DOI 10.1002/ejlt.200300901 *Eur. J. Lipid Sci. Technol.* **106** (2004) 170–182

Mette B. Let[a]
Charlotte Jacobsen[a]
Anne S. Meyer[b]

[a] Danish Institute for Fisheries Research, Department of Seafood Research, Lyngby, Sweden
[b] Food Biotechnology and Engineering Group, Biocentrum-DTU, Technical University of Denmark, Lyngby, Sweden

Effects of fish oil type, lipid antioxidants and presence of rapeseed oil on oxidative flavour stability of fish oil enriched milk

As a part of our ongoing experiments on optimization of the oxidative stability of fish oils in genuine food systems, this study investigated the oxidative deterioration of fish oil enriched milk emulsions during cold storage. The experimental data showed that addition of rapeseed oil to fish oil (1:1) prior to emulsification into milk significantly protected the emulsions against oxidative deterioration. Addition of propyl gallate and a citric acid ester to the fish oil prior to emulsification also protected the fish oil enriched milk during storage. Emulsions containing a rapeseed:fish oil mixture were oxidatively stable during 11 d at 2 °C. Thus, no additional inhibitory effect of the added antioxidants was observed. The peroxide value and concentrations of five selected volatiles derived from *n*-3 PUFA degradation in rapeseed:fish oil mixture emulsions were not significantly different from the corresponding levels in neither the emulsion containing only rapeseed oil nor the milk. It is proposed that the tocopherols in rapeseed oil may be the protective factor. Three-way chemometric exploratory data analysis was implemented in form of a parallel factor analysis (PARAFAC). The PARAFAC model provided an overview of the obtained data with significantly enhanced interpretability, and revealed information about groupings and correlations in our data.

Keywords: Antioxidants, emulsion, fish oil, lipid oxidation, multiway chemometrics, sensory analysis.

Research Paper

1 Introduction

The interest in utilizing marine oils in food products is mainly due to their high content of long chain polyunsaturated fatty acids (PUFA), primarily docosahexaenoic acid (DHA, C22:6*n*-3) and eicosapentaenoic acid (EPA, C20:5*n*-3). The effects on health of these PUFA have been extensively studied, and they are generally accepted to elicit beneficial effects on risk markers of cardiovascular diseases and to possess anti-inflammatory properties [1, 2]. By enriching food products with fish oils, the general consumption of the *n*-3 PUFA in the population may be increased.

Due to their high degree of unsaturation, both DHA and EPA are highly susceptible to oxidation. Thus, when incorporating marine oils into food emulsions the main concern is oxidative deterioration and subsequent development of undesirable off-flavors. The oxidative deterioration of oil-in-water emulsions containing fish oil generates particularly offensive off-flavors even at low levels of oxidation [3, 4]. The oxidation is affected by several parameters, such as the chemical composition of the oils, components of the emulsions, *e.g.* emulsifiers, trace metals, added and naturally occurring antioxidants, as well as storage conditions [5, 6]. In addition, our previous work [7] indicates that the quality of the fish oil itself significantly affects the oxidative stability of fish oil enriched emulsions. The use of a highly unsaturated oil with a very low initial peroxide value (<0.1 meq/kg) resulted in an emulsion of much better sensory quality than an emulsion prepared from a less unsaturated oil with a higher peroxide value (1.5 meq/kg). Therefore, the initial peroxide value of the fish oil seemed to be a more important parameter than its fatty acid composition. The physical and chemical properties of the emulsions affect the partitioning of antioxidant molecules into the different emulsion phases, which in turn alters the effectiveness of the antioxidants [8–10]. It is proposed, that non-polar antioxidants are particularly effective in oil-in-water emulsion systems, and that synergistic effects can be obtained in combination with metal chelating, secondary antioxidants [8]. Accordingly, the tocopherols, which are lipid soluble chain-breaking antioxidants, and also calcium disodium ethylenediaminetetraacetate (EDTA) have been shown to

Correspondence: *Charlotte Jacobsen*, Danish Institute for Fisheries Research, Department of Seafood Research, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark. Phone: +45-4525-2559, Fax: +45-4588-4774; e-mail: cja@dfu.min.dk

reduce oxidation in oil-in-water emulsions [7, 11]. Industrial claims have been made, that addition of vegetable oil to fish oil during production exerts a protective effect on fish oil in bulk [12, 13].

This investigation was undertaken to evaluate whether the addition of rapeseed oil may protect fish oils in emulsions. Secondly, as a part of our ongoing experiments on optimization of the oxidative protection of various genuine fish oil enriched food systems, we also investigated how the mixture of a chain-breaking antioxidant and a metal chelator added to the fish oil before processing of the milk emulsions would affect the oxidation. This mixture of propyl gallate and citric acid ester is commonly used by the industry. The effect of the type of fish oil was investigated by comparing the oxidative deterioration of milk emulsions containing a mixture of rapeseed and cod liver oil with an emulsion containing rapeseed and tuna oil. The oxidative deterioration of emulsions containing oils obtained by two different deodorization procedures was also compared.

Finally, a three-dimensional chemometric approach to data analysis was undertaken in order to explore the data obtained. The multiway parallel factor analysis (PARAFAC) was chosen, as it first of all provides a graphical overview that aims at improving the interpretability of results. Secondly, in our case, this method might disentangle the main parameters responsible for differences observed in the oxidative deterioration of different emulsions. The PARAFAC may also provide information about correlations between the analytical variables determined during storage.

2 Materials and methods

2.1 Materials

Fresh milk with fat contents of 0.5 and 1.5 wt-% was purchased locally and subsequently mixed in a ratio of 1:1. Cod liver oil without added antioxidants, cod liver oil with added antioxidants (2000 ppm citric acid ester (mono- and diglycerides of fatty acids) and 500 ppm propyl gallate), refined rapeseed oil and a 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. A refined non-deodorized cod liver oil without added antioxidants was also provided by Maritex A/S. This oil was subsequently deodorized at Biocentrum-DTU, Technical University of Denmark, Lyngby, DK. The antioxidants propyl gallate (Grindox propyl gallate) and citric acid ester (Grindox TS-G 460) were added to the oils deodorized at Biocentrum-DTU in concentrations corresponding to the levels of the complete citric acid ester in the oils from Maritex A/S. The propyl gallate and citric acid ester added by both Maritex A/S and Biocentrum-DTU were of identical brand from Danisco Ingredients, Brabrand, Denmark. The tuna oil was provided by Roche Vitamins, Heanor, UK. The fatty acid composition [14], peroxide value (PV) and levels of tocopherols [15] for each oil are given in Tab. 1. Chemicals for hydroperoxide determination 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 Deodorization of oils

The industrial scale deodorization of the cod liver oil with and without added antioxidants and of the mixture of cod liver oil and rapeseed oil were done by Maritex A/S, Århus, Denmark. The oils were deodorized at $<$180 °C for 3 h under vacuum ($<$5 hPa). They were flushed with nitrogen, stored for 2 d at room temperature and subsequently for 2 wk at $-$80 °C until use. The pilot plant deodorization was done at the batch deodorization facility of Biocentrum-DTU, Lyngby, Denmark. Two batches (20 kg each) were deodorized, one with refined cod liver oil and one with a mixture of rapeseed oil and refined cod liver oil (1:1). Conditions of deodorization were: temperature, 190 °C; pressure, $<$5 hPa; strip gas, 10 wt-%. After deodorization the batches were split equally into two parts each. The antioxidants propyl gallate and citric acid ester were dissolved in oil (100 mL) at ca. 75 °C and added to one part of the deodorized oil at ca. 75 °C. Oils were immediately covered with nitrogen and stored at $-$80 °C for 1 wk until use.

