability compared to pure fish oil and blends of fish oil and camelina oil. The oxidative stability of skimmed milk emulsion enriched with a blend of 90% fish oil and 10% oat oil also revealed the lowest peroxide values and volatile compounds during storage at 4 °C for 14 days storage. However, sensory analysis of the same emulsion gave the highest scores for undesirable off-flavors, indicating that several methods, including sensory analysis, should be combined to illustrate the complete picture of lipid oxidation in emulsions. It was also demonstrated that lipid oxidation in fish oil enriched skimmed milk could be detected using the non-destructive method of fluorescence-spectroscopy.

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Figure captions

Figure 1. Average intensity [1-9] of sensory descriptions in skimmed milk enriched with fish oil (FO), binary blend of fish oil and camelina oil (FO+CO) and binary blend of fish oil and oat oil (FO+OO) during 8 days of storage at 4 °C.

Figure 2. Fluorescence emission spectra of fish oil enriched skimmed milk emulsion stored in dark at 4 °C for 21 days (*n*=4).

Tables

Table 1. Experimental design over the addition of oils for preparation of enriched emulsions.

	Addition of oil to milk [wt-%]		
Sample name	FO	CO	OO
FO	1.0	—	—
FO + CO	0.9	0.1	—
FO + OO	0.9	—	0.1

FO = Fish oil, FO+CO = Fish oil and camelina oil mixture, FO+OO = Fish oil and oat oil mixture

Table 2. Fatty acid composition, initial peroxide value and tocopherol content of the fish oil (FO), camelina oil (CO) and oat oil (OO), and blends of FO:CO and FO:OO (90 % and 10 % respectively)

Fatty Acids %	FO	CO	OO	90:10 FO:CO	90:10 FO:OO
SFA					
C14:0	3.8	0.1	0.2	3.4	3.4
C16:0	13.5	5.4	15.8	12.7	13.7
C18:0	4.0	2.4	1.3	3.8	3.7
C20:0	–	1.3	0.1	0.1	0.0
Sum SFA	21.3	9.2	17.4	20.0	20.8
MUFA					
C16:1(*n*-7)	5.5	–	0.2	5.0	5.0
C18:1(*n*-7)	4.0	0.7	0.7	3.7	3.7
C18:1(*n*-9)	19.0	12.5	37.7	18.4	20.9
C20:1(*n*-9)	–	14.7	0.7	1.5	0.1
C20:1(*n*-11)	1.3	–	–	–	–
C22:1(*n*-9)	–	3.0	0.1	0.3	0.0
C22:1(*n*-11)	4.6	–	–	4,1	4,1
Sum MUFA	34.4	30.9	39.4	32.9	33.8
PUFA					
C18:2(*n*-6)	4.4	15.6	41.5	5.5	8.1
C18:3(*n*-3)	1.2	37.9	1.4	4.9	1.2
C18:4(*n*-3)	1.9	–	–	1.7	1.7
C20:2(*n*-6)	–	2.2	–	0.2	0.0
C20:4(*n*-3)	0.9	1.9	–	1.0	0.8
C20:5(*n*-3)	10.0	–	–	9.0	9.0

C21:5(*n-3)*	0.6	–	–	0.5	0.5
C22:5(*n*-3)	2.2	–	–	2.0	2.0
C22:6(*n*-3)	11.9	–	–	10.7	10.7
Sum PUFA	33.1	57.6	42.9	35.6	34.1
Other	11.2	2.4	0.3		
Degree of unsaturation	193.4	187.8	126.6	191.7	185.6
Initial PV (meq/kg)	< 0.1	1.5	2.2		
Tocopherols (ppm)					
α-	740	26	90		
γ-	1310	784	13		
δ-	600	13	6,5		

Table 3. Oxidative Stability Index (OSI) and weight gain values of oils and binary mixtures of oils. The values are expressed as hours ± standard deviation (n = 6 for OSI and n = 3 for the weight gain method)

