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Lipid Oxidation in Milk, Yoghurt, and Salad Dressing Enriched with Neat Fish Oil or Pre-Emulsified Fish Oil

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This study compared the oxidative stabilities of fish-oil-enriched milk, yoghurt, and salad dressing and investigated the effects on oxidation of adding either neat fish oil or a fish-oil-in-water emulsion to these products. Milk emulsions had higher levels of a fishy off-flavor and oxidized faster, as determined by the peroxide value and volatile oxidation products, than fish-oil-enriched yoghurt and dressing, despite the fact that dressings had a higher fish oil content and were stored at room temperature. Additionally, fish-oil-enriched yoghurt generally had higher oxidative stability than fish-oil-enriched dressings, irrespective of the mode of fish oil addition. Yoghurt thus seemed to be a good delivery system of lipids containing n-3 polyunsaturated fatty acids. Different effects of adding fish oil either as neat fish oil or as a fish-oil-in-water emulsion were observed for milk, yoghurt, and dressing. Yoghurt and dressing enriched with neat fish oil were more stable than those enriched with a fish-oil-in-water emulsion, whereas milk enriched with neat fish oil was less stable than milk enriched with the fish-oil-in-water emulsion. Overall, it seemed that application of neat fish oil was a good option for preserving the final quality in yoghurt and dressings, but a pre-emulsion may still be considered for the fish oil enrichment of certain food products, for example, milk.

KEYWORDS: Fish oil; lipid oxidation; oil-in-water emulsion; n-3 PUFA; milk; yoghurt; salad dressing

INTRODUCTION

An increasing amount of evidence compiled over the past 30 years supports the nutritional benefits of dietary long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) (*1–3*). The main focus has been on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The intake of n-6 unsaturated fatty acids is generally high in the typical western diet. This prevents synthesis of the n-3 unsaturated fatty acids to a sufficient level in the human body, and the n-3 PUFA thus needs to be provided through the diet (*4*). The U.S. Food and Drug Administration has allowed the use of a qualified health claim for conventional foods containing EPA and DHA in relation to a reduced risk of coronary heart disease (*5*). The permission of this health claim has further intensified the research regarding the incorporation of n-3 PUFA-rich oils into foods.

Due to the high degree of unsaturation of EPA and DHA, triglycerides rich in these fatty acids undergo rapid oxidative deterioration, which furthermore seems to be a particularly prominent problem in emulsions and complex food systems (*6, 7*). In these complex systems, many factors, such as transition metals, interfacial area, processing conditions, the type of emulsifier, and droplet size, can affect the initiation and propagation of oxidation (*8, 9*). Accordingly, the available literature suggests that the particular mechanisms of oxidation can differ significantly between different food emulsion systems.

One strategy to reduce the extent of processing of the fish oil during the production of n-3 PUFA enriched foods is to prepare a stabilized pre-emulsion of the n-3 PUFA oil, which is then to be added to the finished or semifinished food product without requiring significant additional emulsification. Moreover, addition of the fish oil at the latest possible stage can reduce the amount of stresses, such as exposure to light, but also heat and oxygen, which can otherwise be necessary to achieve emulsification of the fish oil into the product or which are part of the production of the particular food product. In addition, contact between n-3 PUFA oil and potential pro-oxidative compounds of the food product during processing is reduced by adding the oil in an already stabilized pre-emulsion as the final step of processing, because of the interfacial membrane surrounding the fish oil in the pre-emulsion.

The pre-emulsification strategy has previously been used for several n-3 PUFA enriched foods. An algae oil emulsion stabilized by whey protein and protected by mixed tocopherols and ethylene diaminetetraacetate (EDTA) has been suggested

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as an oxidatively and physically stable n-3 PUFA delivery system (*10*, *11*). Such an emulsion has successfully been incorporated into products such as yoghurt and meat products without imposing significant losses in sensory attributes of the final product (*12*, *13*). A similar emulsion, which was further stabilized by ascorbyl palmitate and rosemary extract, has also been shown to be relatively stable in surimi (*14*).

This paper presents results from our ongoing research on the fish oil enrichment of genuine food systems. Milk and yoghurt are widely used dairy products, but milk triglycerides have a high relative content of shorter-chain and saturated fatty acids. Salad dressing has a relatively high fat content; thus, substitution of some of the less healthy fats in these three food products with fish oil could provide a more healthy lipid profile for the products. Previous investigations of fish-oil-enriched milk, yoghurt, and dressing have indicated that these products responded very differently with regard to retaining their oxidative stability upon addition of fish oil (*15*–*17*). To further substantiate this preliminary conclusion, the present study directly compared the oxidative stability of fish-oil-enriched milk, yoghurt, and salad dressing during storage. To our knowledge, fish oil enrichment by the addition of neat oil or fish-oil-in-water emulsion has not previously been compared directly. The main purpose of this investigation was to examine the effect on the oxidative stability of adding the fish oil directly or by adding the oil as a fish-oil-in-water emulsion in these three products. The oxidative stability of the individual products was assessed by evaluating the peroxide values, the development of secondary oxidation products, and the sensory off-flavors during storage.