2.3 Production of emulsions

Emulsions were prepared at a milk processing pilot plant (Pasilac Therm, Kolding, Denmark) coupled to a two-valve Rannie homogenizer (APV, Albertslund, Denmark). Each type of milk emulsion was prepared in one batch according to Tab. 2. Milk with 1.0% milk fat was continuously bubbled with nitrogen in a feeding tank (quality 5.0, AGA A/S, Copenhagen, Denmark). The milk (2 L/min) was heated to 50 °C and oil was added continuously through a vacuum chamber (10 g/min), followed by homogenization at a total pressure of 5.0 MPa. The milk emulsion was pasteurized at 72 °C for 15 s, cooled to 13 °C, bottled sterile and sealed in separate pyrex bottles for chemical and sensory analyses for each sampling day. After storage at 2 °C in the dark, the emulsions were subjected to sensory evaluation and separate samples were taken for PV and dynamic headspace GC-MS analyses, respectively. These samples were immediately flushed with nitrogen and stored at $-$40 °C.

Tab. 1. Chemical data of milk fat, rapeseed oil and fish oils: Fatty acid composition, content of natural tocopherols and added antioxidants, PV, AV and content of free fatty acids.

	Fatty acids [%]									
	Milk	Rape-seed	F1	F2	F1+A	F2+A	FR1	FR3	FR1+A	FR2+A
C14:0	11.0		3.6	3.7	3.6	3.7	1.9	3.2	1.9	1.9
C15:0	1.1									
C16:0	36.5	4.4	10.3	10.2	10.3	10.2	7.4	10.4	7.4	7.4
C17:0	0.5									
C18:0	10.2	1.7	2.2	2.1	2.2	2.1	2.0	2.7	2.0	1.9
Total SAT	**59.5**	**6.2**	**16.1**	**16.0**	**16.1**	**16.0**	**11.3**	**16.3**	**11.3**	**11.3**
C14:1(*n*-5)	1.1									
C16:1(*n*-7)	2.0		6.4	6.4	6.4	6.4	3.3	3.0	3.3	3.3
C18:1(*n*-9)	21.8	59.2	17.5	17.2	17.5	17.2	38.6	34.5	38.6	38.0
C18:1(*n*-7)	2.4	3.0	3.9	3.9	3.9	3.9	3.5	2.7	3.5	3.5
C20:1(*n*-9)		1.5	11.0	10.8	11.0	10.8	6.2	1.5	6.2	6.2
C22:1(*n*-11)			7.6	7.3	7.6	7.3	3.8		3.8	3.7
C22:1(*n*-9)			0.7	0.7	0.7	0.7	0.5		0.5	0.5
Total MUFA	**27.4**	**63.7**	**47.1**	**46.3**	**47.1**	**46.3**	**55.9**	**41.7**	**55.9**	**55.3**
C18:2(*n*-6)	2.4	18.9	1.7	1.7	1.7	1.7	10.2	10.2	10.2	10.2
C18:3(*n*-3)		8.3	0.9	0.9	0.9	0.9	4.6	4.7	4.6	4.5
C18:4(*n*-3)	0.6		2.5	2.6	2.5	2.6	1.3	1.4	1.3	1.3
C19:2		0.6								
C20:5(*n*-3)			8.0	8.4	8.0	8.4	4.1	6.5	4.1	4.3
C22:5(*n*-3)			1.0	1.0	1.0	1.0	0.5	0.9	0.5	0.5
C22:6(*n*-3)			10.9	11.7	10.9	11.7	5.5	8.6	5.5	5.8
Total PUFA	**3.0**	**27.8**	**25.0**	**26.3**	**25.0**	**26.3**	**26.2**	**32.3**	**26.2**	**26.7**
other	10.1	2.3	11.8	11.4	11.8	11.4	6.6	9.7	6.6	6.7
Natural tocopherols [μg/g oil]										
α-tocopherol	20	250	368	393	368	393	308	167	308	325
β-tocopherol		88					43	44	43	43
γ-tocopherol		372					176	186	176	188
Total tocopherols	20	710	368	393	368	393	527	398	527	556
Added antioxidants [μg/g oil]										
Citric acid ester					2000	2000			1840	1840
Propyl gallate					500	500			460	460
Peroxide value[†] **[meq/kg]**		0.20 ± 0.01	0.08 ± 0.01	0.50 ± 0.00	0.21 ± 0.00	0.66 ± 0.05	0.03 ± 0.00	0.12 ± 0.02	0.07 ± 0.01	0.06 ± 0.00
Anisidine value		0.8 ± 0.1	2.3 ± 0.4	4.0 ± 0.3	2.0 ± 0.1	3.2 ± 0.1	2.4 ± 0.2	– –	2.0 ± 0.7	1.2 ± 0.1
Free fatty acids [%]		0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.03 ± 0.01	– –	0.10 ± 0.01	0.14 ± 0.01

[†] Double determination. Sample names refer to Tab. 2.

Tab. 2. Experimental design.

Sample name	Fish oil type	Milk fat	Addition of oil [%-wt]			
		[%-wt]	Fish oil	Rapeseed oil	Added oil antioxidants	Deodorisation of oil
Milk	–	1	–	–	–	–
Rapeseed	–	1	0	0.5	–	–
F1	Cod liver	1	0.5	0	–	BioCentrum
F2	Cod liver	1	0.5	0	–	Maritex
F1+A	Cod liver	1	0.5	0	+	BioCentrum
F2+A	Cod liver	1	0.5	0	+	Maritex
FR1	Cod liver	1	0.25	0.25	–	BioCentrum
FR3	Tuna	1	0.25	0.25	–	Roche
FR1+A	Cod liver	1	0.25	0.25	+	BioCentrum
FR2+A	Cod liver	1	0.25	0.25	+	Maritex

F = fish oil, R = rapeseed oil, FR = fish oil and rapeseed oil mixture (1:1), A = antioxidant added to oil after deodorization, 1 = deodorized at Biocentrum-DTU, 2 = deodorized at Maritex A/S, 3 = deodorized at Roche.

2.4 Chemical analysis of oils and emulsions

The anisidine value (AV) of individual oils were determined spectrophotometrically [16]. Contents of free fatty acids (FFA) in the oils were determined by titration with sodium hydroxide [17]. In the emulsions, lipids were extracted by chloroform:methanol (1:1 wt/wt) [18], using reduced amounts of solvent [19]. The PV was measured by colorimetric determination of ferri-thiocyanate [20] directly on the oils and on the lipid extract from the emulsions.

2.5 Dynamic headspace analysis of volatile secondary oxidation products

Volatile secondary oxidation products were purged and trapped on Tenax GR® tubes, desorbed on an automatic thermal desorber (ATD-400, Perkin Elmer, Norwalk, CN, USA) and subsequently separated by gas chromatography (HP 5890 IIA, Hewlett Packard, Palo Alto, CA, USA) as described previously [7]. The individual compounds were analyzed by mass spectrometry (HP 5972 mass-selective detector), identified by both MS-library searches and by spiking of samples with authentic external standards, and quantified through calibration curves.

2.6 Sensory evaluation

The following six emulsions were evaluated by descriptive analysis by 12 panellists trained in descriptive analysis of fishy off-flavors: The emulsion with rapeseed oil (R), the two emulsions containing cod liver oil without added antioxidants (F1, F2), the emulsion with rapeseed and tuna oil (FR3), and both emulsions containing rapeseed and cod liver oil with added antioxidants (FR1+A, FR2+A) (Tab. 2). Descriptors used for flavor and odor assessment were fishy, rancid, milky 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 randomiszed at 5 °C with crisp bread and cold water in blind trials after 1, 4, and 8 d of storage. Data were collected on PSION mini computers (PSION, London, UK).

2.7 Droplet size

Droplet sizes in emulsion samples were determined by laser diffraction in a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) [21]. Droplets (6–7) of milk emulsion were dispensed directly into the circulating water (2800 rpm, 14–17% obscuration). Results are given as surface area mean diameter $D[3,2] = \Sigma d^3/\Sigma d^2$.