% of added oil	OSI values (h) at 70 °C	0.5 % weight increase (h) at 70°C
FO		
100	56.9 ± 2.2	51.1 ± 0.4
CO		
100	139.5 ± 2.5^{v}	123.6 ± 2.5^{v}
50	74.9 ± 2.5^{w}	62.1 ± 0.5^{w}
30	66.4 ± 1.4^{x}	56.6 ± 1.1^{wx}
10	58.1 ± 2.5^{y}	51.8 ± 1.8^{wx}
5	54.1 ± 3.3^{y}	53.1 ± 0.1^{x}
OO		
100	ND	ND
50	ND	841.3 ± 12.7^{v}
30	ND	434.7 ± 7.8^{w}
10	159.4 ± 1.7^{v}	162.6 ± 1.1^{x}
5	113.3 ± 2.3^{w}	117.9 ± 0.9^{y}

$^{v\text{-}z}$ indicate significant differences ($p < 0.05$) within oil types in the column (combinations sharing a letter are not sign. different)
ND = not determined

Table 4. Peroxide value of enriched skimmed milk emulsions during 14 days storage at 4 °C. PV expressed as meq O_2/kg ± standard deviation (n = 2).

Peroxide value (meq O_2/kg oil)				
	Days of storage			
Sample	0	5	8	14
FO	1.4 ± 0.7^{a,x}	47.0 ± 0.4^{b,x}	62.4 ± 1.0^{c,x}	90.6 ± 1.5^{d,x}
FO+CO	5.8 ± 3.1^{a,x}	42.6 ± 0.5^{b,x}	32.3 ± 3.6^{c,y}	17.0 ± 1.0^{d,y}
FO+OO	7.1 ± 1.2^{a,x}	5.8 ± 0.0^{a,y}	10.6 ± 0.2^{a,z}	9.0 ± 0.7^{a,z}

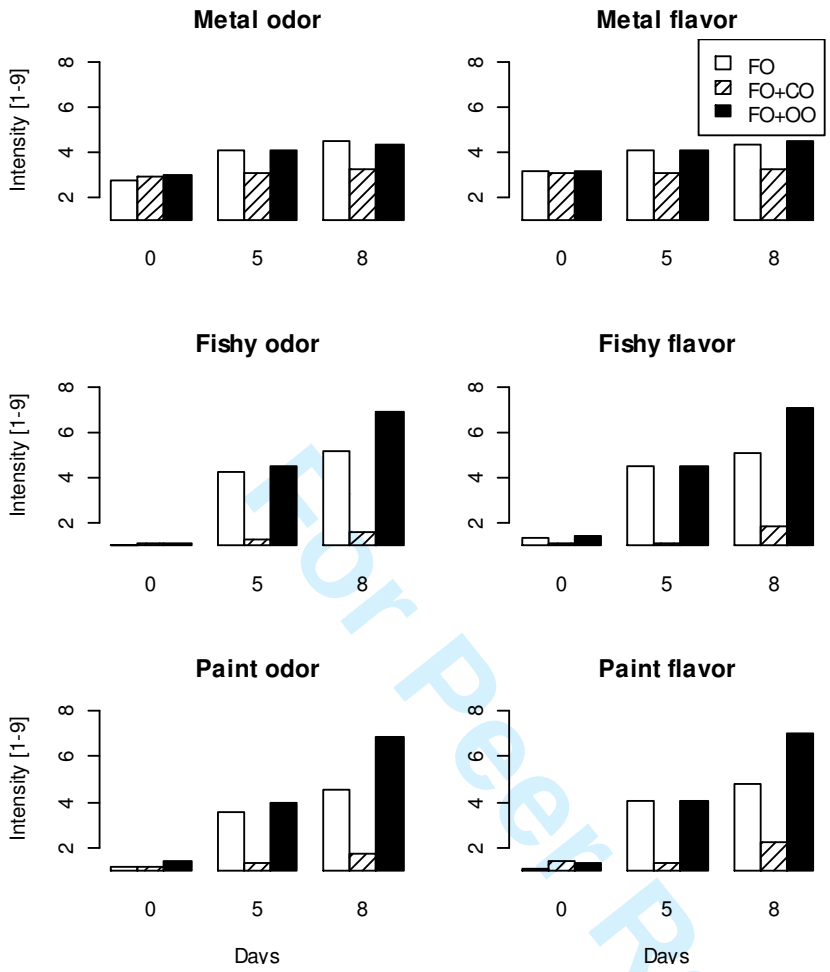
$^{a-d}$ in the row indicate significant difference ($p < 0.05$) between days of storage within sample