MATERIALS AND METHODS

Materials. Refined cod liver oil [10.2% C16:0, 23.2% C18:1(n-9), 4.3% C18:2(n-6), 2.2% C18:3(n-3), 10.8% C20:1(n-9), 8% C20:5(n-3), 11.5% C22:6(n-3)] and a refined rapeseed oil [4.5% C16:0, 60.4% C18:1(n-9), 19.2% C18:2(n-6), 8.6% C18:3(n-3), 1.5% C20:1(n-9), 0% C20:5(n-3), 0% C22:6(n-3)], both without added antioxidants, were provided by Maritex A/S, Norway. The fatty acid composition was determined by preparation of methyl esters (*18*) that were in turn analyzed by gas chromatography (*19*). The peroxide value (PV) and content of tocopherols in the cod liver oil were 0.11 meq/kg of oil, 200 mg α-tocopherol/kg of oil, and 36 mg γ-tocopherol/kg of oil, whereas the rapeseed oil had a PV of 0.16 meq/kg of oil and contained 220 mg α-tocopherol/kg and 365 mg γ-tocopherol/kg of oil. The levels of tocopherols were determined by high-performance liquid chromatography (HPLC) (*20*). Both oils had less than 0.02% free fatty acids and an anisidine value less than 1.7. Milk with 0.5% and 1.5% fat, yoghurt with 1.5% fat, and vinegar (5% acetic acid, De Jydske Eddikebryggerier A/S, Randers, Denmark) were purchased locally. Denatured whey protein emulsifier (Nutrilac DR8080) was provided by Arla Foods, a.m.b.a., Denmark. A mixture of guar gum, xanthan gum, and acetylated distarch adipate (Grindsted FF2110) was used as a stabilizing agent and obtained from Danisco Ingredients, Brabrand, Denmark. All chemicals and external standards for identification of volatile oxidation products were obtained from either Sigma Aldrich, Steinheim, Germany, or Merck, Darmstadt, Germany. All solvents were of HPLC grade from Laboratory-Scan, Dublin, Ireland.

Preparation of the Fish-Oil-in-Water Emulsion. Potassium sorbate (0.1 wt %) and denatured whey protein (1.5 wt %) were dissolved in water, and fish oil (50% wt) was then added slowly (1 min) during mixing (Ultra Turrax, Rose Scientific, Canada). This mixture was subsequently homogenized at 150 MPa (2.5 min recirculation, Panda 2K, Niro Soavi, Parma, Italy), kept at 2 °C, and used within 4 h.

Production of Fish-Oil-Enriched Milks. Milk (3 L) was pasteurized by heating to 72 °C within 3 min and holding it at 72 °C for 15 s. Then, either neat fish oil (1.0% by weight) was added followed by homogenization at a total pressure of 22.5 MPa (two-valve Rannie homogenizer, APV, Albertslund, Denmark) or a 50% fish-oil-in-water emulsion (2.0% by weight of final product) was added (stirred by hand) after a similar heating and homogenization of the milk alone. Aliquots of milk (250 mL) were stored in closed 250 mL pyrex bottles (one bottle per sample for each sampling day).

Production of Fish-Oil-Enriched Yoghurt. Yoghurt (3L) was either supplemented with neat fish oil (1.0% by weight) followed by homogenization at a total pressure of 22.5 MPa (two-valve Rannie homogenizer, APV, Albertslund, Denmark) or the fish-oil-in-water emulsion (2.0% by weight of final product) was gently added (stirred by hand) after a parallel homogenization treatment (22.5 MPa) of the yoghurt alone. Yoghurt (250 mL) were stored in closed 250 mL pyrex bottles (one bottle per sample for each sampling week).

Production of Fish-Oil-Enriched Dressings. Dressing enriched with neat fish oil was produced in a 1000 g batch [15 wt % rapeseed oil, 10 wt % fish oil, 6 wt % vinegar (hereof, 5% acetic acid), 1.2 wt % stabilizing agent (Grindsted FF2110), 0.1 wt % potassium sorbate, 0.08 wt % denatured whey protein emulsifier (Nutrilac DR8080), 67.62 wt % deionized water]. The denatured whey protein emulsifier and the potassium sorbate were dissolved in deionized water. The stabilizer dispersed in rapeseed oil (24 g) was mixed into the water phase in a Stephan Universal Mixer (Stephan, Hameln, Germany). The dressing was cooled with circulating water at 0 °C throughout processing. Fish oil, the remaining rapeseed oil, and vinegar were added slowly during mixing (3 min, 1200 rpm), and the dressing was mixed for an additional 4 min at reduced pressure (40 kPa). The dressing enriched with fish-oil-in-water emulsion was generally produced as described above, but 0.48 g of emulsifier was used, and only the remaining rapeseed oil and vinegar were added slowly during mixing (2.5 min, 1200 rpm). After mixing for an additional 4 min at reduced pressure (40 kPa), the fish-oil-in-water emulsion (20% by weight of final product) was added and mixed in by hand. Dressings (250 mL) were stored in closed 250 mL pyrex bottles (one bottle per sample for each sampling week).

Preparation of Samples for Analyses. Milk and yoghurt were stored in the dark at 5 °C, whereas dressings were stored in the dark at room temperature (21–22.5 °C). Samples for chemical analysis were taken during storage and stored in separate, brown glass bottles, which were immediately flushed with nitrogen and kept at −80 °C until the analyses of PV and secondary volatile oxidation products. Samples were taken at regular intervals: milk; 1, 5, 8, and 12 days; yoghurt 1, 8, 12, 22, and 29 days; dressing: 1, 8, 12, 22, 29, and 43 days.