2.8 Statistical analysis

The structure of the designed storage experiment was three-dimensional (Fig. 1), and therefore the three-dimensional methods of multivariate data analysis were used. The three-dimensional structure is called a three-way array, whereby the three directions in the array are called modes [22]. With i, j, and k variables in the three modes, the array consists of $i \times j \times k$ elements. The analytical data for PV and volatiles and the sensory data were investi-

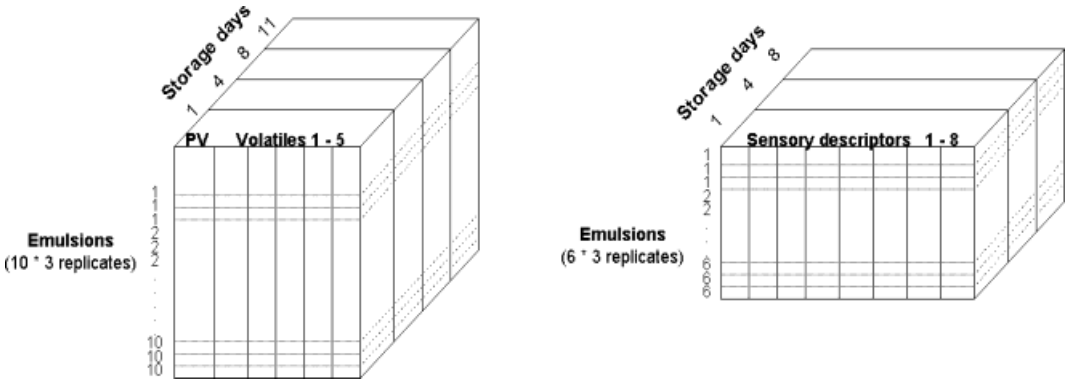

Fig. 1. Arrangement of the experimental data in a three-dimensional structure. Chemical data: First mode: the 10 different emulsions with three analytical replicates each; Second mode: the 5 analytical variables of PV, 2-pentene-1-ol, 1-pentene-3-one, 2-hexenal, 2,4-heptadienal, and 2,6-nonadienal; 3rd mode: storage times day 1, 4, 8 and 11. Sensory data: First mode: the 6 different emulsions with three pseudo-replicates each; Second mode = fishy smell, rancid smell, milky smell, metallic smell, fishy taste, rancid taste, milky taste, metallic taste; 3rd mode: storage times day 1, 4, and 8.

gated by the parallel factor analysis (PARAFAC) using the algorithms in the N-way toolbox for Matlab® [23, 24]. PARAFAC is a three-dimensional extension of the principal component analysis (PCA), which is used in two-dimensional data analysis. As in PCA, the PARAFAC model aims at describing the experimental data by computing latent variables. These latent variables, here referred to as components, are computed in all three modes in a PARAFAC model. Since it was not possible to accomplish sensory evaluation on all emulsions, the analytical data for PV and volatiles were analyzed separately from the sensory data. Prior to modelling, data from this experiment were centered across the first mode in order to remove off-sets of the different emulsions, and scaled within the second mode to normalize the chemical and sensory variables, respectively [22, 23]. The optimum number of components was determined by segmented cross-validation [25]. The principle of this segmented cross-validation in three-way analysis highly resembles cross-validation in two-way analysis as (i) in segmented cross-validation an estimate of the models' predictability is achieved; (ii) the elements in each segment are left out one segment at a time; and (iii) the left-out segment is subsequently estimated from a model on the remaining segments. The number of calibration rounds thus equals the number of segments. The optimum model then contains the number of components giving the maximum explanation of the variance on these missing elements.

The sensory results were pre-processed prior to the PARAFAC analysis. By projecting the average values of the assessors at each sensory session as described previously [7, 26], three pseudo-replicates of the sensory data were constructed and subsequently used in the PARAFAC analysis. The sensory data were arranged similarly to the analytical data (Fig. 1), but with the sensory descriptors (fishy odor, rancid odor, metallic odor, milky odor, fishy taste, rancid taste, metallic taste, milky taste) in the second mode instead of the chemical data. The original data were also analyzed by one-way analysis of variance and Tukey's test using Minitab Statistical software (Addison-Wesley, Reading, MA, USA).

3 Results

The different cod liver oils used in the emulsions all contained 25–26.3 wt-% PUFA. In rapeseed oil the majority of the PUFA consisted of linoleic and linolenic acid (18.9 and 8.3%, respectively), while the PUFA of cod liver oils were mainly EPA and DHA (8.0–8.4% and 10.9–11.7%, respectively). In the tuna and rapeseed oil mixture (FR3), the content of EPA and DHA was higher compared to the mixtures of cod liver and rapeseed oil (FR1, FR1+A, FR2+A), and the total content of PUFA was 32.3 wt-%. Rapeseed oil contained both α-, β-, and γ-tocopherol homologues, while the fish oils contained only the α-tocopherol (Tab. 1). Furthermore, rapeseed oil contained significantly more total tocopherols than the fish oils. The mixtures of rapeseed and cod liver oils thus contained all three tocopherol homologues and more total tocopherols than the cod liver oils alone. The tuna oil mixture contained less α-tocopherol and less total tocopherol than the cod liver oil mixture. All oils had low initial values of PV and AV, and very low contents of FFA. The AV and content of FFA were not available for the mixture of tuna and rapeseed oil. However, since the PV was low and the

tuna oil had been deodorized very carefully on a laboratory scale, these values were expected also to be minimal.

The PV and five volatile secondary oxidation products derived from degradation of *n*-3 fatty acids [8, 27] were chosen as markers of oxidation during storage. The volatiles were *t*-2-hexenal, *t,t*-2,4-heptadienal, *t,c*-2,6-nonadienal, 1-penten-3-one, and *t*-2-penten-1-ol. These particular volatile products were selected because (i) their concentration changed significantly during storage in the most oxidized emulsions containing cod liver oil without antioxidants; (ii) they were present already at day 1 in the most oxidized samples, which were identified by the sensory panel to have fishy off-flavor at day 1; and (iii) these volatiles have previously been identified in the headspace of boiled fish [28], fish oil [4, 29, 30] and have been shown to correlate with the degree of oxidation in fish oil emulsions [3]. The sensory data are discussed separately below.

3.1 PARAFAC model on the chemical variables: PV and volatiles

3.1.1 Optimum number of components and validation of the model

The optimum number of components in the PARAFAC model was initially determined by investigating PARAFAC models containing two to five components. By comparing the core consistency and residual variance of these models, it was indicated that a model with two components would sufficiently describe the data (data not shown). Cross-validation verified that a two-component model was optimal in describing the data. With two components, the PARAFAC model described 92% of variation in the data.

3.1.2 Plot of loadings

In the plot of loadings in Fig. 2, the loadings of both the second mode variables (PV and volatiles) and the third mode variables (storage days) are presented together. PV and the volatiles were all located in the first quadrant. The storage days, moved from just above the origin for day 1 upwards along PC 2 for day 4 and day 8 and then further to the right for day 11. It was thus evident that the effect of storage time was present in the directions of both the first and second component. PV and volatiles were all located in the same direction as the storage days, indicating that the values of these variables increaed during storage. Taken together, a positive correlation between PV, volatiles and storage time was observed.

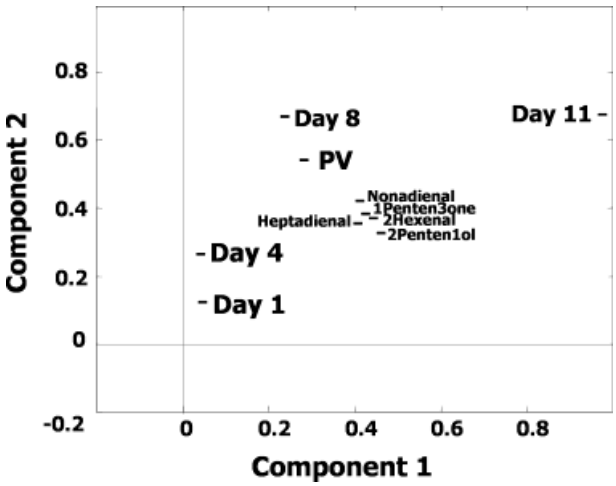

Fig. 2. Loadings plot in the PARAFAC model of the chemical variables (component 1 *vs.* component 2). The loadings in component 1 and component 2 of the PARAFAC model are illustrated for both the second mode (= PV and volatiles) and the third mode (= storage days).

