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# Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality

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**Abstract**

The experiments evaluated the influence of fish oil quality and cold storage temperature on the oxidative stability of milk emulsions containing 1.0% w/w milk fat and 0.5% w/w of either a pure fish oil or a fish oil:rapeseed oil mixture. The results showed that it was possible to produce a pasteurised milk product enriched with the important n-3 PUFA from fish oil with acceptable sensory characteristics if (1) the emulsions were based on a mixture of fish oil and rapeseed oil and (2) the initial peroxide value (PV) of the added oil blend was below 0.5 meq $kg^{-1}$. The sensory analysis showed a clear distinction between emulsions based on oil with PV 0.1 and 0.5 meq $kg^{-1}$, whereas the PV and the gas chromatographic (GC) analysis of volatile oxidation products were not sensitive enough to reveal these differences clearly. The GC analyses showed that the onset of formation of the volatiles was earlier with increased storage temperature in the range of 2–9 °C.


*Keywords:* Fish oil; Lipid oxidation; Milk; Emulsion

## 1. Introduction

Intake of fish and fish oils are known to reduce the risk of coronary heart disease (Kris-Etherton, Harris, & Appel, 2002) and ameliorate inflammatory diseases (Trebble et al., 2003). The long chain n-3 polyunsaturated fatty acids docosahexaenoic acid (DHA, C22:6) and eicosapentaenoic acid (EPA, C20:5) in fish are recognised to contribute to these positive effects, and supplementation with DHA and EPA was recently shown also to favourably influence the serum lipid profiles in women (Laidlaw & Holub, 2003). In addition, notably DHA seems to be an important factor in the mental development and visual acuity in infants (Connor, 2000).

Several investigations have shown that the general intake of fish and fish products in the typical Western diet is inadequate, and thus that the consumption of long chain polyunsaturated fatty acids (PUFA) of the n-3 family is less than the recommended intake (Kris-Etherton et al., 2000; Sanders, 2000; World Health Organisation, 2003). To meet the recommended intake for especially DHA and EPA, efforts have been made to incorporate marine oils, rich in n-3 PUFA, into various food products (Trautwein, 2001). However, the successful production of food products enriched with n-3 PUFA is impeded by the high susceptibility of these PUFA towards oxidative deterioration.

In the complex matrix of a real food emulsion system several factors may influence the initiation and progress of the lipid autoxidation processes (McClements & Decker, 2000; Frankel, 1998). We previously demonstrated (Let, Jacobsen, Frankel, & Meyer, 2003) that the oxidative deterioration of fish oil enriched milk could be controlled if the initial peroxide value (PV) of the fish oil was very low (PV < 0.5), and if gentle processing conditions were employed during the emulsification of

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the fish oil enriched milk. We also found that incorporation of a mixture of rapeseed oil and fish oil rather than pure fish oil gave a better quality of fish oil enriched milk during cold storage at 2 °C (Let, Jacobsen, & Meyer, 2004). However, in practical retail and household handling the cold storage temperature may exceed 2 °C, and despite the well-known sensitivity of fish oil oxidation to temperature, the influence of slightly elevated cold storage temperatures on the stability of fish oil enriched food products has received limited attention.

Oxidation of the PUFA produces a complex mixture of volatile secondary oxidation products, which cause particularly objectionable off-flavours. The highly unsaturated PUFAs are significantly more susceptible towards oxidation than the milk fat, which consists of more saturated fatty acids with shorter chains. (*E*)-2-hexenal, (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, and 2-penten-1-ol have previously been identified as important markers for fish oil oxidation in emulsions, such as mayonnaise and milk emulsions (Hartvigsen, Lund, Hansen, & Hølmer, 2000; Let et al., 2004). The mentioned volatile compounds have also been identified in the headspace of oxidising fish and fish oil (Milo & Grosch, 1996; Karahadian & Lindsay, 1989). These volatiles have been shown to originate from degradation of n-3 fatty acids (Frankel, 1998; Grosch, 1987). Furthermore, Venkateshwarlu, Let, Jacobsen, and Meyer (2004) have shown that (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one were important in relation to formation of fishy off-flavours in fish oil enriched milk, and these components were suggested as potential markers of n-3 PUFA degradation.

This study was undertaken to further improve the understanding of the influence of fish oil quality and product storage temperature on the oxidative stability of real food emulsions containing these oils. The effect of increasing oil PV was investigated in two different oil types: (a) a mixture of rapeseed and fish oil stabilised with the added antioxidants citric acid ester and propyl gallate, and (b) a fish oil without added antioxidants. The aim of the study was not to compare these oil types directly, but to assess the influence of the initial oxidative stage of the oils on the oxidative deterioration of milk enriched with a stabilised fish oil mixture versus milk enriched with an unprotected fish oil. The rapeseed and fish oil mixture with added antioxidants was chosen because it is a commercially available product intended for fish oil enrichment of foods and because it was previously proved to be more stable than the pure fish oil in milk emulsions (Let et al., 2004). The rapeseed and fish oil mixture was also included to evaluate the influence of small changes in the cold storage temperatures at the different initial oxidative stages of the oil mixture in the enriched milk system. The effect of cold storage temperature of the milk emulsions was examined by storing the milk emulsions at 2, 5 and 9 °C, respectively.

## 2. Materials and methods

### 2.1. Materials

Fresh milk with fat contents of 0.5 and 1.5 wt% were purchased locally and mixed in a 1:1 ratio. A refined cod liver oil without added antioxidants and an oil mixture of rapeseed oil and cod liver oil (1:1) with added antioxidants (1840 ppm citric acid ester (mono- and diglycerides of fatty acids) and 460 ppm propyl gallate) were provided by Maritex A/S, Århus, Denmark. The refined cod liver oil was deodorised at Biocentrum-DTU, Technical University of Denmark, Lyngby, DK, as previously described (Let et al., 2004). The oils were described by their fatty acid composition, the peroxide value (PV), anisidine value (AV), the amount of free fatty acids (FFA), and the level of tocopherols of each oil, which are given in Table 1. The fatty acid composition was determined by preparation of methyl esters (AOCS Official method Ce 2-66, 1992a) that were in turn analysed by gas chromatography (AOCS Official method Ce 1b-89, 1992b). The levels of tocopherols were determined by HPLC (AOCS Official method Ce 8-89, 1992c). The AV and the amount of FFA in the oils were determined by the AOCS Official method Cd 18-90 (1994) and AOCS Official method Ca 5a-40 (1998), respectively. Chemicals and external standards for identification of volatile oxidation products were all from Sigma Aldrich, Steinheim, Germany. All solvents were of HPLC grade from Lab-Scan, Dublin, Ireland.