Determination of Droplet Size and Viscosity. Oil droplet sizes of the emulsions were determined during storage (Mastersizer 2000, Malvern Instruments, Worcestershire, U.K.). Milk and yoghurt samples were suspended directly in circulating distilled water (2800 rpm, 14–16% obscuration), and the droplet sizes were determined using the Fraunhofer method. Dressing samples (1 mL) were dissolved in 1% sodium dodecylsulfate buffer (9 mL) and sonicated for 15 min in an ultrasound bath (Branson, Hatfield, PA) prior to suspension in circulating, deionized water (3000 rpm, 14–16% obscuration). Average droplet sizes were determined using a refractive index of 1.4694. Results are presented graphically as the overall size distribution of the droplets and as the surface area mean diameter $D[3,2] = \Sigma n_i d_i^3 (\Sigma n_i d_i^2)^{-1}$. The viscosity of emulsions was determined after 1, 8, and 22 days of storage. Shear stress was exponentially increased in the following ranges: milk, 0.1–2.0 Pa; yoghurt, 0.5–3.0 Pa; dressing, 3–60 Pa (CC25, 5 °C, StressTech HR, Reologica Instruments AB, Lund, Sweden). The apparent viscosities (duplicate measurements) were subsequently determined at a shear rate of 100 L s^{-1} in all three emulsions.

Sensory Evaluation of Emulsion Samples. Sensory evaluations were performed on the sampling days: milk 1, 5, and 8 days; yoghurt 1, 5, 8, 22, and 29 days; dressing 1, 5, 8, 22, and 29 days. The products were evaluated by a descriptive analysis by 9–13 panelists trained in the descriptive analysis of fishy off-flavors. ISO standards 6658, 8586, and 6564 were generally followed for training and sensory analysis methods. Samples (30 mL, 10 °C) were served in randomized order with crisp bread and cold water, and the following attributes were evaluated with respect to both odor and flavor: fishy, milk, yoghurt, sour, rancid/old, other. The intensity of each attribute was determined on a 9 cm unstructured scale. Before sample evaluation, assessors were served a reference sample of fish-oil-enriched milk with a defined

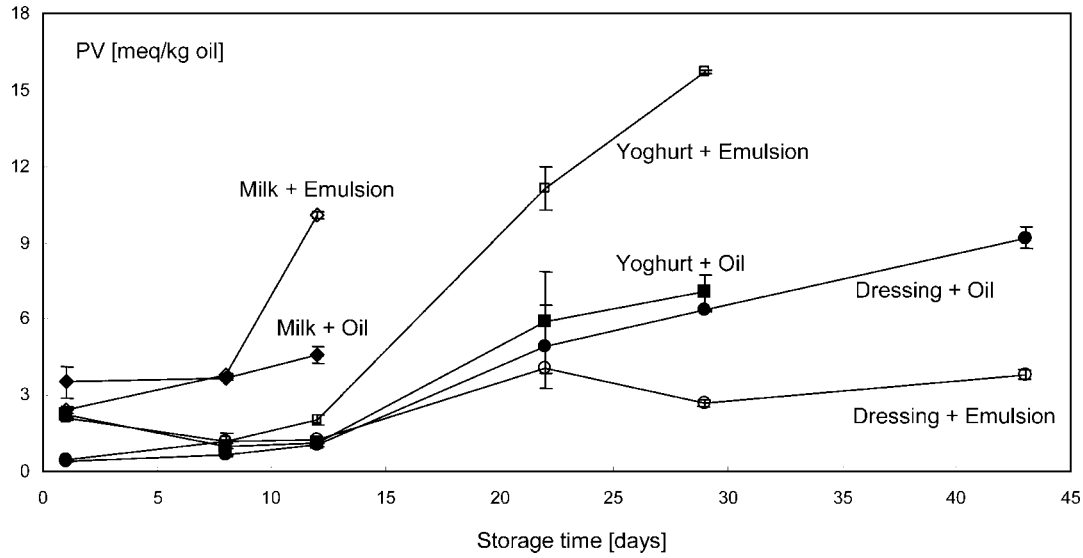

Figure 1. Peroxide values of fish-oil-enriched milk, yoghurt, and dressing emulsions during storage (milk and yoghurt, 5 °C; dressing, room temperature). ◇,◆ milk; □,■ yoghurt; ○,● dressing. Open symbols refer to enrichment with fish-oil-in-water emulsion; solid symbols refer to enrichment with neat fish oil. Data points are averages of duplicate measurements ± 1 standard deviation.

intensity of fishy odor and flavor. For each attribute, the intensities given by the assessors were averaged, and the total off-flavor of each attribute was calculated at each sampling day as the average odor plus average flavor for that attribute.

Analyses of Primary Oxidation Products and Tocopherols. Lipids from the samples were extracted by chloroform/methanol (1:1 v/v), using a reduced amount of solvent (*21, 22*). PV and tocopherols were measured directly on the oils or on the lipid extracts from the samples by a colorimetric determination of iron–thiocyanate (*23*) and by HPLC (*20*), respectively. The PV and tocopherol data reported are averages of duplicate lipid extractions.