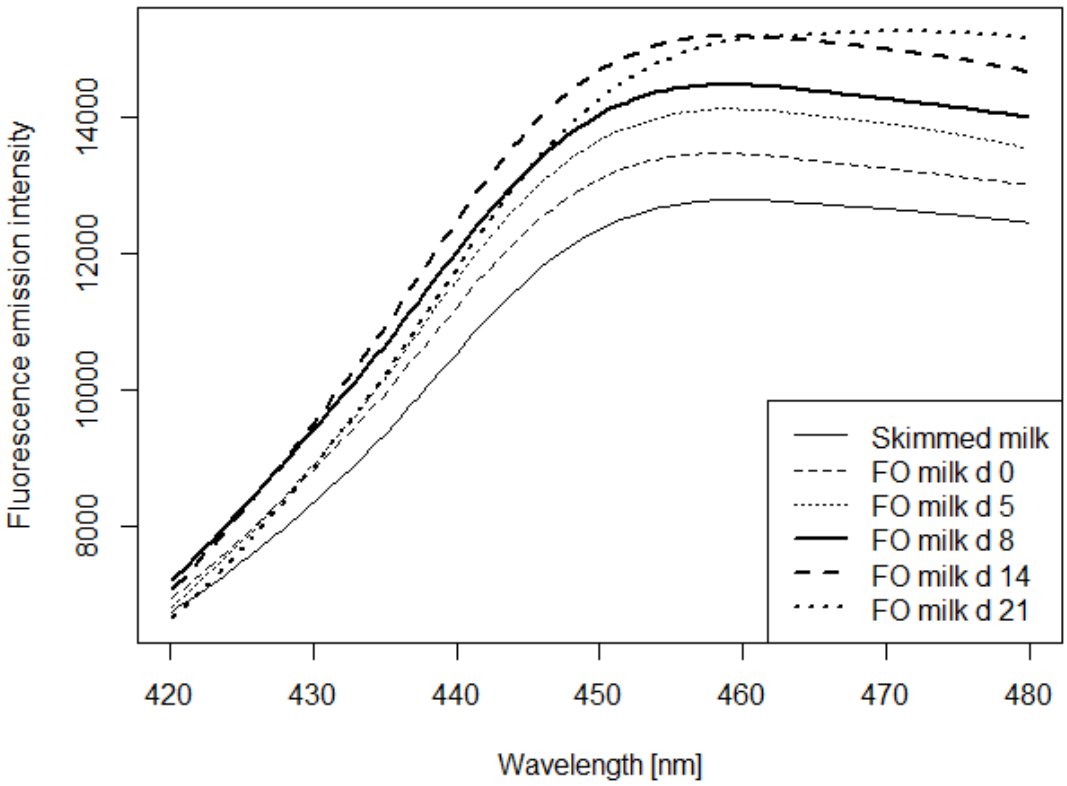
$^{x-z}$ in the columns indicate significant difference ($p < 0.05$) between samples within days

Table 5. Development of selected volatile oxidation products in the enriched skimmed milk emulsions during 14 days of storage at 4°C. Expressed as ng/g of emulsion ± standard deviation (n = 3).