### 2.2. Preparation of oils of different oxidative stage

The fish oil without added antioxidants and the fish oil:rapeseed oil mixture both had initial PVs less than 0.1 meq $kg^{-1}$ and AV was 2.5 and 2.0, respectively. These oils were oxidised in order to obtain peroxide values of approximately 0.5, 1.0 and 2.0 meq $kg^{-1}$. The oils (1.5 kg each) were oxidised at 40–50 °C (protected from light) in the presence of air and with continuous stirring. The PV was measured regularly, and at PVs of 0.5, 1.0, and 2.0 meq $kg^{-1}$ samples of 0.5 kg were taken. The samples were immediately flushed with nitrogen and stored at −80 °C until production of the emulsions. The oils with PV < 0.1 were also kept at −80 °C until preparation of emulsions. At the time of production of emulsions, the PVs of the oils were determined again together with the determination of AV and free fatty acids, and these values are presented in Table 1.

Table 1
Chemical data of the fish oil and the rapeseed and fish oil mixture: fatty acid composition, content of natural tocopherols, peroxide value (PV), anisidine value (AV) and amount of free fatty acids[a]

| Fatty acid (wt%) | Pure fish oil | | | | Rapeseed + fish oil | | | |
|---|---|---|---|---|---|---|---|---|
| | F_0.1 | F_0.5 | F_1.0 | F_2.0 | FR_0.1 | FR_0.5 | FR_1.0 | FR_2.0 |
| 14:0 | 3.6 | 3.8 | 3.8 | 3.8 | 1.9 | 1.9 | 2.0 | 1.9 |
| 16:0 | 10.3 | 10.5 | 10.6 | 10.6 | 7.4 | 7.5 | 7.5 | 7.5 |
| 18:0 | 2.2 | 2.2 | 2.2 | 2.2 | 1.9 | 1.9 | 1.9 | 2.0 |
| **ΣSAT** | **16.7** | **17.1** | **17.2** | **17.2** | **11.8** | **12.0** | **12.0** | **12.0** |
| 16:1(n-7) | 6.4 | 6.6 | 6.7 | 6.7 | 0.1 | 0.1 | 0.1 | 0.1 |
| 18:1(n-9) | 17.5 | 17.7 | 17.7 | 17.7 | 38.0 | 38.2 | 38.3 | 38.3 |
| 18:1(n-7) | 3.9 | 4.0 | 4.0 | 4.0 | 3.5 | 3.6 | 3.7 | 3.7 |
| 20:1(n-9) | 11.0 | 11.1 | 11.1 | 11.1 | 6.2 | 6.2 | 6.2 | 6.3 |
| 20:1(n-11) | 0.3 | 0.4 | 0.4 | 0.4 | 0.2 | 0.2 | 0.2 | 0.2 |
| 22:1(n-11) | 7.6 | 7.9 | 7.9 | 7.8 | 3.7 | 0.2 | 0.2 | 0.2 |
| 22:1(n-9) | 0.7 | 0.0 | 0.0 | 0.0 | 0.5 | 3.8 | 3.8 | 3.9 |
| **ΣMUFA** | **48.2** | **48.4** | **48.5** | **48.4** | **56.0** | **56.1** | **56.1** | **56.2** |
| 18:2(n-6) | 1.7 | 1.7 | 1.7 | 1.8 | 10.2 | 10.4 | 10.4 | 10.4 |
| 18:3(n-3) | 0.9 | 0.9 | 0.9 | 0.9 | 4.5 | 4.7 | 4.7 | 4.6 |
| 18:4(n-3) | 2.5 | 2.6 | 2.6 | 2.6 | 1.3 | 1.4 | 1.4 | 1.3 |
| 20:5(n-3) | 8.0 | 8.2 | 8.2 | 8.2 | 4.3 | 4.3 | 4.3 | 4.3 |
| 22:5(n-3) | 1.0 | 1.0 | 1.0 | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 |
| 22:6(n-3) | 10.9 | 11.0 | 10.9 | 11.0 | 5.8 | 5.8 | 5.7 | 5.8 |
| **ΣPUFA** | **27.4** | **27.6** | **27.7** | **27.7** | **28.3** | **28.2** | **28.2** | **28.0** |
| Other | **7.7** | **6.9** | **6.7** | **6.6** | **3.8** | **3.7** | **3.7** | **3.7** |
| PV (meq $kg^{-1}$) | 0.06 ± 0.01 | 0.48 ± 0.01 | 0.98 ± 0.01 | 2.12 ± 0.01 | 0.08 ± 0.01 | 0.53 ± 0.03 | 0.94 ± 0.03 | 1.99 ± 0.07 |
| AV | 2.5abc ± 0.1 | 2.3ab ± 0.2 | 2.6bc ± 0.0 | 3.1c ± 0.3 | 2.0a ± 0.0 | 2.2ab ± 0.1 | 2.3ab ± 0.0 | 2.8bc ± 0.2 |
| FFA (%) | 0.05 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.06 ± 0.00 | 0.14 ± 0.01 | 0.14 ± 0.00 | 0.14 ± 0.02 | 0.13 ± 0.01 |
| Tocopherols (ppm ± 2 ppm) | | | | | | | | |
| α | 395 | 387 | 391 | 383 | 339 | 337 | 337 | 336 |
| β | | | | | 34 | 36 | 35 | 34 |
| γ | 4 | 5 | 4 | 4 | 173 | 172 | 176 | 173 |

[a]The sample names of the oils refer to the PV aimed at during the production of the oils with the different PVs. The actual values determined are given in the table.