Dynamic Headspace Analysis of Volatile Secondary Oxidation Products. Dressing (10 g) was diluted with 10 g of deionized water, and volatile secondary oxidation products were purged through dry, powdered potassium hydroxide (200 mg) to remove acetic acid (*24*). Volatiles from yoghurt (10 g was diluted with 10 g of deionized water) and 8 g of milk emulsion were purged directly from the samples. Milk, yoghurt, and dressings were purged with nitrogen (150 mL min^{-1}) for 30 min at 45 °C and trapped on Tenax GR tubes, using 4-methyl-1-pentanol as an internal standard. The volatiles were desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin Elmer, Norwalk, CT) and cryofocused on a Tenax GR cold trap. Volatiles were analyzed by gas chromatography with mass spectrometrical detection (GC-MS) as follows: volatiles were separated by gas chromatography (HP 5890 IIA, Hewlett Packard, Palo Alto, CA; column DB-1701) as described previously (*25*) and analyzed by mass spectrometry (HP 5972 mass-selective detector). Oven temperature program: 45 °C held for 5 min, 1.5 °C min^{-1} to 55 °C, 2.5 °C min^{-1} to 90 °C, 12 °C min^{-1} to 220 °C, and finally held at 220 °C for 4 min. The individual compounds were identified by both MS-library searches (Wiley138K, John Wiley and Sons, Hewlett Packard, U.S.A.) and authentic external standards. First, the compounds were quantified through individual calibration curves in the range 2–1500 ng g^{-1}. The compounds were dissolved in ethanol, added directly on the TenaxGR, and analyzed by GC-MS. The quantified amounts were subsequently adjusted for differences in the relative release of each individual compound from each emulsion type. Releases were determined as the amount of standard released by the headspace sampling of each emulsion, as described above, relative to the amount added directly to the sampling tube (both in triplicate). Standard compounds (in ethanol) were added to the emulsions and left to equilibrate for 24 h (2 °C) before headspace sampling and GC-MS.

Statistical Analysis. The obtained data were analyzed by a two-way analysis of variance, and individual samples were compared on a 0.05 level of significance by the Bonferroni multiple comparison or, in the case of pair wise comparison, by a *t* test. All references to significant differences between samples or between sampling times are based on this statistical analysis of data.

RESULTS

The pH of the milk, yoghurt, and dressing emulsions were stable at 6.7, 4.4, and 4.0, respectively, throughout the storage periods. The oxidative status of the different emulsions was compared by peroxide values (**Figure 1**) and development in concentrations of selected, individual volatile oxidation products, as analyzed by GC-MS (**Figure 2**). The volatile compounds quantified included 1-penten-3-ol, 1-penten-3-one, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*,*E*)-2,4-heptadienal, and (*E*,*Z*)-2,6-nonadienal, as these compounds previously were shown to be sensitive indicators of the oxidative deterioration of n-3 PUFA in fish-oil-enriched milk and dressing during storage (*15, 26*).

Milk: Fish Oil versus Fish-Oil-in-Water Emulsion. The PV of milk enriched with the fish-oil-in-water emulsion increased more than the PV of milk enriched with neat fish oil during storage (**Figure 1**). However, the opposite was observed with regard to the volatiles (**Figure 2**). For 1-penten-3-one, 1-penten-3-ol, 2-hexenal, 2,4-heptadienal, and 2,6-nonadienal, the milk with neat fish oil had significantly higher levels than the milk enriched with the fish-oil-in-water emulsion. The lower PV in combination with the higher levels of volatiles for the milk enriched with neat fish oil indicated that the faster development of volatiles was a result of a faster decomposition of peroxides in the milk enriched with neat fish oil than that in milk enriched with the fish-oil-in-water emulsion. Both milks had a pronounced fishy off-flavor already at the beginning of the storage experiment (**Figure 3**). Milk with neat fish oil also had a significantly higher fishy off-flavor than milk with the fish-oil-in-water emulsion after 1 and 5 days of storage, but after 8 days, the milks had similar and high fishy off-flavors. There were no significant differences between the milk emulsions regarding any of the other sensory descriptors throughout storage (**Table 1**).

During storage, the content of α-tocopherol decreased equally fast in the milks enriched with neat fish oil and the fish-oil-in-water emulsion (44%, **Table 2**). The content of γ-tocopherol was low and remained unchanged (13–16 mg kg^{-1} oil) in both

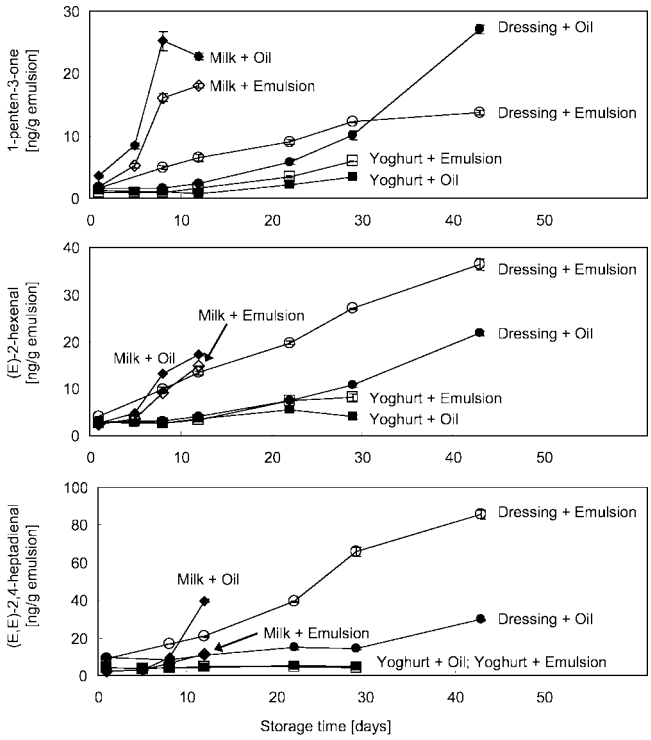

Figure 2. Development in volatile oxidation products as exemplified by 1-penten-3-one, (*E*)-2-hexenal, and (*E*,*E*)-2,4-heptadienal during storage (milk and yoghurt, 5 °C; dressing, room temperature). ◊,♦ milk; □,■ yoghurt; ○,● dressing. Open symbols refer to enrichment with fish-oil-in-water emulsion; solid symbols refer to enrichment with neat fish oil. Data points are averages of triple measurements ± 1 standard deviation.

types of fish-oil-enriched milk. The average droplet size of the fish-oil-in-water emulsion was 1.20 ± 0.01 μm (**Figure 4c**). After mixing the pre-emulsified fish-oil-in-water emulsion into the milk, the droplets in the milk enriched with the emulsion were therefore larger than the droplets in milk enriched with neat fish oil (1.06 ± 0.01 versus 0.41 ± 0.01 μm, respectively). Droplet sizes as well as the apparent viscosities (4.3–4.4 mPas) were stable in both milk types between 2 and 12 days of storage (data not shown).