3.1.3 Plot of scores

The localization of the variables in Fig. 3 shows that the emulsions were arranged in three groups, which were clearly separated. The first group was located in the third quadrant just below the origin. This group comprised the milk sample, the emulsion with rapeseed oil and all four emulsions containing the mixture of rapeseed and fish oil (FR1, FR3, FR1+A, FR2+A). The second group was located above the first group, and comprised the two emulsions containing cod liver oil with added antioxidants (F1+A, F2+A). The third group was located to the right in the first quadrant, and comprised the two emulsions containing cod liver oil without added antioxidants (F1, F2).

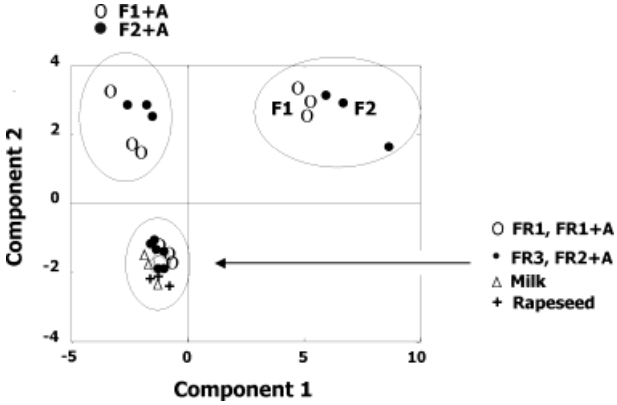

Fig. 3. Scores plot in the PARAFAC model of the chemical variables (component 1 *vs.* component 2). Sample names refer to Tab. 2. Each sample is represented by three analytical replicates.

When comparing the three groups of emulsions in the scores plot (Fig. 3) to the location of the chemical variables in the loadings plot (Fig. 2) it became evident that the distribution of the samples reflected their increasing degree of oxidation. Since the emulsions in the first group were located just below the origin, these emulsions were not influenced by storage time, as the PV and the concentration of the five volatiles did not increase during storage. The emulsions containing cod liver oil only were all influenced by storage time and had increasing levels of PV and volatiles. Among these emulsions, those containing cod liver oil without added antioxidants (F1, F2) were located furthest to the right, and were thus more strongly influenced by storage and apparently more oxidized than the other emulsions.

3.1.4 Comparison with original chemical oxidation data

Our observations from the PARAFAC model regarding the differences in oxidative deterioration were strongly supported by the original data. The developments in PV during storage are shown in Tab. 3. After 1 and 4 d of storage the PV of all the emulsions were generally at the same level. After 14 d of storage distribution of the PV of the emulsions was as reflected by the scores plot, and the three groups were significantly different. The emulsions containing cod liver oil without added antioxidants (F1, F2) oxidized the most. Among these, the emulsion containing cod liver oil from Biocentrum (F1) had a lower PV at days 1, 4, and 8, and a higher PV at days 11 and 14 than the emulsion containing cod liver oil from Maritex (F2). Emulsions containing cod liver oil with added antioxidants (F1+A, F2+A) were less oxidized, and in this case the Maritex emulsion (F2+A) had a higher PV than the Biocentrum emulsion (F1+A) at days 11 and 14. During the entire storage period the PV of the emulsions containing the rapeseed and fish oil mixtures were not significantly different from the PV of the milk sample or the neat rapeseed oil emulsion. The PV of these six emulsions remained stable until day 11, but increased slightly thereafter. As described, there was a significant effect of the added antioxidants on PV in the emulsions containing cod liver oil only (F1 *vs.* F1+A, F2 *vs.* F2+A), however, there was no difference between emulsions containing oil mixtures with and without added antioxidants (FR1 *vs.* FR1+A, FR2 *vs.* FR2+A). Also, there was no significant difference in PV between the emulsions containing rapeseed oil with either cod liver oil or tuna oil (FR1 *vs.* FR3).

The concentrations of all five volatiles increased similarly to PV, as exemplified by the progress of *t*-2-hexenal levels during storage (Fig. 4). The increase in concentrations of

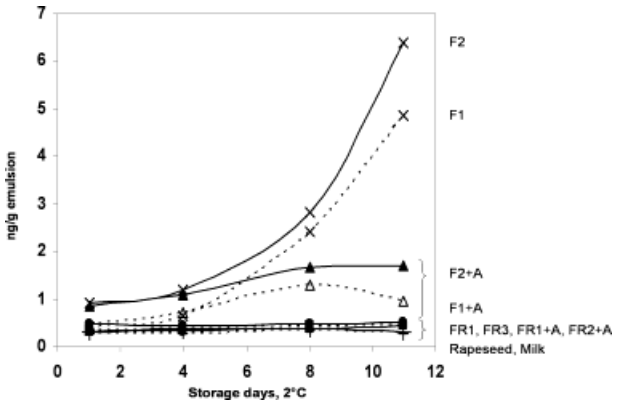

Fig. 4. Concentration of the *t*-2-hexenal in the milk emulsions during storage at 2 °C. Sample names refer to Tab. 2: - - × - - F1, — × — F2, - - △ - - F1+A, —▲— F2+A, - - ○ - - FR1, —●— FR3, - - □ - - FR1+A, —■— FR2+A, — + — Rapeseed, - - + - - Milk.

Tab. 3. Peroxide values [meq/kg] of milk emulsions during storage at 2 °C.

	Day 1[†]	Day 4	Day 8	Day 11	Day 14
Milk	$2.1^{a,x} \pm 0.0$	$0.5^{a,vx} \pm 0.4$	$0.6^{a,vx} \pm 0.3$	$0.4^{a,v} \pm 0.2$	$1.1^{a,v} \pm 1.1$
Rapeseed	$1.3^{a,vx} \pm 0.6$	$0.1^{a,v} \pm 0.2$	$0.2^{a,v} \pm 0.0$	$0.2^{a,v} \pm 0.0$	$0.6^{a,v} \pm 0.3$
F1	$0.8^{a,v} \pm 0.1$	$0.8^{a,vx} \pm 0.1$	$2.2^{b,y} \pm 0.0$	$4.1^{c,z} \pm 0.1$	$7.8^{d,y} \pm 0.0$
F2	$0.8^{a,v} \pm 0.2$	$1.2^{ab,x} \pm 0.1$	$2.2^{b,y} \pm 0.1$	$3.4^{c,z} \pm 0.2$	$5.4^{d,y} \pm 0.5$
F1+A	$0.6^{a,v} \pm 0.0$	$0.4^{a,vx} \pm 0.1$	$2.0^{bc,xy} \pm 0.7$	$1.3^{ab,xy} \pm 0.0$	$3.0^{c,x} \pm 0.0$
F2+A	$0.8^{a,v} \pm 0.1$	$0.8^{a,vx} \pm 0.5$	$1.8^{a,xy} \pm 0.7$	$1.7^{a,y} \pm 0.0$	$3.6^{b,x} \pm 0.2$
FR1	$0.4^{a,v} \pm 0.1$	$0.4^{a,vx} \pm 0.0$	$0.3^{a,v} \pm 0.0$	$0.8^{a,vxy} \pm 0.3$	$0.9^{a,v} \pm 0.3$
FR3	$0.5^{a,v} \pm 0.0$	$0.4^{a,vx} \pm 0.1$	$0.5^{a,vx} \pm 0.1$	$0.8^{a,vxy} \pm 0.4$	$0.9^{a,v} \pm 0.2$
FR1+A	$0.8^{ab,v} \pm 0.3$	$0.4^{a,vx} \pm 0.1$	$0.4^{a,v} \pm 0.0$	$0.7^{ab,vx} \pm 0.2$	$1.2^{b,v} \pm 0.3$
FR2+A	$0.7^{b,v} \pm 0.2$	$0.4^{ab,vx} \pm 0.1$	$0.4^{a,v} \pm 0.0$	$0.5^{ab,vx} \pm 0.0$	$1.1^{c,v} \pm 0.1$

[†] average of duplicate determination on the same sample. Emulsions followed by same letter are not significantly different in Tukey's test using 0.05-level of significance. Rows: a, b, c, d; Columns: v, x, y, z. Sample names refer to Tab. 2.