### 2.3. Production of emulsions and preparation of samples for analyses

Emulsions were prepared at a milk processing pilot plant (Pasilac Therm, Kolding, Denmark) coupled to a two-valve Rannie homogenisor (APV, Albertslund, Denmark), as previously described (Let et al., 2004). One batch of each milk emulsion was prepared according to the experimental plan (Table 2). The pasteurised milk emulsions were bottled under laminar, steril air flow in separate steril bottles for chemical and sensory analyses for each sampling day. After storage at 2, 5, or 9 °C in the dark, the emulsions were subjected to sensory evaluation, PV determination, and dynamic headspace GC-MS analyses. The samples for chemical analysis were stored in separate, brown glass bottles and immediately flushed with nitrogen and stored at −30 °C until analyses, while samples for sensory analyses were evaluated directly at sampling.

### 2.4. Analysis of iron and copper in the milk

The total iron and copper content of the milk was determined by graphite furnace atomic absorption spectrometry using a Perkin Elmer model 5100 instrument with Zeeman background correction. The milk was diluted with 0.2% nitric acid, injected into the furnace, subjected to an optimised temperature program, and the absorbances were then read at 248.3 and 324.8 nm for iron and copper, respectively. Concentrations were determined from calibration curves produced under the same conditions.

### 2.5. Analyses of primary oxidation products

Lipids from the emulsions were extracted by chloroform:methanol (1:1 w/w) as described by Bligh and Dyer (1959), using a reduced amount of solvent, according to the description of Iverson, Lang and Cooper (2001). PV was measured directly on the oils or

Table 2
Experimental design

| Sample | Oil type | Peroxide value (PV) of oil (meq $kg^{-1}$) | Storage temp. (°C) | Sensory analysis |
|---|---|---|---|---|
| Milk + 2C | No oil | — | 2 | |
| Milk + 5C | | — | 5 | × |
| Milk + 9C | | — | 9 | |
| FR_0.1 + 2C | Rapeseed + fish oil | <0.1 | 2 | |
| FR_0.1 + 5C | | <0.1 | 5 | × |
| FR_0.1 + 9C | | <0.1 | 9 | |
| FR_0.5 + 2C | Rapeseed + fish oil | 0.5 | 2 | |
| FR_0.5 + 5C | | 0.5 | 5 | × |
| FR_0.5 + 9C | | 0.5 | 9 | |
| FR_1.0 + 2C | Rapeseed + fish oil | 1.0 | 2 | |
| FR_1.0 + 5C | | 1.0 | 5 | × |
| FR_1.0 + 9C | | 1.0 | 9 | |
| FR_2.0 + 2C | Rapeseed + fish oil | 2.0 | 2 | |
| FR_2.0 + 5C | | 2.0 | 5 | × |
| FR_2.0 + 9C | | 2.0 | 9 | |
| F_0.1 | Fish oil | 0.1 | 2 | |
| F_0.5 | Fish oil | 0.5 | 2 | |
| F_1.0 | Fish oil | 1.0 | 2 | |
| F_2.0 | Fish oil | 2.0 | 2 | |

Sample codes refer to oil type, oil PV, and storage temperature.
×; Sensory analysis was performed on the five samples marked.

on the fat extract from the milk emulsions by colorimetric determination of iron-thiocyanate according to the method described by the International IDF Standards (1991).

### 2.6. Dynamic headspace analysis of volatile secondary oxidation products

Volatile secondary oxidation products from 8 g of emulsion were purged and trapped on Tenax GR® tubes with nitrogen (150 mL $min^{-1}$) for 30 min at 45 °C using dodecane as internal standard. The volatiles were desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin Elmer, Norwalk, CN) and cryofocused on a Tenax GR cold trap. Volatiles were separated by gas chromatography (HP 5890 IIA, Hewlett Packard, Palo Alto, CA) as described previously by Timm, Xuebing, Nielsen, and Jacobsen (2003) and analysed by mass spectrometry (HP 5972 mass-selective detector). Oven temperature programme: 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 hold at 220 °C for 4 min. The individual compounds were identified by both MS-library searches (Wiley138K, John Wiley and Sons, Hewlett Packard, US) and by authentic external standards. The individual compounds were quantified through calibration curves. The limit of detection and the limit of quantification were determined at a signal-to-noise ratio of 2 and 5, respectively, for each of the compounds at the given conditions (8 g emulsion, 45 °C, 30 min purge with $N_2$ at 150 mL $min^{-1}$).

### 2.7. Sensory evaluation

The milk reference sample and the four emulsions containing the rapeseed and fish oil mixture, which were stored at 5 °C (Table 2: Milk + 5C, FR_0.1 + 5C, FR_0.5 + 5C, FR_1.0 + 5C, FR_2.0 + 5C), were evaluated by descriptive analysis by 12 panellists trained in descriptive analysis of fishy off-flavours. ISO Standard 6658, 8586, 6564 (1985a, b, 1993) were generally followed for training and sensory analysis methods, respectively. The descriptors used for odour and flavour assessment were fishy, rancid, milk and metallic, and they were evaluated on a continuous intensity scale ranging from zero intensity to a maximum intensity of 9. Samples (40 mL) were served randomised at 5 °C with crisp bread and cold water in blind trials after 1, 4, and 8 days of storage. Data were collected on PSION mini computers (PSION, London, UK). Separately from the descriptive analysis a triangular test was performed. The difference between a milk sample and an emulsion containing the fish oil:rapeseed oil mixture with PV 0.1 meq $kg^{-1}$ similar to FR_0.1 + 2C was tested at the time of production and after 8 days of storage by 14 untrained panellists.

