Eur Food Res Technol (1999) 210:13–30

ORIGINAL PAPER

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Oxidation in fish-oil-enriched mayonnaise

1. Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis

Received: 16 November 1998

Abstract A number of different analytical techniques (HPLC, GC-MS, sensory analysis, laser diffraction droplet size determination, confocal laser scanning microscopy and rheological measurements) were employed to elucidate both chemical, sensory, structural and rheological aspects of the oxidation process in mayonnaise containing 16% fish oil. The primary focus of the study was on the antioxidative effect of two different types of commercial propyl gallate mixtures: an oil-soluble and a water-soluble preparation. The effect of adding extra emulsifier (Panodan TR), used to manipulate the physical structure of the fish-oil-enriched mayonnaise and in turn affect the antioxidative activity of the propyl gallate mixtures, was also investigated. Mayonnaise with fish oil did not oxidise faster than mayonnaise without fish oil when judged from the chemical parameters tested. However, the fish-oil-enriched mayonnaises developed unpleasant off-odours and off-flavours much faster than the mayonnaise without fish oil. Addition of the two different propyl gallate mixtures not only influenced negatively the sensory qualities but also affected the structure and the rheological properties of the mayonnaise. Propyl gallate thus, in particular, promoted the development of fishy and rancid off-flavours during the storage of mayonnaise with fish oil, and this effect was especially pronounced for the water-soluble propyl gallate mixture. Four volatile oxidation compounds, namely 3-furaldehyde, 2,4-heptadienal, 2,4-decadienal and ethyl benzene, appeared to correlate to the fishy and rancid off-flavours that developed in mayonnaises with propyl gallate. Addition of propyl gallate also resulted in increased peroxide values, and a less viscous mayonnaise with bigger droplets. The data thus demonstrated that the propyl gallate mixtures employed did not protect mayonnaise with fish oil against flavour deterioration due to oxidation during storage. In addition, the data showed that several structural and rheological parameters were affected by the addition of propyl gallate.

Key words Mayonnaise · Propyl gallate · Volatiles · Oxidation · Sensory analysis

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Introduction

Evidence from several investigations suggests that n-3 polyunsaturated fatty acids, especially C20:5 n-3 (eicosapentaenoic acid; EPA) and C22:6 n-3 (docosahexaenoic acid; DHA) are beneficial to the human body. The physiological benefits have mainly been associated with a reduced risk of cardiovascular diseases [1, 2] and with the visual and neural development of the child [3–5]. These polyunsaturated fatty acids are abundant in fish and sea mammals. As the intake of fish and fish products in the western world is relatively low, efforts have been made to increase the consumption of marine fatty acids by incorporating fish oil into different food products such as bread, yoghurt drinks, salad dressing and mayonnaise [2, 6–9]. However, n-3 fatty acids are highly susceptible to oxidation due to their high degree of unsaturation. Efficient antioxidative measures are therefore required to protect fish-oil-enriched foods from oxidative deterioration.

The current knowledge on oxidation mechanisms and antioxidant efficacy in emulsions is largely based on studies in model systems. However, even in such relatively well-defined model emulsions, the prediction of antioxidative activity is difficult as the antioxidative efficiency depends on a number of chemical and physical factors [10–12]. Furthermore, the antioxidant activity appears to be affected by the physical structure of the emulsion system. Data recently reported in the literature have thus highlighted the following complex phenomena:

1. In oil-in-water (o/w) emulsions the efficiency of antioxidants is inversely correlated to their polarity. Thus, the less polar antioxidants such as ascorbyl palmitate and alpha-tocopherol are superior in antioxidant activity to their more polar counterparts, ascorbic acid and trolox, in o/w emulsions containing 10% corn oil [10, 12]. This "polar paradox" was suggested to be due to differences in the affinity of the antioxidants for the different phases in the system including the interaction of the antioxidants with oxygen at the water/air surface [10].
2. In o/w emulsions polar antioxidants may partially partition into the oil/water interface [13]. This partitioning depends on the type of emulsifier and the pH of the system [13], but the degree of partitioning is not immediately predictable only from chemical principles.
3. The localisation of antioxidants at the interface may change the overall antioxidant efficacy, but the degree of influence on antioxidant activity depends on the antioxidant [12]. From this it can be inferred that the apparent activity of antioxidants in emulsions may also depend on the droplet size as this variable governs the interfacial area in emulsions.

Taken together, the available data thus suggest that in heterophasic emulsion systems, the activity of antioxidants may depend, at least in part, on their effective concentrations in the different phases of the emulsion. However, the relationship between partitioning of antioxidants and their efficacy in food emulsion systems has not been clarified to a degree that enables prediction of the efficacy of different antioxidants in such systems. Furthermore, the antioxidant activity may also depend on the structural composition of the particular system considered. At present, however, the significance of the physical structure of food emulsions on their antioxidant protection is not clearly understood.

The overall purpose of our current work is to get a better understanding of the oxidation mechanisms in real food emulsions containing fish oil. Mayonnaise has been chosen as our model for o/w food emulsions [14]. Furthermore, our aim is to elucidate both chemical, sensory, structural and rheological aspects of oxidation in real food emulsions. The techniques employed in our analyses include sensory assessment, determination of lipid hydroperoxides by HPLC, measurements of secondary oxidation products by dynamic headspace GC-MS, determination of droplet size distribution, evaluation of physical properties by confocal laser scanning microscopy (CLSM) and droplet size distribution by laser diffraction, in addition to the evaluation of rheological properties. This paper is the first in a series reporting our results from these studies.