the five volatiles in the emulsions during storage also categorized the three groups of emulsions as identical to the groups of samples in the scores plot (Fig. 3). The levels of volatiles in all the emulsions after eleven days of storage are given in Tab. 4. It was evident that the three groups were significantly different. The first group comprised the most oxidized emulsions containing cod liver oil without added antioxidants (F1, F2). Within this group, the emulsion containing cod liver oil from Maritex A/S (F2) had significantly higher concentrations of four of the five volatiles than the emulsion containing cod liver oil deodorized at Biocentrum-DTU (F1). Secondly, the addition of antioxidants significantly reduced oxidation in the emulsions containing cod liver oil (F1 *vs.* F1+A, F2 *vs.* F2+A). In the third group, the emulsions with mixtures of fish oil and rapeseed oil (FR samples) were not significantly different from either the emulsion containing only rapeseed oil or the milk sample. The only exception was the emulsion containing the mixture of rapeseed and cod liver oil (FR1), which had a higher concentration of *t,t*-2,4-heptadienal than the milk sample at day 11. The emulsion containing rapeseed and tuna oil tended to have slightly higher levels of volatiles than the milk sample, although the differences were not statistically significant. As the emulsions containing rapeseed and fish oil were oxidatively stable up to 11 d of storage, there was no additional inhibitory effect of the added oil antioxidants in these emulsions with regard to development of any of the volatiles. Finally, there were no differences in the concentrations of any of the volatiles between emulsions containing the rapeseed oil mixtures with either tuna oil or cod liver oil (FR1 *vs.* FR3).

3.2 PARAFAC model of preprocessed sensory data

Analyses of the average data from sensory evaluations are often biased by the assessors' different use of the intensity scale. By using multivariate analysis on the sensory data, it was possible to eliminate this bias [25]. Hence, the interpretations and conclusions of the sensory results processed in this way are more reliable than by simply using the averages of the original data. After preprocessing three pseudo-replicates for each emulsion were used as input data. These data were arranged (Fig. 1) and modelled by PARAFAC similarly to the modelling of the chemical variables as described earlier.

3.2.1 Optimum number of components and validation of the model

Investigation of the core consistency and residual variance of models with two to five components suggested that a model with two components described the data in the best way. Cross-validation of the data array verified that a two-component model was optimum, and 57% of the variation in the data was described by this PARAFAC model (data not shown).

3.2.2 Plot of loadings

In the plot of the loadings (Fig. 5) obtained in the PARAFAC model, the taste and odor of milk were located in the third quadrant. All the undesirable off-flavors were

Tab. 4. Concentrations of volatiles [ng/g emulsion] in milk emulsions after 11 d of storage at 2 °C[†].

	t-2-hexenal	*t,t*-2,4-heptadienal	*c,t*-2,6-nonadienal	1-penten-3-one	*t*-2-penten-1-ol
Milk	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.6^{a} \pm 0.0$	$0.1^{a} \pm 0.1$
Rapeseed	$0.3^{a} \pm 0.0$	$0.4^{ab} \pm 0.4$	$0.2^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
F1	$4.9^{c} \pm 0.0$	$3.7^{c} \pm 0.1$	$3.1^{d} \pm 0.1$	$9.3^{c} \pm 0.5$	$6.0^{c} \pm 0.2$
F2	$6.4^{d} \pm 0.8$	$4.0^{c} \pm 0.2$	$4.2^{e} \pm 0.2$	$11.2^{d} \pm 1.0$	$8.3^{d} \pm 0.8$
F1+A	$0.9^{ab} \pm 0.1$	$0.7^{b} \pm 0.1$	$0.7^{b} \pm 0.1$	$2.4^{b} \pm 0.3$	$0.8^{ab} \pm 0.6$
F2+A	$1.7^{b} \pm 0.2$	$0.7^{b} \pm 0.1$	$1.2^{c} \pm 0.3$	$2.6^{b} \pm 0.5$	$1.7^{b} \pm 0.3$
FR1	$0.5^{a} \pm 0.0$	$0.7^{b} \pm 0.4$	$0.4^{ab} \pm 0.1$	$1.3^{a} \pm 0.0$	$0.1^{a} \pm 0.1$
FR3	$0.5^{a} \pm 0.0$	$0.5^{ab} \pm 0.0$	$0.4^{ab} \pm 0.1$	$1.5^{ab} \pm 0.1$	$0.2^{a} \pm 0.2$
FR1+A	$0.4^{a} \pm 0.0$	$0.4^{ab} \pm 0.2$	$0.3^{a} \pm 0.1$	$1.2^{a} \pm 0.2$	$0.2^{a} \pm 0.1$
FR2+A	$0.4^{a} \pm 0.1$	$0.3^{ab} \pm 0.1$	$0.2^{a} \pm 0.1$	$1.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$

[†] Average of triple determinations ± standard deviation of the same sample. Emulsions followed by same letter (columnwise) are not significantly different in Tukey's test using 0.05-level of significance. Sample names refer to Tab. 2.

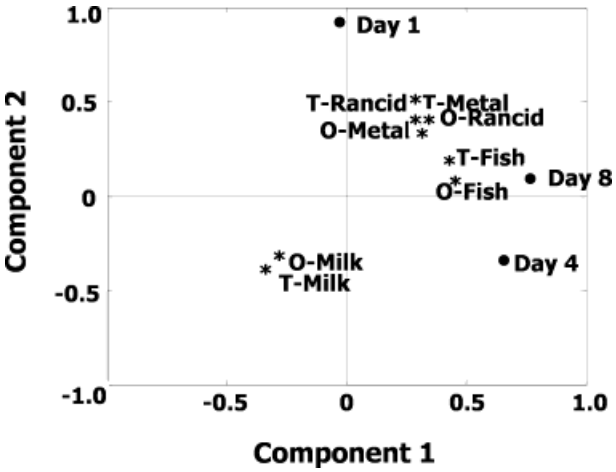

Fig. 5. Loadings plot in the PARAFAC model of the sensory data (component 1 *vs.* component 2). The loadings in component 1 and component 2 of the PARAFAC model are illustrated for both the second mode (= sensory descriptors) and the third mode (= storage days).

located in the first quadrant, with the taste and odor of metallic and rancid located above and slightly to the left of the taste and odor of fish. The storage variable day 1 was located near the second (vertical) axis, while days 4 and 8 were located to the right, below and above the first (horizontal) axis, respectively. This distribution indicated that storage time was mainly represented in the horizontal direction.

3.2.3 Plot of scores

In the plot of scores (Fig. 6) three groups of samples were evident. In the third quadrant rapeseed oil emulsion and the emulsions containing mixtures of rapeseed and cod liver oil with added antioxidants comprised the first group. Above this group the emulsion containing the rapeseed and tuna oil without added antioxidants was located. The third group was located in the first quadrant and consisted of the emulsions containing cod liver oil without added antioxidants. Together with the loadings plot, this distribution of emulsions showed that the emulsions containing cod liver oil only (F1, F2) had higher levels of the undesirable off-flavors than the remaining emulsions. It also showed that the odor and flavor of the emulsion containing rapeseed oil as well as those containing rapeseed and fish oil with added antioxidants were highly correlated to the odor and flavor of milk.

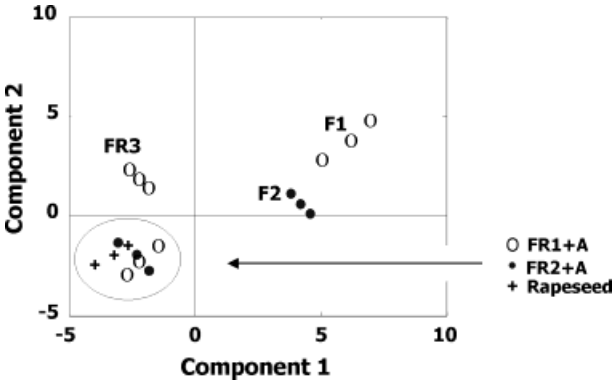

Fig. 6. Scores plot in the PARAFAC model of the sensory data (component 1 *vs.* component 2). Sample names refer to Tab. 2. Each sample is represented by three pseudo-replicates of the pre-processed sensory data.