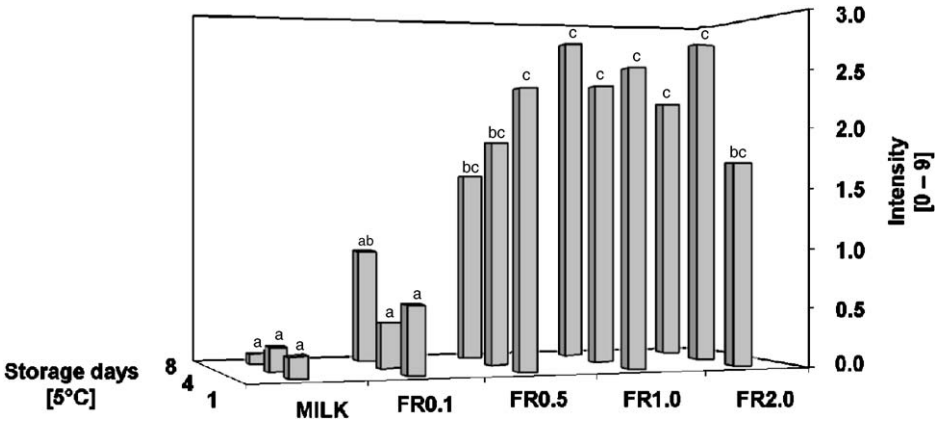



Fig. 1. Results (Average of all 12 assessors' determinations. The emulsions followed by a same letter were not significantly different in the Bonferroni multiple comparison test using 0.05-level of significance. Sample codes are explained in Table 2 of the sensory evaluation of fishy off-flavours of the milk emulsions during storage at 5 °C).

### 2.8. Statistical analysis

The data were analysed by one- or two-way analysis of variance and individual samples were compared on a 0.05-level of significance by the Bonferroni multiple comparison test using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA).

## 3. Results

### 3.1. Results of sensory analysis

The sensory results are presented as average scores of the 12 assessors (Fig. 1). The pure milk and the milk emulsions enriched with the fish oil:rapeseed oil mixture were evaluated, but because of the practical limitations of the sensory analyses, only emulsions stored at 5 °C were compared. The intensities of fishy, rancid and metallic off-flavours were all less than three on a scale from zero to nine in all the emulsions. A sensory score of three corresponds to a mild but distinct off-flavour. The emulsions containing oil with PVs of 0.5, 1.0, and 2.0 meq $kg^{-1}$, respectively, thus clearly had fishy off-flavours already at day 1. The emulsion containing oil with PV 0.5 meq $kg^{-1}$ had less fishy off-flavour than the emulsion containing oil with PV 1.0 meq $kg^{-1}$ at all days, and this difference increased during storage. At day 1 the emulsion containing oil with 2.0 meq $kg^{-1}$ happened to have less fishy off-flavour than the emulsions containing oil with PV 1.0 meq $kg^{-1}$. None of these three emulsions were significantly different at any of the days.

The fishy odour and taste of the PV 0.1 emulsion (FR_0.1) were lower than the fishy odour and taste of the other three emulsions (FR_0.5, FR_1.0, FR_2.0) throughout storage, and at day 1 and 4 this difference was significant. The fishy odour and taste of the PV 0.1 emulsion was around the limit of detection. The average scores in this sample were slightly higher than those of the milk sample, though they did not differ significantly throughout the storage period. Results with an untrained panel, resembling a consumer panel (data not shown), also showed that in triangular tests (days 1 and 8) the milk sample and the milk emulsion containing fish oil:rapeseed oil with PV of 0.1 meq $kg^{-1}$ could not be discerned ($p > 0.05$).

The milk taste scores were from 2.0 to 2.6 for the PV 0.5, 1.0, and 2.0 emulsions (FR_0.5 + 5C, FR_1.0 + 5C, FR_2.0 + 5C), while the milk taste for the PV 0.1 emulsion was constant at 3.0 throughout storage. The milk taste of the pure milk increased from 3.0 at day 1 to 3.8 at days 4 and 8 (data not shown). The increase in the scores of the milk sample could indicate an increased difference between the pure milk and the emulsions containing the fish oils during storage.

### 3.2. Results of chemical analysis

The oxidation of the regular milk (M), the milk emulsions containing fish oil (F) or the fish and rapeseed oil mixture (FR) was chemically assessed by analysis of PV and the concentration of five selected volatile oxidation products: (*E*)-2-hexenal, (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, and 2-penten-1-ol.

#### 3.2.1. Changes in PV of the emulsions

The PV in all the emulsions containing the pure fish oil increased significantly during storage, and increased more than the PV in the emulsions based on the fish oil:rapeseed oil mixture (Table 3). Compared with the other fish oil emulsions, the emulsion based on the PV 0.1 oil (F_0.1) had the lowest PV after 1 day of storage. The PV at day 1 in the emulsions increased in the order F_0.1 < F_0.5 < F_1.0 ~ F_2.0, although differences were

Table 3
Peroxide values (meq $kg^{-1}$) of the milk emulsions without added oil, with the fish oil:rapeseed oil mixture and with fish oil during storage at different temperatures[a]