Propyl gallate is widely used as an antioxidant in the food industry [15]. It has poor fat solubility, but is partially soluble in water [15]. Huang et al. [16] studied the partitioning of pure propyl gallate in a 10% corn oil o/w emulsion. In 10% oil/water mixtures without emulsifier more than 90% propyl gallate was localised in the water phase after equilibration. However, when the same system was emulsified with 1% Tween 20, only 17% propyl gallate equilibrated in the water phase [16]. Based on these findings, the low antioxidant activity of propyl gallate previously observed in emulsions was proposed to be due to the partitioning of the antioxidant into the oil/water interface and into Tween 20 micelles [16].

In the present investigation, the objective was to study the antioxidative effects of two different types of commercial propyl gallate mixtures in fish-oil-enriched mayonnaise. In these mixtures propyl gallate had been made either oil soluble (Grindox 370) or water dispersible (Grindox 413) by the incorporation of different carriers in the preparations [17]. A secondary aim therefore was to investigate whether different carriers affected the efficacy of the propyl gallate. Furthermore, in an attempt to manipulate the physical structure of the mayonnaise and in turn affect the antioxidant activity, the effect of adding extra emulsifier (Panodan TR) [17] to the mayonnaise was studied.

Materials and methods

Materials

Refined rapeseed oil was obtained from Aarhus Olie (Aarhus, Denmark). The composition of unsaturated fatty acids was 18:1, 54.1%, 18:2, 25.2%, 18:3, 8.9%, 20:1, 1.3%. The peroxide value was 0.7 mEq/kg, the anisidine value 3.3, the tocopherol content: alpha, 210 μg/g; gamma, 471 μg/g. Raw fish oil was obtained from Esbjerg Fiskeindustri (Esbjerg, Denmark). The fish oil was refined and deodorised at the pilot plant of the Department of Biotechnology (Technical University of Denmark, Lyngby). The composition of unsaturated fatty acids was 16:1, 15.6%, 18:1, 7.6%, 18:2, 3.6%, 18:3, 1.5%, 18:4, 4.1%, 20:1, 6.9%, 20:5, 8.4%, 22:1, 8.7%, 22:6, 8.4%. The peroxide value was <0.3 mEq/kg, the anisidine value 3.8, the tocopherol content: alpha, 88 μg/g; gamma, 10 μg/g. Egg yolk with 3% salt (sodium chloride) was obtained from Sanovo Foods (Odense, Denmark). Tarragon vinegar (7%) was purchased from Lagerberg (Hamburg, Germany). The lemon juice was obtained from Borden (Gent, Belgium). Potassium sorbate was purchased from Merck (Darmstadt, Germany). The propyl gallate systems, Grindox 413 (20% propyl gallate and 80% acetic acid esters of mono- and diglycerides plus diacetyl tartaric acid esters of mono- and diglycerides) and Grindox 370 (20% propyl gallate and 80% propylene glycol), as well as Grindsted FF DC stabiliser (guar gum and sodium alginate) and Panodan TR emulsifier (containing diacetyl tartaric acid ester of mono- and diglycerides of fatty acids) were donated by Danisco Ingredients (Brabrand, Denmark).

Production of mayonnaises

Mayonnaise batches of 40 kg were produced in a continuous process on a Schröder Combinator pilot plant (Schröder, Lübeck, Germany). Each batch contained by weight 16% fish oil, 64% rapeseed oil, 9.25% water, 1.2% lemon juice, 4% vinegar, 0.3% salt (sodium chloride), 1.0% sugar, 0.1% potassium sorbate, 4% egg yolk and 0.15% Grindsted FF DC. In mayonnaises with propyl gallate, the antioxidant was added either to the oil phase (Grindox 370) or to the water phase (Grindox 413) before mayonnaise production. In mayonnaises with extra emulsifier, Panodan TR was mixed with vinegar and lemon juice before addition to the other ingredients.

Determination of lipid hydroperoxides by HPLC

Sample preparation. Mayonnaise was frozen at –80 °C until analysis, thawed and centrifuged at 2500 *g* for 10 min at 4 °C. The resulting upper phase was a clear oil phase, of which 100 mg was dissolved in 4.0 ml cold, degassed hexane containing 0.002% BHT. The headspace was flushed with nitrogen, and the samples were kept at –80 °C until further analysis by HPLC.

High performance liquid chromatography. Peroxide values (PV) were determined by HPLC by a method adapted from Akasaka et al. [18]. The mobile phase (4% 1-butanol in glass-distilled hexane) was delivered by a multi-HPLC pump with a flow rate of 0.6 ml/min (model 600E; Waters, Milford, Mass.), and passed through an autosampler (Waters 717). Subsequently, the eluent was mixed in a stainless-steel T-connector with a diphenyl-1-pyrenylphosphine (DPPP; Dojindo Laboratories, Kumamoto, Japan) reagent solution (3.8 mg DPPP in 500 ml degassed 0.05% BHT in methanol) delivered by a HPLC pump (Bioclean Model 350; Waters) with a flow rate of 0.3 ml/min. The mixture reacted in a stainless-steel reaction coil (20 m × 0.5 mm i.d.) at 80 °C, and was then cooled to 30 °C by passing it through another stainless-steel coil (1 m × 0.5 mm i.d.) before reaching a fluorescence detector (model LC 9070; Varian, Walnut Creek, Calif.). The detection was performed by monitoring the fluorescence intensity of the DPPP oxides, at 380 nm, with excitation at 352 nm, formed by the reaction between lipid hydroperoxides and DPPP. The HPLC system was controlled by Millennium 2010 (Waters) software. Lipid hydroperoxides were quantified by comparison to an external trilinolein mono-hydroperoxide standard. Reported PV are means of triplicate determinations and are reported as μmmol lipid hydroperoxide/g oil.