Yoghurt: Fish Oil versus the Fish-Oil-in-Water Emulsion. During storage, the PV of the yoghurt enriched with the fish-oil-in-water emulsion increased significantly more than the PV of the yoghurt enriched with neat fish oil (15.7 vs 7 meq kg^{-1} oil, respectively, after 29 days, **Figure 1**). Likewise, the levels of volatiles in yoghurt enriched with the fish-oil-in-water emulsion were slightly higher than the levels determined in yoghurt enriched with neat fish oil (**Figure 2**). Still, the levels of volatiles in both yoghurts were low and relatively stable during storage, and only small increases were observed after 22 and 29 days of storage despite the relatively high PVs.

Accordingly, the fishy off-flavor of yoghurt enriched with the fish-oil-in-water emulsion tended to increase during storage, whereas no changes in fishy off-flavors were observed during storage of the yoghurt enriched with neat fish oil. However, none of these changes were significant. The content of α-tocopherol decreased in both yoghurts (**Table 2**), but these changes were not significant either. The content of γ-tocopherol remained low and unchanged in both types of fish-oil-enriched yoghurts (11–14 mg kg^{-1} of oil; data not shown).

In both yoghurts, small, but significant, increases in the droplet sizes were observed during storage. Between day 1 and day 29, the average droplet size in yoghurts with neat oil and emulsion increased from 5.0 to 6.1 ± 0.2 μm and from 3.2 to 3.7 ± 0.1 μm, respectively. Initially, the apparent viscosity of yoghurt with neat fish oil was higher than that of yoghurt with the fish-oil-in-water emulsion (33.7 vs 22.3 mPa s), indicating a thinning effect caused by the addition of the pre-emulsion. During storage, the apparent viscosity of yoghurt with neat fish oil decreased the most, and at the end of the storage, both yoghurts had comparable viscosities (21.7 vs 20.0 mPa s; data not shown).

Dressing: Fish Oil versus Fish-Oil-in-Water Emulsion. Up to 22 days of storage, the PVs of the two dressing types were similar, but at the end of the storage period, the PV of the dressing enriched with neat fish oil was significantly higher than that in the dressing enriched with the fish-oil-in-water emulsion (9.2 vs 3.8 meq kg^{-1} of oil after 43 days, respectively, **Figure 1**). In contrast, the levels of volatiles were much higher in dressing enriched with the fish-oil-in-water emulsion from day 1 and until day 29 (1-penten-3-one, 1-penten-3-ol, pentanal, hexanal, and 2-pentenal) or throughout the entire storage period (2-hexenal, 4-heptenal, and 2,4-heptadienal; **Figure 2**). Taken together, these data indicated a better stability of the dressing with neat fish oil than that with the fish-oil-in-water emulsion. The precursors of pentanal and hexanal are n-6 unsaturated fatty acids; thus, these compounds were only evaluated in dressing, as the dressings contained 15% rapeseed oil rich in 18:2(n-6). Likewise, the contents of both α- and γ-tocopherol were higher in the dressings compared to the other products because of the rapeseed oil addition. Overall, similar but insignificant decreases (5–11%) in both α- and γ-tocopherol were observed in the two dressing types during storage (**Table 2**, data for γ-tocopherol not shown). The sensory evaluation was generally in accordance with the observations for the volatiles. Initially, dressing enriched with the fish-oil-in-water emulsion had only a slightly higher fishy off-flavor than dressing enriched with neat fish oil, but after 29 days, a significantly higher fishy off-flavor was observed in the dressing with the fish-oil-in-water emulsion compared to the dressing with neat fish oil.

The average droplet size of the dressing with neat fish oil was 24.9 ± 0.3 μm after 1 day of storage and remained stable throughout the storage period (**Figure 4a**). On the other hand, the average droplet size in the dressing enriched with the fish-oil-in-water emulsion increased from 1.6 μm ± 0.1 at day 1 to 11.3 μm at day 43 (**Figure 4b**). In particular, between day 8 and day 22, a decrease in the group of smaller droplets was observed, indicating coalescence of the droplets.

The apparent viscosity of the dressing enriched with neat fish oil (284 mPa s) was lower than the viscosity of the dressing with the fish-oil-in-water emulsion (347 mPa s), which furthermore increased between 2 and 12 days of storage (data not shown). After 12 days of storage, the viscosity remained stable. A minor decrease was observed during storage for dressing with neat fish oil; thus, after 29 days, the dressing with the fish-oil-in-water emulsion had a much higher viscosity than dressing with neat fish oil (371 vs 273 mPa s; data not shown).