| | Day 1 | Day 4 | Day 8 | Day 11 | Day 14 |
|---|---|---|---|---|---|
| Milk + 5C | 0.9a,v ± 0.2 | 0.5a,v ± 0.0 | 0.9a,v ± 0.2 | 0.6a,v ± 0.0 | 0.6a,v ± 0.1 |
| FR_0.1 + 2C | 1.3a,v ± 0.1 | 1.7b,vx ± 0.4 | 2.7cd,y ± 0.8 | 2.4c,xy ± 0.5 | 2.7d,y ± 0.5 |
| FR_0.1 + 5C | 1.2a,v ± 0.1 | 1.9b,vx ± 0.1 | 2.9d,y ± 0.4 | 3.0cd,y ± 0.7 | 2.5cd,xy ± 0.0 |
| FR_0.1 + 9C | 1.0a,v ± 0.0 | 2.3b,x ± 0.1 | 2.7cd,xy ± 0.1 | 3.2cd,y ± 0.4 | 3.0d,xy ± 0.1 |
| FR_0.5 + 2C | 1.6ab,v ± 0.1 | 2.1b,vx ± 0.0 | 1.5b,v ± 0.3 | 2.7c,x ± 0.3 | 2.3bcd,vx ± 0.0 |
| FR_0.5 + 5C | 1.5a,v ± 0.1 | 2.0b,vx ± 0.3 | 1.3b,v ± 0.0 | 2.8c,x ± 0.3 | 1.9bc,vx ± 0.0 |
| FR_0.5 + 9C | 1.7ab,v ± 0.0 | 1.8b,v ± 0.0 | 2.2bcd,v ± 0.2 | 3.7d,x ± 1.0 | 3.2d,x ± 0.0 |
| FR_1.0 + 2C | 1.4a,v ± 0.0 | 2.2b,v ± 0.1 | 1.9bc,v ± 0.1 | 4.7e,x ± 0.3 | 2.4cd,x ± 0.1 |
| FR_1.0 + 5C | 2.3bc,v ± 0.2 | 2.1b,v ± 0.2 | 2.5cd,v ± 0.5 | 2.3bc,v ± 0.8 | 2.4cd,v ± 0.0 |
| FR_1.0 + 9C | 3.3d,x ± 0.3 | 1.9b,v ± 0.1 | 2.6cd,x ± 0.1 | 1.7b,v ± 0.1 | 2.9d,x ± 0.3 |
| FR_2.0 + 2C | 3.0d,x ± 0.6 | 2.1b,v ± 0.1 | 2.3bcd,vx ± 0.6 | 1.6b,v ± 0.1 | 1.6b,v ± 0.0 |
| FR_2.0 + 5C | 3.1d,y ± 0.5 | 2.1b,x ± 0.1 | 1.7bc,v ± 0.1 | 1.1ab,v ± 0.1 | 1.9bc,vx ± 0.0 |
| FR_2.0 + 9C | 2.6cd,v ± 0.1 | 2.0b,v ± 0.1 | 2.0bc,v ± 0.2 | 2.1bc,v ± 0.1 | 2.3bcd,v ± 0.2 |
| F_0.1 + 2C | 2.0b,u ± 0.2 | 3.8b,v ± 0.9 | 7.1c,x ± 0.2 | 9.1c,y ± 0.1 | 13.2d,z ± 0.1 |
| F_0.5 + 2C | 2.3bc,u ± 0.0 | 4.4bc,v ± 0.1 | 6.9c,x ± 0.1 | 8.6c,y ± 0.5 | 11.2c,z ± 0.1 |
| F_1.0 + 2C | 3.2c,u ± 0.0 | 5.0c,v ± 0.2 | 6.9c,x ± 0.0 | 9.1c,y ± 0.1 | 11.5c,z ± 0.2 |
| F_2.0 + 2C | 3.1c,u ± 0.4 | 4.1b,v ± 0.2 | 4.1b,v ± 0.0 | 6.2b,x ± 0.1 | 8.5b,y ± 0.3 |

[a]Average of duplicate determination on the same sample. The individual emulsions were compared to the milk emulsion and to the other emulsions containing the same type of oil. Emulsions followed by a same letter are not significantly different in the Bonferroni multiple comparison test using 0.05-level of significance. The samples were compared each day (columns): a, b, c, d, and the changes during storage for each sample were also compared (rows): u, v, x, y, z. Sample codes are explained in Table 2.

only significant between F_0.1, F_1.0 and F_2.0 (Table 3). This order was also observed after day 4, except for the emulsion containing oil with PV 2.0 meq $kg^{-1}$. At day 8 and 11 the emulsions based on PV 0.1, 0.5, and 1.0 oils had similar PVs. At day 14 the PV 0.1 emulsion had the highest PV. From day 4 and onwards, the emulsion containing oil with PV of 2.0 (F_2.0 + 2C) deviated significantly from the other emulsions, as its PV increased less than in the other three emulsions.

Among the fish oil:rapeseed oil mixtures, the PVs of the emulsions at day 1 corresponded to the PVs of the oils used (Table 3). Hence, the order of the emulsions regarding PV at day 1 was FR_0.1 < FR_0.5 < FR_1.0 < FR_2.0, irrespective of the storage temperature, although these differences were not overall significant.

Generally the PV in the emulsions developed similarly for the three temperatures 2, 5, and 9 °C (e.g. FR_0.1 + 2C vs. FR_0.1 + 5C vs. FR_0.1 + 9C). The emulsion containing oil with PV 0.1 meq $kg^{-1}$ increased significantly during storage at both 2, 5 and 9 °C (FR_0.1). The emulsions based on oil with PV of 0.5 meq $kg^{-1}$ increased only slightly during storage (FR_0.5), while the emulsions based on PV 1.0 oil did not change significantly during storage at the three temperatures (FR_1.0). The PV of the emulsions with PV 2.0 oil decreased during storage at 2, 5 and 9 °C (FR_2.0).

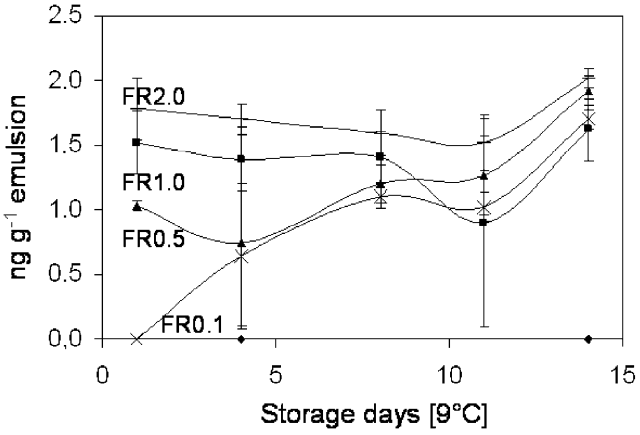



Fig. 2. Development of (E,E)-2,4-heptadienal (ng g−1 emulsion) in the milk emulsions containing rapeseed and fish oil mixture with antioxidants during storage at 9 °C. —◆—, milk; —×—, FR_0.1 + 9C; —▲—, FR_0.5 + 9C; —■—, FR_1.0 + 9C; — – —, FR_2.0 + 9C. Sample codes are explained in Table 2.