Determination of secondary volatile oxidation products by dynamic headspace GC-MS

Collection of volatiles by dynamic headspace. Mayonnaise (4 g) was weighed into a pear-shaped glass flask and headspace volatiles were collected by a dynamic headspace sampling method modified after that of Olafsdottir et al. [19]. *n*-Undecane was added as an internal standard. The samples were purged in triplicate with nitrogen (Hydro Plus 5,5) for 30 min at 60 °C at a flow rate of 150 ml/min, and the volatile compounds were trapped by a Tenax tube (225 mg TENAX GR; Chrompack, Middelburg, The Netherlands). Water was subsequently removed by blowing nitrogen through the Tenax tube for 20 min at 50 ml/min.

Trapped volatiles were desorbed by heating the tube for 2 min at 200 °C in an automatic thermal desorber (ATD 400; Perkin Elmer, Norwalk, Conn.) by passing He (Hydro Plus 5,5) at 60 ml/min through the tube. The volatiles were then trapped in a cold trap (–30 °C), which was rapidly heated to 250 °C to inject the volatiles onto the gas capillary column.

Gas chromatography. The volatiles were separated in a gas chromatograph (Fisons Instruments, Manchester, UK) on a 30-m DB1701 fused silica capillary column (0.25 mm i.d., 0.25-μm film thickness; J&W Scientific, Folsom, Calif.). The column temperature was held at 35 °C for 3 min, then increased at a rate of 3 °C/min to 120 °C, then by 7 °C/min to 160 °C, and finally by 15 °C/min to 200 °C, which was held for 4 min.

Mass spectrometry. The mass spectrometer was directly connected to the gas chromatograph (MD 800; Fisons Instruments) and operated in electron ionisation mode with continuous scanning of masses from 30 to 350, with a scan time of 0.55 s and an interscan time of 0.05 s. Electron ionisation was at 70 eV and the ion-source temperature was 200 °C. Quantification was based on a characteristic ion for each component. Data were acquired and processed using the Fisons Masslab data system and were normalised against the internal standard.

Sensory analysis

Fifteen assessors were selected for the panel. The assessors were trained during 15 sessions on profiling mayonnaise with fish oil prior to the evaluation of our mayonnaise samples. Before each session, the panel was calibrated by presenting a freshly prepared reference mayonnaise to each assessor. The reference sample did not contain fish oil or antioxidant. For each profiling of mayonnaise the following attributes were evaluated: (1) aroma (vinegary/acidic, fishy/train oil, rancid, oily, dusty, miscellaneous); (2) texture (appearance and mouthfeel); (3) flavour (vinegary/acidic, fishy/train oil, rancid, oily, dusty/dry, synthetic, metallic, nutty, egg yolk and miscellaneous). Mayonnaise was presented to the panelists in small, transparent, disposable plastic cups with a white plastic lid. The panelists evaluated seven different samples per session. Distilled water, heated to 50 °C, as well as crisp bread, were provided for oral rinsing at the beginning of sessions and between mayonnaise samples. The order of presentation of samples to the panelists was balanced to minimise possible carry-over effects between samples. The panel rated all attributes for each sample on separate 9-cm unstructured scales using a PSION mini computer (PSION, UK).

Rheological measurements

Stress sweep. Stress sweep analyses were carried out on a Stresstech controlled stress rheometer (Reologica, Lund, Sweden) mounted with a 40-mm upper polycarbonate plate and a 50-mm lower stainless-steel plate operating at 2-mm gaps. The following parameter set-up was used: the stress was increased at oscillations of 1 Hz from 0.1500–200.0 Pa in 30 logarithmic steps, delay time constant 11 s, integration time 11 periods, continuous shear on, initial equilibrium time 300 s. G^*_{lin} was defined as the value of the complex modulus (G*) in the linear viscoelastic region. The critical stress (scrit) was defined as the shear stress applied at $0.9 \times G^*_{lin}$.

Flow curves. Flow curves were measured on a Bohlin VOR controlled-rate rheometer (Bohlin Instruments, Cirencester, UK) at 5 °C mounted with 30-mm stainless-steel upper and lower plates operating at 2 mm gaps. The shear rate was 0.919–58.1 1/s up/down in 13 logarithmic steps. The parameter set-up was torsion bar 0.33 g/cm, delay time constant 10 s, integration time 10 s, continuous shear on, initial equilibrium time 300 s. The viscosities at 1.57 1/s [V(1.57)] and 39.3 1/s [V(39.3)] were used for further calculations.

Yield stress. The yield stress was measured at 5 °C on a Haake VT550 viscometer (Haake, Karlsruhe, Germany) mounted with a 20 mm × 20 mm four-blade vane, using the following parameter set-up: CD-test, shear rate 0.212 1/s, 120 s. The yield stress (S0) was defined as the peak stress value [20].