3.2.4 Comparison with original sensory data

The average values of the sensory evaluations showed that the two emulsions containing cod liver oil without added antioxidants (F1, F2) generally had higher levels of off-flavor both fishy and metallic than the other emulsions (Tab. 5). In these emulsions, and not in the emulsions containing the rapeseed and fish oil mixture (FR3, FR1+A, FR2+A), an increase in the fishy off-flavor was observed during storage. An increase in the metallic off-flavor was not observed in any of the emulsions. After 4 and 8 d the difference between F1 and F2 and the other emulsions was significant for both the fishy odor and fishy taste, and at day 8 also significant for the metallic odou. For metallic odor at day 4 and metallic taste at day 4 and 8 only the emulsion containing cod liver oil without added antioxidants from Biocentrum (F1) was significantly different from the rapeseed oil emulsion and the three emulsions containing rapeseed and fish oil mixtures (FR3, FR1+A, FR2+A). At day 1 the emulsion containing rapeseed and tuna oil mixture (FR3) had significantly higher intensities of fishy and metallic taste than the emulsion containing rapeseed oil and the emulsions containing rapeseed and cod liver oil from Maritex with added antioxidants (FR2+A). The PARAFAC model reflected these differences because, in the loadings plot, the emulsions containing cod liver oil (F1, F2) were located furthest to the right, indicating a higher degree of oxidation than the other emulsions. Day 1 was located near the second axis and the off-flavors also had positive values in this vertical direction. In the scores plot the tuna emulsion (FR3) was separated from the rapeseed oil emulsion and mixture emulsions only in the PC2-direction, reflecting this elevated intensity of off-flavors at day 1. The emulsions containing cod liver and rapeseed oil mixtures with added antioxidants could not consistently be distinguished from the emulsion containing only rapeseed oil.

Tab. 5. Results of the sensory evaluation of fishy and metallic off-flavors of milk emulsions during storage at 2 °C.

	Fish odor			Fish taste		
	Day 1[†]	Day 4	Day 8	Day 1	Day 4	Day 8
Rapeseed	0.4 [a] ± 0.7	0.0 [a] ± 0.1	0.2 [a] ± 0.4	0.2 [a] ± 0.5	0.1 [a] ± 0.3	0.2 [a] ± 0.3
FR1+A	0.3 [a] ± 0.5	0.1 [a] ± 0.3	0.3 [a] ± 0.5	0.6 [ab] ± 0.8	0.5 [a] ± 0.7	0.3 [a] ± 0.5
FR2+A	0.1 [a] ± 0.3	0.3 [a] ± 0.5	0.2 [a] ± 0.3	0.2 [a] ± 0.2	0.4 [a] ± 0.5	0.5 [a] ± 0.5
FR3	0.7 [a] ± 0.9	0.2 [a] ± 0.4	0.3 [a] ± 0.5	1.0 [bc] ± 0.7	0.5 [a] ± 0.6	0.5 [a] ± 0.7
F1	0.4 [a] ± 0.4	1.3 [b] ± 1.1	1.9 [b] ± 1.7	1.0 [bc] ± 0.8	2.3 [b] ± 1.4	2.4 [b] ± 1.5
F2	0.4 [a] ± 0.4	1.5 [b] ± 1.4	1.4 [b] ± 1.1	1.3 [c] ± 1.0	2.1 [b] ± 1.2	2.4 [b] ± 1.4
	Metallic odor			**Metallic taste**		
	Day 1	Day 4	Day 8	Day 1	Day 4	Day 8
Rapeseed	0.1 [a] ± 0.2	0.1 [a] ± 0.2	0.1 [a] ± 0.2	0.3 [a] ± 0.8	0.1 [a] ± 0.2	0.3 [a] ± 0.6
FR1+A	0.2 [a] ± 0.4	0.1 [a] ± 0.4	0.0 [a] ± 0.1	0.5 [ab] ±0.8	0.3 [ab] ± 0.5	0.2 [a] ± 0.4
FR2+A	0.1 [a] ± 0.3	0.2 [a] ± 0.3	0.1 [a] ± 0.2	0.3 [a] ± 0.4	0.1 [a] ± 0.3	0.3 [a] ± 0.4
FR3	0.7 [ab] ± 0.9	0.1 [a] ± 0.2	0.1 [a] ± 0.2	1.4 [b] ± 1.3	0.1 [a] ± 0.3	0.2 [a] ± 0.4
F1	1.1 [b] ± 1.2	0.8 [b] ± 0.7	1.0 [b] ± 1.1	1.4 [b] ± 1.1	0.8 [b] ± 1.0	1.2 [b] ± 1.2
F2	0.5 [ab] ± 1.0	0.5 [ab] ± 0.6	0.7 [b] ± 1.0	0.8 [ab] ± 0.7	0.6 [ab] ± 0.6	1.0 [ab] ± 1.0

[†] average of all twelve assessors' determinations. The six emulsions were compared at each day (columnwise) in Tukey's test using 0.05-level of significance, and emulsions followed by same letter are not significantly different. Sample names refer to Tab. 2.

3.3 Droplet size determinations

The results of the droplet size determinations showed that the droplet sizes did not change from day 2 of storage to day 15 of storage, indicating that the emulsions were stable during the 2 wk of storage (data not shown). The average droplet size in all the emulsions containing 0.5% oil was 1.30 μm ± 0.03, while the droplet size in the milk sample was only 0.98 μm ± 0.00 μm. Thus, the results showed that the droplets in emulsions containing 0.5% added oil, irrespective of the oil type, were significantly larger than the droplets in the original milk sample.

4 Discussion

As described previously, this experiment served to investigate the effect on oxidative deterioration of fish oil enriched milk of (i) rapeseed oil addition; (ii) addition of antioxidants to fish oil; (iii) fish oil type; and finally (iv) oils obtained by two different deodorization procedures.

Based on PV and on the concentrations of *t*-2-hexenal, *t,t*-2,4-heptadienal, *t,c*-2,6-nonadienal, 1-pentene-3-one, and *t*-2-penten-1-ol, it was not possible to distinguish the original milk sample and the emulsion containing rapeseed oil from the emulsions containing rapeseed and fish oil with and without added antioxidants (FR1, FR3, FR1+A, FR2+A). Based on PV and volatiles these mixture emulsions were stable throughout the 11-d storage period, and they did not develop significant fishy off-flavors during 8 d of storage at 2 °C.

The results consistently showed that the emulsions containing only cod liver oil were more oxidized than the emulsions containing rapeseed oil and cod liver oil mixtures. The mixture emulsions contained only half the amount of cod liver oil, but the PV and volatile concentrations increased less than 50% of those of the corresponding emulsions based only on cod liver oil without antioxidants. Both the cod liver oil (F1) and the rapeseed and cod liver oil mixture produced at Biocentrum-DTU (FR1) had very low PV (< 0.1 meq/kg). Therefore, in this comparison of the emulsion stabilities, the effect of the initial oil PV could be ignored. Nevertheless, the oxidative deterioration of these emulsions (F1, FR1) was significantly different. The cod liver oil emulsion had a PV of 4.1 meq/kg after 11 d of storage, while the mixture emulsion had a PV of only 0.8 meq/kg, which corresponds to approximately 20% of the PV of 4.1 meq/kg. The emulsion containing only rapeseed oil had a PV of 0.2 meq/kg after 11 d of storage. A similar pattern was observed for the concentration of the five volatiles derived from *n*-3 PUFA degradation. Concentrations of the volatiles in the mixture emulsion were between 3 to 19% of the corre-

sponding concentrations in the fish oil emulsion after 11 d of storage, while the concentrations in the emulsion containing rapeseed oil were 3–11%. This indicates a protective effect of rapeseed oil that exceeds a simple dilution of the cod liver oil. Comparing the emulsions containing cod liver oil with added antioxidants (F1+A) and the emulsion containing rapeseed and cod liver oil also with added antioxidants (FR1+A), the effect of rapeseed oil addition was less pronounced. In these, the PVs and volatile concentrations of the mixture emulsion were 45–55% of the corresponding levels in the emulsion containing only cod liver oil, which could be expected from a simple dilution of the cod liver oil with rapeseed oil. Thus, as the emulsions containing rapeseed and cod liver oil without added antioxidants were already stable throughout the 11-day storage period, an improved stability caused by additional antioxidants could not be identified. The very similar droplet sizes of all the emulsions containing the 0.5% added oil indicates that this parameter did not differentially influence the oxidation between the emulsions.