DISCUSSION

Milk, yoghurt, and dressing differ significantly with respect to both chemical composition and emulsion structure, including the viscosity, and especially the composition and the area of the interfacial membrane surrounding the emulsion droplets are very different. The emulsification can impact the oxidative stability significantly, and the milk and yoghurt emulsions were therefore homogenized at the same pressure. The fish-

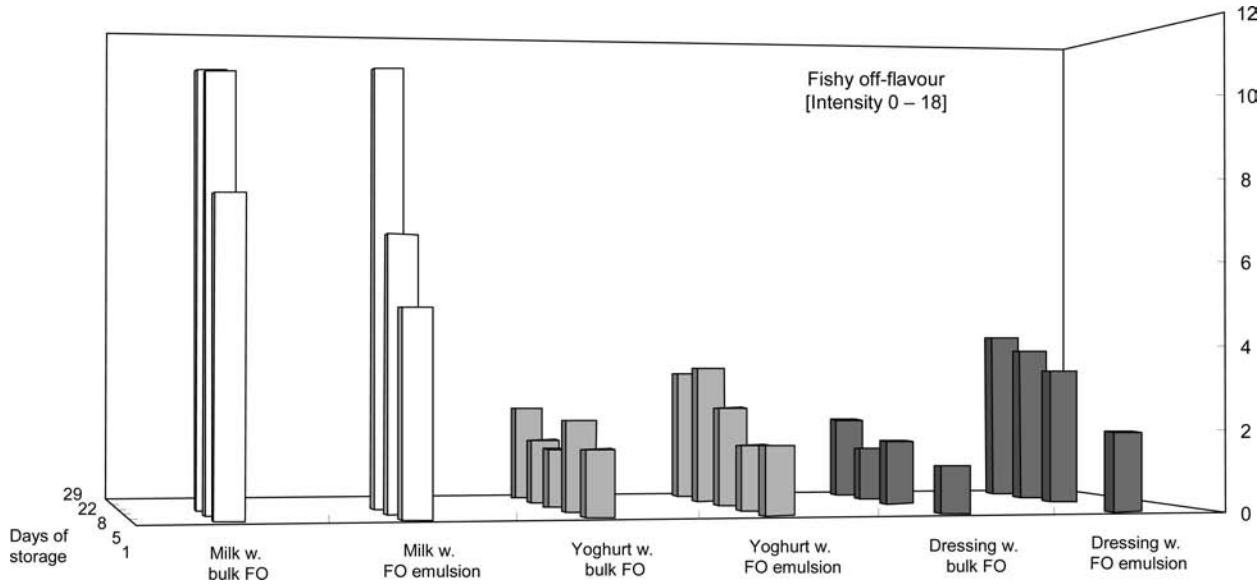

Figure 3. Summarized intensity of fishy odor and flavor of milk, yoghurt, and dressing enriched with either neat fish oil or the fish-oil-in-water emulsion. Average standard deviations were 1.9, 1.3, and 1.4 for milk, yoghurt, and dressing, respectively.

Table 1. Average Intensity [0–18] of Sensory Descriptors in Milk, Yoghurt, and Dressing Enriched with Either Neat Fish Oil or the Fish-Oil-in-Water Emulsion

odor + flavor at day 8	fishy	rancid/old	milk	yoghurt	sour
milk + oil	10.7 ± 1.0	0.8 ± 0.7	4.7 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
milk + emulsion	10.8 ± 1.4	0.5 ± 0.6	5.4 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
yoghurt + oil	1.4 ± 0.8	0.9 ± 0.8	0.1 ± 0.2	9.6 ± 2.1	1.0 ± 1.2
yoghurt + emulsion	2.4 ± 1.4	0.9 ± 0.7	0.1 ± 0.2	10.2 ± 2.0	0.9 ± 0.8
dressing + oil	1.6 ± 1.1	1.0 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	9.8 ± 2.3
dressing + emulsion	3.3 ± 1.6	0.9 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	8.2 ± 2.0

Table 2. Contents of Tocopherol in the Milk, Yoghurt, and Dressing During Storage[a]

	fat content [wt %]		α-tocopherol [μg g^{-1} oil] storage time		
	fish oil	additional fat	start	middle	end
			Day 1	Day 8	Day 12
milk + oil	1.0%	milk fat: 1.0%	91(0) a	70 (23) b	51 (44) c
milk + emulsion			97 (0) a	88 (10) a	54 (44) b
			Day 1	Day 8	Day 22
yoghurt + oil	1.0%	milk fat: 1.5%	77 (0) ab	86 (−12) a	65 (15) b
yoghurt + emulsion			75 (0) ab	81 (−8) a	59 (21) b
			Day 1	Day 22	Day 43
dressing + oil	10%	rapeseed oil: 15%	211 (0) a	200 (5) ab	187 (11) b
dressing + emulsion			218 (0) a	207 (5) a	207 (5) a

[a] Average standard deviation was 7 μg g^{-1} of oil, and tocopherol amounts [μg g^{-1} of oil] in rows followed by same letter are not significantly different. Contents are given in μg g^{-1} of oil followed by the percent decrease relative to day 1 presented in parentheses.

oil-in-water emulsion was homogenized at a higher pressure due to the higher fat content and in order to minimize the oil droplet size of the pre-emulsion. However, the same fish-oil-in-water emulsion was added to all three products; thus, the effect of pre-emulsion homogenization was identical in all three products.

The high oxidative susceptibility observed for the fish-oil-enriched milk is in accordance with several previous observations (*27, 28*) and may be due to various factors in the complex medium of cow's milk. It has been suggested that both transition metals and unsaturated phospholipids present in the milk fat globule membrane can promote oxidation in milk (*29*) and therefore could contribute to the observed high oxidative susceptibility of the fish-oil-enriched milk.