Confocal laser scanning microscopy

Mayonnaises were dyed with Nile Red (Sigma-Aldrich, Steinheim, Germany) approximately 24 h before microscopy. Mayonnaises were stored at 5 °C until examined by microscopy, which was carried out on a confocal laser scanning microscope from Leica (Heerbrugg, Switzerland). For each mayonnaise, four scans were made at three different positions. The clearest and sharpest scan for each position was selected for further analysis. Subsequently, droplets were counted manually. Different droplet mean diameters were calculated using the method of Rawle [21]:

volume mean diameter, $D[4,3] = \Sigma d^4/\Sigma d^3$;
surface mean diameter, $D[3,2] = \Sigma d^3/\Sigma d^2$;
number volume mean, $D[3,0] = \sqrt[3]{(\Sigma d^3/n)}$;
number surface mean, $D[2,0] = \sqrt{\Sigma(d^2/n)}$;

where d was the diameter of a droplet (μm) and n was the total number of droplets. In order to discriminate between diameters obtained from the laser diffraction measurements (see below) and from microscopy, the microscopy data corresponding to D [4,3], D[3,2], D[3,0] and D[2,0] will be termed C(4,3), C(3,2), C(3,0) and C(2,0) respectively, whereas the conventional abbreviations will be used for the laser diffraction data [21]. The diameter of a sphere of a volume equivalent to a given oil droplet with an arbitrary shape is D[4,3]. Likewise the diameter of a sphere of a surface area equivalent to this droplet is D[3,2]. D[3,0] and D[2,0] are also related to volume and surface areas of the average particle, respectively. However, as these values are calculated on the basis of the number of particles, it means that small droplets count as much as big droplets, i.e. these diameters do not reflect where the largest mass of the droplet lies.

Particle size measurements by laser diffraction analysis

The samples were measured using a Malvern Mastersizer S (Malvern Instruments, Malvern, UK). Particle sizes were reported as: D[4,3], D[3,2] and the 10%, 50% and 90% percentiles (D[v,0.1], D[v,0.5] and D[v,0.9]) respectively of the droplet distribution. The parameter set-up was ALPHA configuration, 3000 sweeps; and the presentation was 3OHD, volume distribution, lens 300 RF, small sample cell. Before measurement the emulsions were diluted in de-gassed distilled water (obscuration 10–30%).

Experimental design

As previously described, two different propyl gallate systems were used: a water-dispersible system (Grindox 413) and an oil-soluble system (Grindox 370). In addition, the effect of adding extra emulsifier (Panodan TR) to the mayonnaise was tested. Table 1 shows the design of the experiment and the doses of antioxidant and emulsifier used. Mayonnaises were stored at 5 °C for 14 weeks. Samples were taken for sensory analysis and PV measurements after 0, 5, 8, 11 and 14 weeks of storage and for GC-MS measurements after 0, 5, 8 and 14 weeks of storage. Rheological measurements were made after 1 week of storage, and particle size measurements as well as CLSM were carried out after 4 weeks of storage. Samples for PV and GC-MS measurements were kept at –80 °C until analysis, while all other analyses were made directly after sampling.

Table 1 Experimental design. *R* Rapeseed oil mayonnaise without fish oil, *F* fish oil mayonnaise with 20% fish oil, *E* emulsifier addition, *1* no antioxidant, *2* propyl gallate in the oil phase, *3* propyl gallate in the water phase

Code	Antioxidant	Phase[a]	Emulsifier
R1	–		–
FE1	–		2000 μg/g Panodan TR
FE2	200 μg/g Grindox 370	Oil	2000 μg/g Panodan TR
FE3	200 μg/g Grindox 413	Water	2000 μg/g Panodan TR
F1	–		–
F2	200 μg/g Grindox 370	Oil	–
F3	200 μg/g Grindox 413	Water	–

[a] To which antioxidant system was added

Data analysis

To correlate the different analytical data, discriminant partial least squares regression (DPLSR) was employed [22]. The software programme Unscrambler version 6.11b (CAMO, Oslo) was used as an aid for this analysis.

ANOVA PLSR on sensory data. Prior to the main data analysis, a preliminary ANOVA partial least squares regression (PLSR) analysis was made on the sensory data to determine differences in the sensory score levels of the assessors [22]. The differences were subsequently projected away using the ANOVA PLSR as a projection tool. The means of the residuals obtained after the optimal number of principal components were used for the subsequent discriminant PLSR (DPLSR) analysis. Three different mean residual values were calculated for each sample. The two initial mean values were obtained by separating the data randomly into two groups. The means were calculated for each group and were denoted replicate 1 and 2, respectively. The third mean value was calculated on all data for each sample.

DPLSR analysis. Subsequently, two different DPLSR analyses were performed. In both analyses sample codes and replicates were used as design variables. In the first analysis sensory data (i.e. residuals obtained from the preliminary PLSR), data from hydroperoxide determination, rheology, particle size measurements and CLSM were used as x-variables and design variables were used as y-variables. In the second DPLSR analysis, GC-MS and sensory data (i.e. residuals from the preliminary PLSR) were used as x-variables and design variables as y-variables. Full cross-validation was used in both cases [23]. Both x and y values were standardised by 1/SD. Replicates as well as means for each sample were used as sample codes in both analyses.