The protective effect of rapeseed oil may partly be ascribed to its high content of tocopherols [8, 11]. As chain-breaking antioxidants, the tocopherols have been shown to retard oxidation in bulk fish oils. Rapeseed oil contained both the α-, β-, and the γ-tocopherols, while the fish oils contained only the α-tocopherol homologue. The activities of the individual α-, β-, γ-, and δ-tocopherols depend on the concentration of these homologues in the oil. Studies have shown that at lower concentrations (100 ppm) the order of antioxidant activity was $\alpha > \gamma > \delta$ in fish oil [31]. This corresponds to the ability of tocopherols to donate a hydrogen to the peroxyl radical [32]. At higher concentrations of tocopherols, prooxidant effects have been observed [31, 33, 34]. The antioxidant and prooxidant properties of tocopherols are influenced by oil type, oil quality and the chemical composition of the fish oil. Thus, no general distinction between antioxidant and prooxidant concentrations of tocopherols in bulk fish oils has been given in the literature. In emulsions, the factors influencing the overall oxidative stability as well as the effect of tocopherols are even more complex [35]. Emulsifiers, surface-active agents, partitioning of antioxidants into the different phases and also synergistic and antagonistic effects between antioxidants affect the stability of emulsions. The antioxidative effects of tocopherols in emulsions have been less intensively investigated, and again both prooxidative and antioxidative effects have been observed [36]. In a study on antioxidant activity of tocopherols in emulsions it was shown that α-tocopherol had prooxidant effects at 500, 750, and 1000 ppm in the early stage of oxidation in oil-in-water emulsions, while γ-tocopherol did not exert any prooxidant effects [12]. In the later stages of oxidation the γ-tocopherol was slightly more protective than α-tocopherol. On the other hand, one study has shown that the content of tocopherols in the oils tended to be less important for oxidative stability when these oils were emulsified [37].

Minor constituents of the rapeseed oil, such as phenolic substances (*e.g.* sinapic acid) have been shown to protect rapeseed oil against oxidation in bulk [38]. Traces of these components present in refined oils may also influence the stability of emulsions containing the rapeseed and fish oil mixture.

Milk contains ca. 1.8 g/L citric acid [39], and citric acid is generally recognized as a metal chelator. The chelating properties of citric acid have been proposed to protect tocopherols during oxidation [8]. Citric acid in milk could therefore enhance the antioxidant activity of tocopherols in both the emulsions containing cod liver oil and the emulsions containing rapeseed and tuna or cod liver oil in milk.

As mentioned above, addition of oil antioxidants (500 ppm propyl gallate and 2000 ppm citric acid ester) to the cod liver oil reduced oxidation in the emulsions containing the cod liver oils. PV after 11 and 14 d of storage and concentrations of volatiles were significantly reduced in the emulsions containing cod liver oil with added antioxidants (F1+A, F2+A) compared to the emulsions containing only cod liver oil (F1, F2). This protective effect of the added antioxidants was absent in emulsions containing the mixture of rapeseed and cod liver oil. As the emulsions were stable throughout 11 d of storage, it was not possible to identify any additional protective effect of the added oil antioxidants or any synergistic effects between the added antioxidants and the rapeseed oil on the progress of oxidation during storage in these emulsions. It seemed that the addition of rapeseed oil to both the tuna and cod liver oil sufficiently prevented these fish oils from oxidizing during storage. Again, the low initial PV of the oils imply that while comparing emulsion stabilities this parameter could be ignored.

Tuna oil had a significantly higher level of PUFA and also a lower level of tocopherols than cod liver oil, but the processing procedures of tuna oil were different from those of cod liver oils. Hence, the effects of different PUFA and tocopherol contents cannot be distinguished from the possible differences in oil composition, *e.g.* content of trace metals, caused by different processing procedures. In the sensory evaluation (Figs. 5 and 6, Tab. 5) the emulsion containing tuna and rapeseed oil (FR3) had a higher content of metallic and fishy off-flavors at day 1 than the

emulsions containing rapeseed and cod liver oil with added antioxidants (FR1+A, FR2+A). This could indicate that the sensory panel was very sensitive to minor changes during the early stage of oxidation. The difference between the emulsions was not present after 4 and 8 d of storage.

The pilot plant and industrial deodorizations at Biocentrum-DTU and Maritex A/S, respectively, resulted in slightly different PV and AV for the cod liver oils with and without added antioxidants (Tab. 1). The PV of the cod liver oils with and without added antioxidants from the industrial deodorization (0.66 and 0.50 meq/kg) were significantly higher than the PV of the corresponding oils from the pilot plant deodorization (0.2 and 0.08 meq/kg). Similar differences were observed for AV. These differences in initial oil PV and AV could have been caused by the deodorization procedure itself as well as the different handling procedures of the oils after deodorization, as the Maritex' oils were not frozen until after 2 d of storage. Therefore, the effect of the different deodorization procedures, including the possible resulting differences in oil composition, PV and AV, was confounded with the different handling procedures of the oils. The PV and sensory properties of emulsions containing only the Maritex cod liver oils were not significantly different from the emulsions containing the oils from Biocentrum, although the concentration of volatiles in the emulsion containing Maritex' cod liver oil without added antioxidants (F2) was slightly higher than the concentration of volatiles in the Biocentrum emulsion (F1) from day 1 and throughout the storage period. In accordance, the Maritex emulsion (F2) was located to the right of the Biocentrum emulsion (F1) in the scores plot (Fig. 3), indicating a slightly higher degree of oxidation of the Maritex emulsion. In the scores plot from the model of the sensory data, the Biocentrum emulsion was located above the Maritex emulsion (F2), which was caused by the higher level in metallic off-flavors especially at day 1 in the Biocentrum emulsion (F1). The emulsions containing cod liver oil with added antioxidants were located close to each other (F1+A, F2+A), and the differences in oil PV and AV were therefore not reflected in the oxidative deterioration of these emulsions during storage. Thus, the differences in PV and AV of the emulsions caused by the different deodorization and handling procedures seemed more pronounced in cod liver oil without added antioxidants than in cod liver oil with added antioxidants. Regarding the deodorization of the mixtures of rapeseed and cod liver oil, no difference in oil PV was observed (PV 0.06 and 0.07 meq/kg), whereas the oil mixture deodorized at Maritex had lower AV. Nevertheless, no differences in neither chemical nor sensory data of the emulsions were observed.

5 Conclusion

In conclusion, these experiments showed that rapeseed oil efficiently protected fish oil from oxidation during emulsification and storage of the fish oil enriched milk emulsions. It was possible to produce milk emulsions containing a fish oil and rapeseed oil mixture which were stable to oxidative deterioration during storage, and which had no undesirable fishy off-flavors. Secondly, the addition of antioxidants had a significantly protective effect on the emulsions containing only cod liver oil. However, in emulsions containing rapeseed and cod liver oil, the addition of the antioxidants citric acid ester and propyl gallate to the oil mixture did not improve the oxidative stability of the emulsions. The emulsions containing rapeseed oil with either cod liver oil or tuna oil were equally stable towards oxidation during storage. Hence, the combination of differences in fatty acid composition and levels of tocopherols and different processing procedures for the oils did not seem to affect the oxidative stability of the emulsions prepared with these oils. Fourthly, the oxidative deterioration of the emulsions during storage showed, that emulsions containing cod liver oil without added antioxidants was more sensitive to the differences in deodorization and handling procedures, including the minor differences in initial oil PV and AV, than cod liver oil with added antioxidants. More work is required to evaluate the exact influence of individual rapeseed oil components on the oxidative stability.