During the first 8 days of storage, the summarized scores of fishy odor and flavor of the fish-oil-enriched milks were between 4.9 and 10.8, whereas the corresponding fishy scores in the yoghurt and dressing were between only 1.4 and 3.4 and 1.1 and 3.8, respectively. Clearly, the milk, yoghurt, and sour flavors varied in intensity between the products (**Table 1**). However, even if the yoghurt and sour flavor notes were more pronounced in the yoghurt and dressing, and thus could affect the perceived intensity of the fishy off-flavor as compared to what was perceived with milk, both the chemical and sensory analyses corroborated that milk emulsions oxidized more than yoghurt and dressing during the first 12 days of storage. The dressings even had higher fish oil and total fat contents than milk (10 vs 1.0 % fish oil, **Table 2**) and were stored at room temperature,

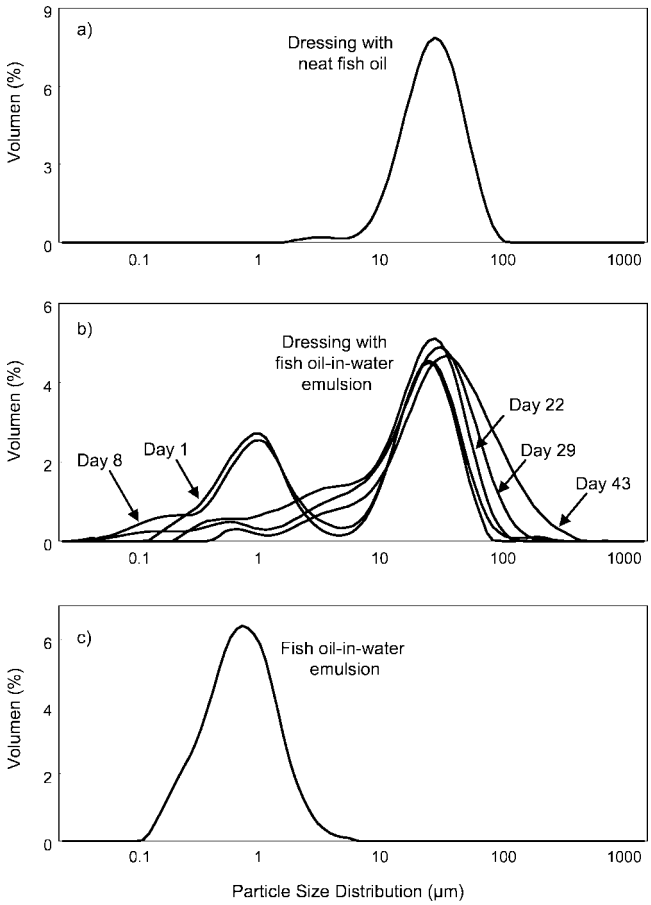

Figure 4. Droplet size distribution (volume %) of the fish-oil-in-water emulsion and dressing enriched with neat fish oil after 1 day of storage, and of the dressing enriched with the fish-oil-in-water emulsion during storage.

whereas milk emulsions were stored at 5 °C. The release of volatile compounds from a food matrix is affected by many factors (*30*), but the differences in the release of the individual compounds from milk, yoghurt, and dressing were included in the calculations before the comparison of oxidation in these products.

Previously, rapeseed oil has been shown to protect fish oil against oxidation in milk emulsions, where the protective effect was proposed to be due to the content of tocopherols in rapeseed oil (*27, 28*). Radical scavenging properties provided by the sulfhydryl group of cysteine amino acids have been proposed for denatured whey protein (*31*). Both these antioxidant mechanisms are likely to improve the oxidative stability of the fish oil in the salad dressing system compared to that in the milk system.

The yoghurt and dressing enriched with neat fish oil had similar PVs throughout the 29 days of the storage period of the yoghurt (**Figure 1**), but the yoghurt had significantly lower and more stable levels of all volatiles (**Figure 2**). The yoghurt enriched with the fish-oil-in-water emulsion had an even higher PV, but still the levels of volatiles were as low and stable as those of the yoghurt with neat fish oil (**Figure 2**). These findings indicated that fish-oil-enriched yoghurts had higher oxidative stability than fish-oil-enriched dressings, irrespective of the type of fish oil addition, and in particular, the lipid hydroperoxides present in the dressing were more easily decomposed than those formed in the yoghurt.

It is generally accepted that lipid hydroperoxides coming from n-3 fatty acids are especially susceptible to decomposition, and it is also well known that such degradation is significantly promoted by transition metals through Fenton-like reactions (*8*). The fact that previous experiments have pointed towards transition metals as the most important pro-oxidant factor in fish-oil-enriched dressing (*15*) supports the statement that the differences observed between yoghurt and dressing could be due to transition metals catalyzing faster degradation of the hydroperoxides in dressings. Thus, it is hypothesized that transition metals are less important as pro-oxidants in the yoghurt system, even though yoghurt was also a low-pH emulsion.

Effects of Viscosity and Droplet Interface. It has been proposed that viscosity can affect oxidation by reducing the diffusion of potential pro-oxidative molecules, such as ferrous ions or lipid hydroperoxides, but the currently available data on effects of viscosity on lipid oxidation in oil-in-water emulsions are not clear-cut (*32, 33*). Overall, the results showed that the least viscous milks were most oxidized. However, the dressings were more viscous than the yoghurts but were also more oxidized than the yoghurts. Thus, no direct relationship between viscosity and oxidation was indicated in the data.

The results also showed that the milk emulsions had the smallest droplets and thus largest interfacial surface area and, again, were the most oxidized. This is in agreement with the general expectation that a large interfacial area can lead to increased oxidation, due to an increased contact area between the oil and possible pro-oxidants present in the water phase of the emulsion. On the other hand, the yoghurts had much larger interfacial area than the dressings, which nonetheless were more oxidized than the yoghurts. No direct relationship between droplet size or interfacial surface area and the degree of oxidation could be drawn from these data, indicating that factors other than the surface area itself are important determinants for oxidation stability. This observation is in accordance with previous investigations of protein-emulsified oil-in-water emulsions (*34*).