Results

Sensory analysis

Sensory scores for fishy aroma and fishy flavour during storage were almost constant in mayonnaise without fish oil, and were all between 0.5 and 1 (Table 2). The values for fishy aroma and fishy flavour obtained for mayonnaise without fish oil were therefore regarded as background values. In the mayonnaises with fish oil the intensity of the fishy aroma and flavour increased gradually during storage and sensory scores were between 0.2 and 1.6 for fishy aroma, and between 0.7 and 4.7 for fishy flavour (Table 2). Surprisingly, mayonnaise without fish oil developed more intense rancid off-odours and off-flavours than did mayonnaises with fish oil. There was a tendency for mayonnaises with propyl gallate (F2, F3, FE2 and FE3) to have higher scores for fishy and rancid off-flavour than the mayonnaises without propyl gallate (FE1 and F1; Table 2). However, as

Table 2 Sensory scores for fishy and rancid aromas and flavours for mayonnaises during storage (sensory scale 0–9; mean ± SD). For abbreviations, see Table 1

Code	Fishy aroma					Rancid aroma				
	0 Weeks	5 Weeks	8 Weeks	11 Weeks	14 Weeks	0 Weeks	5 Weeks	8 Weeks	11 Weeks	14 Weeks
R1	0.52 ± 1.27	0.69 ± 0.78	0.79 ± 1.16	0.59 ± 1.18	0.70 ± 1.07	0.48 ± 0.82	0.93 ± 0.97	1.06 ± 1.44	1.10 ± 1.30	0.66 ± 0.91
FE1	0.43 ± 0.81	0.81 ± 1.04	0.98 ± 0.96	1.33 ± 1.57	1.13 ± 1.86	0.29 ± 0.84	0.73 ± 1.25	0.64 ± 1.60	0.75 ± 0.96	0.64 ± 0.73
FE2	0.60 ± 1.30	0.39 ± 0.86	1.10 ± 1.36	1.58 ± 1.45	1.64 ± 1.43	0.62 ± 1.10	0.76 ± 1.02	0.71 ± 1.54	0.57 ± 0.92	0.35 ± 0.56
FE3	0.31 ± 0.86	0.73 ± 1.40	0.70 ± 0.89	1.05 ± 1.52	0.98 ± 1.29	0.87 ± 1.36	0.84 ± 1.41	0.71 ± 0.76	0.59 ± 0.76	0.68 ± 0.81
F1	0.46 ± 1.26	0.54 ± 0.85	0.52 ± 0.83	1.52 ± 1.91	0.40 ± 0.62	0.49 ± 1.26	0.44 ± 1.21	0.65 ± 1.06	0.57 ± 0.97	0.43 ± 0.69
F2	0.62 ± 0.96	1.09 ± 1.54	1.06 ± 1.05	1.56 ± 1.58	0.94 ± 0.79	0.38 ± 0.80	0.51 ± 0.73	0.96 ± 1.44	0.61 ± 1.12	0.91 ± 1.32
F3	0.24 ± 0.72	0.67 ± 0.90	0.83 ± 1.06	1.47 ± 1.38	1.17 ± 1.58	0.50 ± 0.86	0.49 ± 1.24	0.53 ± 1.07	0.24 ± 0.70	0.73 ± 1.05
Code	Fishy flavour					Rancid flavour				
	0 Weeks	5 Weeks	8 Weeks	11 Weeks	14 Weeks	0 Weeks	5 Weeks	8 Weeks	11 Weeks	14 Weeks
R1	0.99 ± 1.64	0.62 ± 0.82	0.65 ± 0.95	0.75 ± 0.95	0.67 ± 0.71	0.64 ± 1.14	1.58 ± 1.54	2.29 ± 1.90	2.25 ± 1.88	1.87 ± 2.46
FE1	1.65 ± 2.25	1.80 ± 1.42	2.03 ± 1.59	2.75 ± 1.82	2.37 ± 2.57	0.93 ± 1.33	0.78 ± 1.17	1.03 ± 1.67	1.20 ± 1.56	1.05 ± 1.39
FE2	0.69 ± 1.43	1.00 ± 1.12	2.33 ± 1.29	3.54 ± 2.01	3.04 ± 2.00	1.08 ± 1.87	1.20 ± 1.56	1.41 ± 1.73	0.66 ± 1.45	1.08 ± 1.38
FE3	0.90 ± 1.50	2.08 ± 1.41	2.42 ± 1.96	3.71 ± 1.96	2.83 ± 1.70	1.27 ± 1.54	0.89 ± 1.38	1.04 ± 1.34	1.19 ± 1.61	1.36 ± 1.32
F1	1.18 ± 1.36	1.69 ± 1.09	2.08 ± 1.48	3.17 ± 2.37	2.05 ± 1.87	0.97 ± 1.85	0.94 ± 1.09	0.88 ± 1.68	1.05 ± 1.25	1.59 ± 1.33
F2	0.81 ± 1.09	2.09 ± 1.86	1.94 ± 1.67	3.64 ± 2.05	2.18 ± 1.45	1.28 ± 1.69	1.06 ± 1.28	1.52 ± 1.38	1.28 ± 1.54	1.85 ± 1.57
F3	1.07 ± 1.70	1.67 ± 1.47	2.04 ± 1.71	4.70 ± 1.97	3.13 ± 2.08	1.17 ± 1.85	0.79 ± 1.36	0.86 ± 1.22	1.15 ± 1.65	1.59 ± 1.37