Finally these data provide an example of how the three-dimensional chemometric analysis can be used to analyze oxidation data from storage experiments. These methods give an easily interpretable overview of the results and they provide information about differences between the emulsions as well as about correlations between the analytical variables determined during storage.

Acknowledgements

This study was financed by a grant from the Danish Føtek III program, Maritex A/S, Denmark, and Arla Foods a.m.b.a. We thank *Bert Nielsen* and *Xu Xuebing*, Biocentrum-DTU, Denmark, for deodorization of the oils, and *Lis Berner* for skilful work in the laboratory. We also thank Central Soya for allowing us to utilize their pilot plant, and Maritex A/S, Århus, DK, and Roche Vitamins, Heanor, UK, for providing the oils.

References

[1] *L. A. Horrocks, Y. K. Yeo*: Health benefits of docosahexaenoic acid (DHA). Pharmacol. Res. **40** (1999) 211–225.

[2] *M. T. Arts, R. G. Ackman, B. J. Holub*: "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. **58** (2001) 122–137.

[3] *K. Hartvigsen, P. Lund, L. F. Hansen, G. Hølmer*: Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. J. Agric. Food Chem. **48** (2000) 4858–4867.

[4] *C. Karahadian, R. C. Lindsay*: Evaluation of compounds contributing to characterizing fishy flavors in fish oils. J. Am. Oil Chem. Soc. **66** (1989) 953–960.

[5] *D. J. McClements, E. A. Decker*: Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. **65** (2000) 1270–1282.

[6] *C. Jacobsen, K. Hartvigsen, P. Lund, M. K. Thomsen, L. H. Skibsted, J. Adler-Nissen, G. Hølmer, A. S. Meyer*: Oxidation in fish oil-enriched mayonnaise 3: Assessment of the influence of the emulsion structure on oxidation by discriminant partial least squares regression analysis. Eur. Food Res. Technol. **211** (2000) 86–98.

[7] *M. B. Let, C. Jacobsen, A. S. Meyer*: Oxidative flavor deterioration of fish oil enriched milk. Eur. J. Lipid Sci. Technol. **105** (2003) 518–528.

[8] *E. N. Frankel*: Lipid Oxidation. The Oily Press, Dundee, Scotland, UK, 1998.

[9] *C. Jacobsen, K. Schwarz, H. Stöckmann, A. S. Meyer, J. Adler-Nissen*: Partitioning of selected antioxidants in mayonnaise. J. Agric. Food Chem. **47** (1999) 3601–3610.

[10] *K. Schwarz, S.-W. Huang, J. B. German, B. Tiersch, J. Hartmann, E. N. Frankel*: Activities of antioxidants are affected by collodial properties of oil-in-water and water-in-oil emulsions and bulk oils. J. Agric. Food Chem. **48** (2000) 4874–4882.

[11] *S.-W. Huang, E. N. Frankel, J. B. German*: Antioxidant activity of α- and γ-tocopherols in bulk oils and in oil-in-water emulsions. J. Agric. Food Chem. **42** (1994) 2108–2114.

[12] *F. B. Padley, I. P. Freeman, R. G. Polman, G. J. van Lookeren*: European patent 304 115 (1989).

[13] Product data sheet: Maritex 43-02, marine oil and vegetable oil blend. Maritex A/S, Århus, Denmark.

[14] AOCS Official method Ce 1b-89. Fatty acid composition by GLC. Marine oils. AOCS Champaign. IL. 1992.

[15] AOCS Official method Ce 8-89. Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. AOCS Champaign, IL, 1992.

[16] AOCS Official method Cd 18-90. p-Anisidine value. AOCS Champaign. IL. 1994.

[17] AOCS Official method Ca 5a-40. Free fatty acids. AOCS Champaign. IL. 1998.

[18] *E. G. Bligh, W. J. Dyer*: A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37** (1959) 900–917.

[19] *S. J. Iverson, S. L. C. Lang, M. H. Cooper*: Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids **36** (2001) 1283–1287.

[20] International IDF Standards. International Dairy Federation, IDF – Square Vergote 41, Brussels, Belgium. 1991, section 74A: 1991.

[21] *A. Rawle*: 1996. The basic principles of particle size analysis. Malvern Instruments Ltd., Worcestershire, UK.

[22] *H. A. L. Kiers*: Towards a standardized notation and terminology in multiway analysis. J. Chemometrics **14** (2000) 105–122.

[23] *R. Bro*: PARAFAC: Tutorial and applications. Chemom. Intell. Lab. Syst. **38** (1997) 149–171.

[24] *C. A. Anderson, R. Bro*: The N-way Toolbox for MATLAB. Chemom. Intell. Lab. Syst. **52** (2000) 1–4.

[25] *D. J. Louwerse, A. K. Smilde, H. A. L. Kiers*: Cross-validation of multiway component models. J. Chemometrics **13** (1999) 491–510.

[26] *C. Jacobsen, K. Hartvigsen, P. Lund, A. S. Meyer, J. Adler-Nissen, J. Holstborg, G. Hølmer*: Oxidation in fish oil-enriched mayonnaise 1: Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis. Eur. Food Res. Technol. **210** (2000) 13–30.

[27] *W. Grosch*: Reactions of Hydroperoxides – Products of low molecular weight. In: Autoxidation of unsaturated lipids. Eds. *H. W.-S. Chan*, Academic Press, London (UK) 1987, pp. 95–139.

[28] *C. Milo, W. Grosch*: Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. J. Agric. Food Chem. **44** (1996) 2366–2371.

[29] *N. Macfarlane*: The FAST Index – a fishy scale. Inform **12** (2001) 244–249.

[30] *E. Kulås, E. Olsen, R. G. Ackman*: Effect of α-, γ-, and δ-tocopherol on the distribution of volatile secondary oxidation products in fish oil. Eur. J. Lipid Sci. Technol. **104** (2002) 520–529.

[31] *E. Kulås, R. G. Ackman*: Properties of α-, β- and δ-tocopherol in purified fish oil triacylglycerols. J. Am. Oil. Chem. Soc. **78** (2001) 360–367.

[32] *G. W. Burton, K. U. Ingold*: Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J. Am. Chem. Soc. **103** (1981) 6472–6477.

[33] *C. Karahadian, R. C. Lindsay*: Action of tocopherol-type compounds in directing reactions forming flavor compounds in autoxidizing fish oils. J. Am. Oil. Chem. Soc. **66** (1989) 1302–1308.

[34] *R. J. Hamilton, C. Kalu, G. P. McNeill, F. B. Padley, and J. H. Pierce*: Effects of tocopherols, ascorbyl palmitate, and lecithin on autoxidation of fish oil. J. Am. Oil. Chem. Soc. **75** (1998) 813–822.

[35] *C. Jacobsen, K. Hartvigsen, P. Lund, J. Adler-Nissen, G. Hølmer, A. S. Meyer*: Oxidation of fish oil enriched mayonnaise: 2. Assessment of the efficacy of different tocopherol antioxidant systems by discriminant partial least squares regression analysis. Eur. Food Res. Technol. **210** (2000) 242–257.

[36] *C. Jacobsen, K. Hartvigsen, P. Lund, M. K. Thomsen, L. H. Skibsted, G. Hølmer, J. Adler-Nissen, A. S. Meyer*: Oxidation in fish oil-enriched mayonnaise: 4. Effect of tocopherol concentration on oxidative deterioration. Eur. Food Res. Technol. **212** (2001) 308–318.

[37] *E. N. Frankel, T. Satué-Gracia, A. S. Meyer, J. B. German*: Oxidative stability if fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. J. Agric. Food Chem. **50** (2002) 2094–2099.

[38] *A. Koski, S. Pekkarinen, A. Hopia, K. Wähälä, M. Heinonen*: Processing of rapeseed oil: Effects of sinapic acid derivative content and oxidative stability. Eur Food Res. Technol. **217** (2003) 110–114.

[39] *H.-D. Belitz, W. Grosch*: Food chemistry. Springer-Verlag, Berlin, Heidelberg, Germany, 1999.

[Received: September 22, 2003; accepted: November 25, 2003]