It has previously been suggested that oil droplets with a positive charge can repel potentially pro-oxidative transition metal ions and, in turn, reduce oxidation (*11*). Hence, the droplet surface charge, described by the z-potential of the emulsion, may influence the oxidative stability of oil-in-water emulsions. The pH of the milk was 6.7, and thus above the pI of both casein and whey protein, whereas the pHs in yoghurt and dressing were 4.4 and 4.0, respectively, and thus both below the pI of casein and whey proteins (*11, 34, 35*). The results are therefore in accordance with the proposed hypothesis, that emulsions with negatively charged droplets, as in milk, are more susceptible to oxidative deterioration than emulsions with positively charged droplets due to the attraction of the positively charged transition metal ions, mainly Fe^{2+}.

However, in many protein-stabilized emulsions, the droplet surface charge does not seem to be the only factor affecting the oxidative stability (*34*). Studies with fish-oil-enriched mayonnaise also showed that decreasing the pH increased oxidation during storage and also that metal ions promoted oxidation significantly (*31, 36*). In model emulsions with polyoxyethylene 10 lauryl ether as an emulsifier, iron showed a highly increased pro-oxidative effect at pH 3 compared to that at pH 7, presumably because of the increased solubility of iron at a low pH (*37*). Furthermore, in a fish-oil-enriched dressing system with a low pH, similar to the present system, it was shown that EDTA was more efficient in inhibiting oxidation than both γ-tocopherol and ascorbyl palmitate (*15*). Such high efficiency of the EDTA suggested that oxidation was

promoted by metal-mediated degradation of the lipid hydroperoxides, and that this mechanism was the most important cause of oxidative deterioration during storage of the dressings despite the low pH. Overall, it seems likely that the low pH, and thus positive droplet surface charge, was not the only important factor for the good oxidative stability of the fish-oil-enriched yoghurt and dressing.

The droplet membrane in yoghurt is likely to contain more whey protein than the droplet membrane in pasteurized milk, due to the higher temperature during pasteurization (*38*). Previous investigations in fish-oil-enriched milk have shown that increasing the temperature during homogenization also led to an increased content of the main whey protein, β-lactoglobulin, which in turn reduced oxidation (*39*). The initial pasteurization may also change the binding or association between β-lactoglobulin and pro-oxidative transition metals. Furthermore, conformational changes in the proteins due to the lower pH, the gel structure, or the microbial activity in the yoghurt may decrease the diffusion of potential pro-oxidative molecules in the yoghurt and may also affect the availability of the transition metal ions to oxidation reactions. Overall, the good oxidative stability observed is in accordance with previous results obtained with both fish-oil-enriched yoghurt and fish-oil-enriched drinking yoghurt, which have shown good oxidative stability (*16*, *40*), but more investigations are needed in order to completely reveal the protective mechanisms in yoghurt.

Addition of Neat Fish Oil versus Addition of Fish-Oil-in-Water Emulsion. The results showed different effects of adding fish oil either as neat fish oil or as a fish-oil-in-water emulsion to the three different product types. The differences within individual product types with neat oil or emulsion became increasingly evident during the last part of their storage periods. The conclusions are mainly based on the data of the volatiles and sensory analyses.

The increased oxidation in yoghurt and dressing enriched with the fish-oil-in-water emulsion could be due to increased oxidation in the fish-oil-in-water emulsion itself, which was caused by the initial temperature increase (65 °C, 3 min) during homogenization of this emulsion.

In dressing enriched with neat fish oil, the oils were mixed before homogenization; thus, it seemed very likely that the protective effects of rapeseed oil, presumably the tocopherols, (*27*) as mentioned above, lead to a better oxidation compared to dressing with the fish-oil-in-water emulsion.

Milk with neat fish oil was slightly more oxidized than when the fish-oil-in-water emulsion was added; thus, the increased content of lipid hydroperoxides in the fish-oil-in-water emulsion did not seem to have a great impact on oxidation in this product. It has previously been shown that fish-oil-enriched milk is a very sensitive system towards oxidative deterioration; thus, the fish oil seemed to be better protected in the droplets emulsified by denatured whey protein than in the droplets emulsified by protein material present in the milk. First of all, this was likely due to the higher concentration of whey protein in the fish-oil-in-water emulsion (1.5 wt %) compared to pasteurized milk (around 0.35 wt %), which could lead to an improved surface coverage, which previously had been shown to improve the oxidative stability (*34*). Second, denaturation of the whey protein exposes the hydrophobic regions, which makes it adsorb even better to the oil–water interface (*38*).

In conclusion, the present experiment concerned the addition of a pre-emulsion of fish-oil-in-water versus the addition of neat fish oil, which was not stabilized by antioxidants, and thus enabled a comparison of effects on oxidation mediated by the different fish oil supplementation methods and food systems. The results showed that yoghurt could be a suitable delivery system for fish oil enrichment due to its high oxidative stability. It was also shown that possible benefits of pre-emulsification of fish oil prior to addition to food systems depends on the systems to which it is added. It seems likely that the composition of the fish oil pre-emulsion, including stabilization with antioxidants, needs to be considered and optimized for each individual food product. Finally, knowledge of the possible interactions or interchange between the droplets in the milk, yoghurt, or dressing and the droplets in the fish-oil-in-water emulsion after mixing could facilitate such optimization.

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