the SDs were relatively large, sensory differences between the mayonnaises were not significant. Thus, it was not possible to compute a model describing the variation between samples using traditional statistical methods such as ANOVA. However, by employing modern multivariate statistical methods it was possible to compute such models; these are discussed below. The reason for the high SDs may have been that the mayonnaises had a very complex and sour taste, which made it difficult to assess precisely the intensity of the fishy off-odour and off-flavours. Furthermore, the assessors may have used the sensory scale differently, which is not unusual [24]. However, even though the assessors may have used the sensory scale differently, the different assessors may have ranked the different mayonnaises similarly. The average scores shown in Table 2 were not adjusted to compensate for possible differences in scaling levels between assessors. However, by employing multivariate statistical methods such as ANOVA PLSR we were able to compensate for this (see Materials and methods).

Lipid hydroperoxides

PV were generally low and varied between 0.09 and 1.02 μmol/g (equivalent to mEq/kg) during the storage period (Table 3). The PV developed slightly differently in the different mayonnaise samples. The mayonnaise without fish oil (R1) developed higher PV during storage than mayonnaise with fish oil (Table 3). Among mayonnaises with fish oil, PV were lowest in mayonnaises without antioxidant after 8 and 11 weeks.

Table 3 Hydroperoxide values measured after 0, 5, 8, 11 and 14 weeks of storage (μmol lipid hydroperoxide/g oil ± SD). For abbreviations, see Table 1

Code	0 Weeks	5 Weeks	8 Weeks	11 Weeks	14 Weeks
R1	0.10 ± 0.01	0.13 ± 0.00	0.16 ± 0.00	0.55 ± 0.02	1.02 ± 0.01
FE1	0.39 ± 0.01	0.17 ± 0.00	0.23 ± 0.00	0.39 ± 0.00	0.39 ± 0.00
FE2	0.09 ± 0.00	0.21 ± 0.00	0.37 ± 0.00	0.47 ± 0.00	0.43 ± 0.03
FE3	0.36 ± 0.00	0.30 ± 0.00	0.54 ± 0.00	0.60 ± 0.00	0.38 ± 0.00
F1	0.28 ± 0.00	0.18 ± 0.00	0.29 ± 0.00	0.43 ± 0.00	0.39 ± 0.01
F2	0.45 ± 0.00	0.38 ± 0.01	0.46 ± 0.00	0.44 ± 0.00	0.42 ± 0.00
F3	0.38 ± 0.00	0.28 ± 0.00	0.50 ± 0.00	0.48 ± 0.00	0.32 ± 0.00

Volatile secondary oxidation products

From the GC-MS analysis more than 100 compounds were observed. Thirty-two of these compounds were tentatively identified by mass spectrometry and used for further data analysis. These compounds are listed in Table 4. The increase in the levels of the 32 compounds during storage of the seven different mayonnaises is also indicated in Table 4. Thus, the compounds were rated with respect to the relative increase in their concentrations between 5 and 14 weeks. The concentrations of hexanal (peak 1), cyclohexanone (peak 8), β-myrcene (peak 9), D-limonene (peak 12), octanal (peak 19), 1-octen-3-ol (peak 20), nonanal (peak 24), linalyl propanoate (peak 29) and 2-decenal (peak 30) either decreased or only increased slightly between weeks 5 and 14 (Table 4). Thus, these compounds apparently did not play any significant role in the development of the fishy and rancid off-flavours during storage. Some of the compounds, including for example D-limonene,

Table 4 Volatile compounds identified in mayonnaise and ratings of their relative increase (calculated relative to their concentration after 5 weeks of storage) between 5 and 14 weeks of storage at 5 °C. *MS* Identification by MS library, *Std* identification by retention time and spectra of spiked standard in mayonnaise samples, – no increase or decrease, *1* 1–49% increase in concentration during storage, *2* 50–99% increase during storage, *3* 100–200% increase during storage, *4* >200% increase during storage. For other abbreviations, see Table 1