### 3.2.2. Changes in the concentrations of volatiles

The levels of volatiles in the fish oil:rapeseed oil emulsions at day 1 also corresponded to the PV of the oils. At day 1 the concentrations of volatiles in the emulsions were generally in the order FR_0.1 < FR_0.5 < FR_1.0 < FR_2.0 (data not shown). This order was maintained throughout the storage period, although the emulsion based on PV 2.0 meq $kg^{-1}$ oil deviated for

Table 4
Concentrations of volatiles (ng $g^{-1}$ emulsion) in the milk emulsions without added oil, with the fish oil:rapeseed oil mixture and with the fish oil after 8 days at different temperatures[a]

| | 1-penten-3-one | 2-penten-1-ol | (*E*)-2-hexenal | (*E*,*E*)-2,4-heptadienal | (*E*,*Z*)-2,6-nonadienal |
|---|---|---|---|---|---|
| Milk | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.0a ± 0.0 |
| FR_0.1 + 2C | 0.6ab ± 0.1 | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.0a ± 0.0 |
| FR_0.1 + 5C | 0.9ab ± 0.2 | 0.0a ± 0.0 | 0.0a ± 0.0 | 0.7ab ± 0.6 | 0.0a ± 0.0 |
| FR_0.1 + 9C | 0.9ab ± 0.1 | 1.0b ± 0.9 | 0.0a ± 0.0 | 1.1bc ± 0.1 | 0.0a ± 0.0 |
| FR_0.5 + 2C | 1.1abc ± 0.1 | 0.7ab ± 1.2 | 1.2bc ± 0.7 | 1.2bc ± 0.2 | 0.0a ± 0.0 |
| FR_0.5 + 5C | 1.1abc ± 0.1 | 2.3cd ± 0.3 | 0.7ab ± 0.9 | 1.1bc ± 0.1 | 0.0a ± 0.0 |
| FR_0.5 + 9C | 1.0abc ± 0.1 | 1.8bc ± 0.9 | 1.8c ± 0.1 | 1.2bc ± 0.1 | 0.0a ± 0.0 |
| FR_1.0 + 2C | 1.5c ± 0.1 | 2.6cd ± 0.8 | 1.6c ± 1.0 | 1.6c ± 0.1 | 0.0a ± 0.0 |
| FR_1.0 + 5C | 1.8c ± 0.1 | 3.2d ± 0.5 | 2.5d ± 0.2 | 1.5bc ± 0.1 | 0.0a ± 0.0 |
| FR_1.0 + 9C | 1.1abc ± 0.1 | 2.8d ± 0.5 | 1.4bc ± 1.3 | 1.4bc ± 0.2 | 0.0a ± 0.0 |
| FR_2.0 + 2C | 1.5c ± 0.0 | 2.4cd ± 0.3 | 2.3cd ± 0.4 | 2.0c ± 0.1 | 0.4a ± 0.7 |
| FR_2.0 + 5C | 1.3bc ± 0.1 | 1.9bc ± 0.8 | 2.5d ± 0.3 | 1.8c ± 0.1 | 0.4a ± 0.6 |
| FR_2.0 + 9C | 1.0abc ± 0.1 | 2.2cd ± 0.4 | 2.3cd ± 0.1 | 1.6c ± 0.2 | 0.4a ± 0.6 |
| F_0.1 | 7.9c ± 0.5 | 10.7c ± 1.4 | 9.3c ± 0.5 | 5.8c ± 0.6 | 3.5b ± 0.6 |
| F_0.5 | 11.0d ± 1.0 | 17.5d ± 2.4 | 13.4d ± 0.6 | 7.8d ± 0.8 | 5.5c ± 0.8 |
| F_1.0 | 13.4e ± 0.7 | 21.7e ± 1.6 | 16.7e ± 0.5 | 9.5d ± 0.8 | 6.2c ± 1.0 |
| F_2.0 | 2.1b ± 0.1 | 4.5b ± 0.4 | 5.2b ± 0.3 | 3.9b ± 0.3 | 2.0b ± 0.3 |

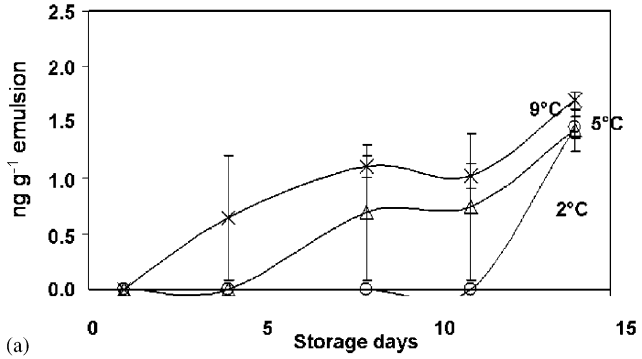
[a]Average of triple determinations ± standard deviation on the same sample. The emulsions were compared to the milk emulsion and to the other emulsions containing the same type of oil. Emulsions followed by a same letter (columnwise) are not significantly different in the Bonferroni multiple comparison test using 0.05-level of significance. Emulsions followed by same letter are not significantly different in the Bonferroni multiple comparison test using 0.05-level of significance. The samples were compared each day (columns): a, b, c, d, and the changes during storage for each sample were also compared (rows): u, v, x, y, z. Sample codes are explained in Table 2.

the 2-penten-1-ol and (*E*)-2-hexenal. Fig. 2 shows the concentrations of (*E*,*E*)-2,4-heptadienal in the emulsions stored at 9°C. The concentration of (*E*,*E*)-2,4-heptadienal increased significantly during storage in the emulsion containing oil with a PV of 0.1, while it increased less in the emulsions containing oils with PVs of 0.5, 1.0, and 2.0 meq $kg^{-1}$ (Fig. 2). Patterns were similar in emulsions stored at 2 and 5 °C (data not shown). None of the selected volatiles were detected in the milk samples stored at either 2, 5, or 9 °C.