	Storage time (days)			
	0	**5**	**8**	**14**
1-Penten-3-one				
FO	-	4.7 ± 1.1 ^{a,x}	6.4 ± 1.1 ^{a,x}	6.2 ± 0.4 ^{a,x}
FO+CO	-	4.7 ± 1.1 ^{a,x}	3.6 ± 0.8 ^{a,y}	-
FO+OO	-	3.88 ± 0.81 ^{a,x}	4.1 ± 0.8 ^{a,y}	1.3 ± 0.2 ^{b,y}
1-Penten-3-ol				
FO	0.5 ± 0.0 ^{a,x}	7.4 ± 1.1 ^{b,x}	18.8 ± 1.9 ^{c,x}	39.1 ± 2.8 ^{d,x}
FO+CO	1.1 ± 0.1 ^{a,x}	7.4 ± 0.4 ^{b,x}	16.4 ± 1.6 ^{c,x}	24.2 ± 1.5 ^{d,y}
FO+OO	1.9 ± 0.1 ^{ab,x}	4.02 ± 0.13 ^{b,x}	6.3 ± 0.7 ^{b,y}	18.7 ± 2.0 ^{c,z}
(E)-2-Pentenal				
FO	-	4.7 ± 0.9 ^{a,x}	8.1 ± 1.5 ^{b,x}	10.3 ± 1.0 ^{b,x}
FO+CO	-	4.5 ± 1.0 ^{a,x}	4.8 ± 1.1 ^{a,y}	2.7 ± 0.2 ^{a,y}
FO+OO	1.6 ± 0.0 ^{a,x}	3.49 ± 0.48 ^{a,x}	3.9 ± 0.5 ^{a,y}	2.6 ± 0.1 ^{a,y}
2-Penten-1-ol				
FO	-	-	-	-
FO+CO	-	-	-	-
FO+OO	-	-	-	-

Hexanal				
FO	125850 ± 2335 ^{a,x}	137256 ± 8043 ^{a,x}	219224 ± 10914 ^{b,x}	272187 ± 11629 ^{c,x}
FO+CO	203317 ± 20938 ^{a,y}	144327 ± 10376 ^{b,x}	109798 ± 6468 ^{b,y}	31997 ± 3591 ^{c,y}
FO+OO	557172 ± 40888 ^{a,z}	314844 ± 11756 ^{b,y}	332771 ± 15715 ^{b,z}	57733 ± 1617 ^{c,y}
(E)-2-Hexanal				
FO	-	6.8 ± 0.2 ^{a,x}	11.8 ± 0.6 ^{b,x}	19.6 ± 1.0 ^{c,x}
FO+CO	-	6.3 ± 0.6 ^{a,x}	8.4 ± 1.0 ^{b,y}	8.0 ± 0.5 ^{b,y}
FO+OO	-	4.4 ± 0.2 ^{a,y}	5.1 ± 0.5 ^{ab,z}	6.6 ± 0.5 ^{b,y}
2,4-Heptadienal				
FO	-	208 ± 31 ^{a,x}	525.5 ± 61.1 ^{b,x}	385.6 ± 73.5 ^{ab,x}
FO+CO	10.1 ± 1.6 ^{a,x}	324.2 ± 121.0 ^{b,x}	460.0 ± 140.0 ^{b,xy}	365.5 ± 131.5 ^{b,x}
FO+OO	14.4 ± 8.4 ^{a,x}	165.8 ± 28.0 ^{b,x}	262.5 ± 23.04 ^{b,y}	355.0 ± 70.5 ^{b,x}
(E,E)-2,4-Heptadienal				
FO	-	17.8 ± 0.7 ^{a,x}	27.2 ± 2.1 ^{b,x}	28.8 ± 6.4 ^{b,x}
FO+CO	0.7 ± 0.1 ^{a,x}	20.1 ± 3.1 ^{b,x}	37.0 ± 3.2 ^{c,y}	15.0 ± 4.4 ^{b,y}
FO+OO	1.8 ± 1.4 ^{a,x}	9.0 ± 2.0 ^{b,y}	11.3 ± 1.0 ^{b,z}	22.8 ± 4.9 ^{c,xy}
(E,Z)-2,6-Nonadienal				
FO	-	10.9 ± 0.9 ^{a,x}	25.2 ± 3.9 ^{b,x}	41.7 ± 3.5 ^{c,x}
FO+CO	-	9.0 ± 2.1 ^{a,x}	14.0 ± 4.6 ^{a,y}	14.2 ± 5.2 ^{a,y}
FO+OO	-	-	-	-




Fluorescence emission intensity
8000
10000
12000
14000
420
430
440
450
460
470
480
Wavelength [nm]
Skimmed milk
FO milk d 0
FO milk d 5
FO milk d 8
FO milk d 14
FO milk d 21