Compound		Identification by	Mayonnaise code						
			R1	FE1	FE2	FE3	F1	F2	F3
Peak 1	Hexanal	MS + Std	1	1	1	1	1	2	2
Peak 2	Ethylbenzene	MS + Std	3	3	4	3	3	3	3
Peak 3	*p*-Xylene	MS + Std	3	2	4	3	3	3	4
Peak 4	*t*-2-Hexenal	MS + Std	4	4	4	3	4	4	4
Peak 5	2-Hexenal	MS	–	2	4	2	4	2	4
Peak 6	3-Furaldehyde	MS + Std	3	3	4	4	4	4	4
Peak 7	Heptanal	MS + Std	3	3	–	1	1	3	3
Peak 8	Cyclohexanone	MS	1	1	–	–	–	1	–
Peak 9	Beta-myrcene	MS	1	1	2	–	1	1	–
Peak 10	2-Pentyl-furan	MS + Std	2	3	3	3	3	4	4
Peak 11	*t,t*-2,4-hexadienal	MS + Std	4	4	–	3	4	4	–
Peak 12	D-Limonene	MS + Std	3	–	–	–	1	–	–
Peak 13	6-Methyl-5-nonen-4-one	MS	4	1	4	3	4	4	4
Peak 14	*t*-2-Heptenal	MS + Std	2	3	3	2	3	3	–
Peak 15	1-Octen-3-one	MS + Std	3	3	–	–	–	2	–
Peak 16	1-Methyl-4-(1-methylethyl)-benzene	MS	2	2	3	–	2	3	3
Peak 17	1,1-Dimethyl-2-(3-methyl-1,3-butadienyl)-cyclopropane	MS	3	2	4	3	4	4	4
Peak 18	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	MS	3	2	4	3	4	4	4
Peak 19	Octanal	MS + Std	1	1	–	–	–	1	1
Peak 20	1-Octen-3-ol	MS + Std	–	2	–	2	2	2	–
Peak 21	*t,c*-2,4-Heptadienal	MS	4	4	4	4	4	4	4
Peak 22	*t,t*-2,4-Heptadienal	MS + Std	4	4	4	4	4	4	4
Peak 23	*t*-2-Octenal	MS + Std	3	4	2	–	1	2	2
Peak 24	Nonanal	MS + Std	1	2	–	–	1	3	1
Peak 25	1-Methyl-2-octyl-cyclopropane	MS	–	4	2	3	–	–	3
Peak 26	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	MS	3	2	2	1	2	3	3
Peak 27	*t,c*-2,6-Nonadienal	MS + Std	2	4	3	3	3	4	4
Peak 28	*t*-2-Nonenal	MS + Std	1	2	4	3	2	4	2
Peak 29	Linalyl propanoate	MS	2	2	2	–	1	2	2
Peak 30	*t*-2-Decenal	MS + Std	–	1	–	–	–	1	2
Peak 31	*t,t*-2,4-Decadienal	MS + Std	3	3	–	4	4	4	4
Peak 32	*t*-2-Undecenal	MS + Std	–	2	–	–	3	4	4

did not even originate from the oxidation of lipids, but from the other ingredients which were added to the mayonnaise. For the remaining compounds, their concentrations during storage increased by more than 100% for most of the mayonnaise samples. As mentioned above, the mayonnaise without fish oil developed higher PV than the mayonnaises with fish oil (Table 3). Although it is tempting to conclude that this finding may have been due to a faster hydroperoxide breakdown in mayonnaise with fish oil, the GC-MS data did not support this conclusion. Thus, the relative increase in the concentrations of the volatiles in the mayonnaise without fish oil was similar to that in the mayonnaises with fish oil for all but four compounds: 2-hexenal (peak 5), 2-pentyl furan (peak 10), *t*, *c*-2,6-nonadienal, (peak 27) and *t*-2-nonenal (peak 28). The relative increase in these compounds was higher in mayonnaises containing fish oil as compared to R1. It was observed that the areas of peaks 9, 14, 17, 23, 26 and 29 were generally higher for R1 than for the samples with fish oil, whereas peaks 4, 5, 6, 20 and 21 had smaller areas (data not shown). Samples with fish oil obviously developed unpleasant off-odours and off-flavours faster and to a higher extent than the sample without fish oil (Table 2). Thus, although R1 apparently oxidised at approximately the same rate as mayonnaise with fish oil, certain differences in the development of volatiles distinguished samples containing fish oil from R1. This result was in accordance with those of our previous studies that indicated that mayonnaises with a highly intense fishy off-flavour were not necessarily highly oxidised as judged from chemical oxidation parameters [25]. In a recent study, we showed that some volatile off-flavour compounds produced by fish oil oxidation apparently partition into the water phase of mayonnaise [26]. The present results support our hypothesis that unpleasant off-flavours that develop in fish-oil-enriched mayonnaise may not be due to the mayonnaise being highly oxidised, but may be ascribed to other factors. Thus, we propose that unpleasant off-flavours in fish-oil-enriched mayonnaise may be caused by the partitioning into the water phase of very sensorically potent volatile compounds stemming from slightly oxidised EPA and/or DHA. The reason for their high

flavour intensity may be that these compounds have a much higher volatility and lower flavour threshold value in the water phase than in the oil phase [27]. Such compounds will therefore have a major influence on the sensory perception of fish-oil-enriched mayonnaise, even if the mayonnaise is not highly oxidised [26].

Several of the compounds identified in the present study (Table 4) have previously been reported as being present in mayonnaise with fish oil, namely: hexanal; 2-hexenal; 1-octen-3-one heptanal; octanal; *t*,*t*-2,4-hexadienal; 2,4-heptadienal and decenal [28]. *t*-2-Octenal, nonanal and *t*-2-nonenal, found in our study, have also been identified in oxidised fish oil [28]. Several of the products identified were cyclic compounds that probably stemmed from polyunsaturated fatty acids. Several furan derivatives have been reported to be responsible for flavour defects in soybean oil [29].