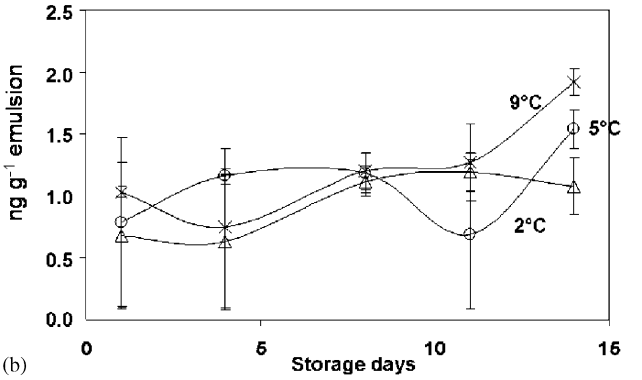
In Table 4 is presented the concentrations of the selected volatiles determined at day 8, which was the last day of sensory analysis. (*E*,*Z*)-2,6-nonadienal was only detected in the emulsions containing PV 2.0 oil at day 8 and onwards. (*E*)-2-hexenal and 2-penten-1-ol were only detected in the emulsion containing the oil with PVs of either 0.5, 1.0, or 2.0 meq $kg^{-1}$, but not in the emulsion containing PV 0.1 oil, except when stored at 9 °C, where 2-penten-1-ol was also detected. Finally, (*E*,*E*)-2,4-heptadienal was detected in the PV 0.1 emulsions stored at 5 or 9 °C, but not in the emulsion stored at 2 °C. Taken together, these findings indicate that an effect of both the initial oil PV and the storage temperature could be observed regarding the formation of these volatiles.

The development of (*E*,*E*)-2,4-heptadienal in the emulsion containing PV 0.1 oil (Fig. 3a) illustrated the effect of storage temperature. (*E*,*E*)-2,4-heptadienal was not detected in any of the PV 0.1 emulsions at day 1 (FR_0.1). After 4 days it was detected in the emulsion stored at 9 °C, after 8 days it was detected also in the emulsion stored at 5 °C, while it was not detected until day 14 in the PV 0.1 emulsions stored at 2 °C. In the emulsions containing oil with PV 2.0, this effect of storage temperature was not evident (Fig. 3b). (*E*,*E*)-2,4-heptadienal was detected in all these emulsions at day 1, although the levels were around the limit of quantification (1.3 ng $g^{-1}$ emulsion), and at days 4 and 11 the concentrations of (*E*,*E*)-2,4-heptadienal were not significantly different (Fig 3b). However, after 14 days the emulsions stored at 9 °C had significantly higher levels of (*E*,*E*)-2,4-heptadienal.

In the emulsions based on fish oil only, a similar pattern was observed regarding the overall development of volatiles, which is exemplified by (*E*,*E*)-2,4-heptadienal in Fig. 4. The concentrations of the selected volatiles at day 8 are given in Table 4. From day 1 and throughout the storage period, the concentrations of volatiles were in the order F_0.1 < F_0.5 < F_1.0 for all the compounds. At day 1 the differences between the emulsions were significant with regard to 1-pentene-3-one, 2-pentene-1-ol, and (*E*)-2-hexenal (data not shown). The deviating behaviour of the PV 2.0 emulsion was again evident with respect to the volatiles. The concentrations of all the selected volatiles increased less in the PV 2.0 emulsion compared to the corresponding concentrations in the other emulsions.




(a)




(b)

Fig. 3. Development of (*E*,*E*)-2,4-heptadienal (ng g$^{-1}$ emulsion) in the milk emulsions containing rapeseed and fish oil mixtures with antioxidants and with (a) PV of 0.1 meq kg$^{-1}$ and (b) PV 2.0 meq kg$^{-1}$ during storage at 2, 5, and 9 °C. (a) —○—, FR_0.1 + 2C; —△—, FR_0.1 + 5C; — × —, FR_0.1 + 9C; (b) —○—, FR_2.0 + 2C; —△—, FR_2.0 + 5C; — × —, FR_2.0 + 9C. Sample codes are explained in Table 2.

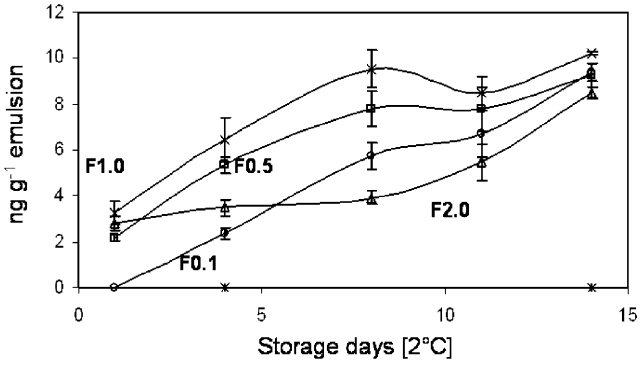



Fig. 4. Development of (*E*,*E*)-2,4-heptadienal (ng g$^{-1}$ emulsion) in the milk emulsions containing fish oil without antioxidants during storage at 2 °C. —∗—, milk, —○—, F_0.1 + 2C; —△—, FR_0.5 + 9C; —□—, FR_0.1 + 9C; —△—, FR_2.0 + 2C. Sample codes are explained in Table 2.

## 4. Discussion

First of all these results showed that the emulsions containing fish oil were significantly more oxidised than the corresponding emulsions based on the fish oil:rapeseed oil (F_0.1 > FR_0.1, etc.). This supports our previous findings in Let et al. (2004), where a significant protective effect of rapeseed oil on the fish oil was observed during storage of fish oil enriched milk. The specific mechanisms responsible for this observed protective effect of the rapeseed oil, still need to be identified.

### 4.1. Effect of oil PV on oxidative stability

Our previous work (Let et al., 2003, 2004) on fish oil enriched milk emulsions has indicated that the initial PV of the oil, and thus the level of lipid hydroperoxides present at the time of production of the emulsions seemed important determinants of the subsequent oxidative stability of the emulsions during storage. It was also shown that the initial PV influenced the antioxidant effect of EDTA, which is recognised as a metal chelating agent. When lipid hydroperoxides decompose, reactive free radicals are formed. These radicals can facilitate further oxidation. These reactions are enhanced by the presence of trace metal ions. We therefore assume, that the presence of even the small levels of hydroperoxides in oils with low PV, can promote oxidation in a milk system, which contains natural iron and copper.

The present results showed that the emulsions prepared with pure fish oil containing PV 0.1 oil were less oxidised than the emulsion containing PV 0.5 oil, which again had less volatiles, and thus was less oxidised than the emulsion based on PV 1.0 fish oil (F_0.1 < F_0.5 < F_1.0). This corroborates our hypothesis that small differences in hydroperoxide concentrations affect the oxidative stability of fish oil enriched emulsions.

A similar pattern was observed for the emulsions based on the rapeseed and fish oil mixture. However, the emulsions based on fish oil alone had at least 5–10 times the concentrations of volatiles after 8 days of storage as compared to the emulsions based on fish oil:rapeseed oil. Therefore, the PV and the concentrations of the volatiles revealed only very small differences between the emulsions based on the four different fish oil:rapeseed oil mixtures. In contrast, the sensory analysis showed a clear discrimination between the PV 0.1 emulsion and the PV 0.5, 1.0 and 2.0 emulsions, and thus revealed differences that were not detectable by chemical analyses. These latter emulsions had equally intense fishy off-flavours, while the off-flavours of the emulsion with PV 0.1 oil were less intensive. In a milk model system, Venkateshwarlu et al. (2004) showed that the formation of fishy off-flavour was a result of interactions between the compounds present rather than due to the presence of a single compound. The present experiment corroborates the hypothesis that the most important factor regarding development of fishy off-

flavour is the interaction between the compounds overall present in the emulsions. The volatiles were selected because they developed significantly in the fish oil enriched emulsions. The very small differences in the concentrations of all these individual compounds were insignificant as analysed by the GC-MS, but in the emulsions these volatiles obviously did result in an overall perceivable sensory difference. Jacobsen et al. (1999) has previously shown that fish oil enriched mayonnaises with intense fishy off-flavours was not necessarily highly oxidised when evaluated from the chemical analysis. Finally, the results obtained thus substantiated that the sensory panel evaluation was more sensitive to these minor differences than analyses of PV and individual volatile compounds.

The results also showed that the AVs of the different oils were low (2.0–3.1), and not directly related to especially the sensory characteristics of the emulsions produced. There were no differences in AV between the PV 0.1 and PV 0.5 emulsions (FR_0.1 vs. FR_0.5, FR_0.1 vs. FR_0.5), whereas the fishy off-flavours were significantly different, as described above. An increase in oil AV was observed around PV 2.0, and at that point the prepared emulsions were already unacceptable with respect to fishy off-flavour. Thus, the AV is not a sufficiently sensitive analysis to provide information about the oil quality required to prepare sensory acceptable emulsions of this type.

In the milk model system described by Venkateshwarlu et al. (2004) it was also shown that the combination of 1-pentene-3-one, (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal and (*Z*)-4-heptenal resulted in fishy off-flavour in a milk model system. These compounds should therefore be important compounds to monitor during the lipid oxidation of the milk emulsions. Surprisingly, we could not detect any (*E*,*Z*)-2,6-nonadienal in the emulsions with PV 0.5 and 1.0 oils even though these emulsions had distinct fishy off-flavours. Nevertheless, these results support that the small differences in oxidative stability actually was reflected in the development profiles of these selected compounds, especially (*E*,*E*)-heptadienal. Further more, this experiment also suggests that the 2-penten-1-ol and the (*E*)-2-hexenal are important volatiles to monitor during storage and could be used as additional markers of oxidation.

The emulsions containing either the pure fish oil or the fish oil:rapeseed oil with PVs of 2.0 meq $kg^{-1}$ (F_2.0, FR_2.0) both deviated significantly from the pattern described for the emulsions based on oils with PVs of 0.1, 0.5, and 1.0. The initial levels of PV and volatiles in these emulsions as well as the development of PV and volatiles during storage were less than expected from the results of the other emulsions.

The lipid hydroperoxides formed during oxidation of the n-3 fatty acids in fish oil are especially susceptible to trace metal mediated degradation. The milk contained approximately 175 ppb iron and 48 ppb copper, and these values were not affected by the addition of oil to the milk. Apart from an increased level of hydroperoxides, these oils also had increased levels of secondary oxidation products, as determined by the AV. The presence of trace metals in the milk emulsions based on oil with PV 2.0 could therefore have accelerated the degradation of the lipid hydroperoxides as well as the degradation of the secondary oxidation products into shorter chain volatiles. This would result in PVs lower than expected, and in higher concentrations of short chain aldehydes, such as propanal, which were not quantified here.

### 4.2. Effect of storage temperature on oxidative stability

The effect of storage temperature was investigated in the range from 2 to 9 °C in the emulsions based on the rapeseed and fish oil mixture. This temperature range corresponds to storage in regular household refrigerators. It was evident that the storage temperature affected the oxidative stability during storage, but only in the emulsions based on the oil with a PV of 0.1 meq $kg^{-1}$. In these emulsions an increasing temperature accelerated the initial formation of volatiles, but within the 14 days storage period, the final levels were not significantly different. The different storage temperatures did not affect the development of volatiles in any of the emulsions containing oil with PVs of 0.5, 1.0, and 2.0 meq $kg^{-1}$.

As described, the selected volatiles have previously been shown to originate from degradation of n-3 PUFA. None of the volatiles were determined in the milk during storage at any of the storage temperatures. Therefore, we conclude that the development of these compounds and the differences observed between the emulsions were caused by lipid oxidation and not by microbial growth in the milk samples stored at the different temperatures.

## 5. Conclusion

First of all, the present experiment showed that it was possible to produce a pasteurised milk product enriched with the important n-3 PUFA from fish oil with acceptable sensory characteristics. However, it was also evident that (1) it seems necessary to use a mixture of fish oil and rapeseed oil and (2) the PV of the oil needs to be below 0.5 meq $kg^{-1}$. The results showed that the storage temperature, in the range 2–9 °C, had small, but important, effects on the oxidation of the fish oil enriched milk emulsions. The determination of PV in the emulsions was not sensitive enough to reveal the small differences between the emulsions, whereas the GC analysis provided information about the early stages

of the formation of the volatiles. It was evident that an increasing temperature promoted the onset of formation of the volatiles. The oil AVs were generally low, and had no correlation to the sensory characteristics of the resulting emulsions.

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