Rheological measurements

G*, which expresses the gel strength of the sample, varied between 408 and 572 Pa among mayonnaises after 1 week of cold storage. The mayonnaise without fish oil had the lowest gel strength and F1 (the mayonnaise with fish oil, no antioxidant, no extra emulsifier) the highest (Table 5). Mayonnaises with antioxidants (F2, F3 and FE2, FE3) had lower gel strengths than the corresponding mayonnaises without antioxidant. A higher gel strength indicates a more "rigid" consistency of the mayonnaise. Scrit, which expresses the force necessary to initiate the breakdown of the structure of a sample, was almost the same (16.7 Pa; Table 5) for all samples, but Scrit values for R1, FE2 and F1 were somewhat lower than for the others samples (i.e. 10.8, 13.1 and 14.9 Pa respectively; Table 5). Thus, the rapeseed oil mayonnaise (R1) had the lowest Scrit. The yield stress (S0), which expresses the force necessary to make the mayonnaise flow, varied markedly for the different mayonnaises. In accordance with the G* data, the lowest S0 was observed for R1 (75.5 Pa) and the highest for F1 and FE1 (120.5 and 102.0 Pa, respectively; Table 5). V(1.57) data showed that the viscosities were highest for mayonnaises without antioxidant (F1 and FE1) and that F3 (with antioxidant, no extra emulsifier) had the lowest viscosity (Table 5). Mayonnaise without fish oil and without antioxidant (R1) had the second lowest viscosity (42.8 Pas).

Droplet size measurements

From the droplet size data obtained by laser diffraction it was observed that R1 generally had the highest droplet sizes. Thus, R1 apparently had bigger droplets than mayonnaise with fish oil. However, the D[v,0.1] value for R1 was the second lowest. Only FE1 had a lower value (Table 5). This indicated that the bigger droplets were also more equal in size, at least in R1. The mayon-

Table 5 Results of rheological, laser diffraction and confocal laser scanning microscopy (*CLSM*) measurements (±SD)

Mayonnaise Code	Gel strength (Pa)	Critical stress (Pa)	Yield stress (Pa)	V(1.57) (Pas)	V(39.3) (Pas)	D[4,3] (μm)	D[3,2] (μm)	D[v,0.1] (μm)	D[v,0.5] (μm)	D[v,0.9] (μm)	C(4,3) (μm)	C(3,2) (μm)	C(2,0) (μm)	C(3,0) (μm)
R1	407.5±3.5	10.8±8.4	75.5±34.4	42.8±0.1	5.7±0.0	5.68±0.30	2.57±0.04	1.01±0.04	4.93±0.01	9.33±0.71	12.7±8.3	8.2±4.4	3.8±0.4	4.8±0.2
FE1	512.5±10.6	16.7±0.0	102.0±7.2	48.5±1.7	5.6±0.3	3.81±0.03	2.10±0.10	0.89±0.06	3.62±0.10	7.19±0.11	12.6±4.8	7.4±1.8	3.4±0.3	4.4±0.6
FE2	454.5±26.2	13.1±0.0	83.5±18.1	44.3±0.5	5.5±0.1	3.43±0.00	2.20±0.03	1.06±0.02	3.13±0.03	6.24±0.05	26.9±26.4	16.7±17.2	3.1±0.6	5.2±2.5
FE3	485.5±3.5	16.7±0.0	101.5±8.9	47.9±1.3	5.9±0.1	4.45±0.06	2.34±0.13	1.07±0.07	3.47±0.14	7.24±0.10	11.4±8.4	6.1±3.7	2.6±0.4	3.4±1.1
F1	571.5±17.7	14.9±2.6	120.5±4.0	52.2±1.3	6.2±0.0	3.44±0.05	2.19±0.07	1.04±0.04	3.17±0.07	6.29±0.01	8.2±1.0	5.1±0.3	2.8±0.1	3.4±0.1
F2	435.5±2.1	16.7±0.0	81.5±14.8	43.8±0.8	5.3±0.1	3.57±0.16	2.24±0.13	1.04±0.06	3.31±0.18	6.51±0.18	9.1±1.8	5.7±1.0	2.9±0.2	3.6±0.4
F3	422.5±16.3	16.7±0.0	81.0±8.6	40.7±1.3	5.2±0.2	3.67±0.16	2.25±0.15	1.03±0.08	3.43±0.20	6.71±0.18	8.1±1.8	5.9±1.1	3.3±0.4	4.0±0.6

V(1.57) Viscosity at 1.57 1/s, *V(39.3)* V at 39.3 1/s, *D[4,3]* volume mean diameter, *D[3,2]* surface mean diameter, *D[v,0.1]* 10% percentile of droplet distribution, *D[v,0.5]* 50% percentile of droplet distribution, *D[v,0.9]* 90% percentile of droplet distribution, *C(4,3)* volume mean diameter determined by CLSM, *C(3,2)* volume mean diameter determined by CLSM, *C(2,0)* number surface mean determined by CLSM, *C(3,0)* number volume mean determined by CLSM; for other abbreviations, see Table 2

naise without antioxidant and without emulsifier (F1) had lower mean D[4,3] and D[3,2] than the corresponding mayonnaises with antioxidants (F2 and F3). Values of D[v,0.5] and D[v,0.9] were also lower for F1 than for F2 and F3. The trend was not as clear for the three mayonnaises with emulsifier (FE1, FE2 and FE3), as D[3,2] and D[v,0.1] were bigger for FE2 and FE3 than for FE1, whereas FE1 had higher D[4,3], D[v,0.5] and D[v,0.9] values (Table 5) than FE2.

When assessing the droplet size of all the mayonnaises by microscopy, the data confirmed that R1 generally had bigger droplets than mayonnaises with fish oil, with the exception of FE2, which had bigger droplets (Table 5). The results also indicated that F1 had smaller droplets than F2 and F3, although C(4,3) was at the same level for F1 as for F3. The microscopy data for FE1, FE2 and FE3 were not fully in accordance with the data obtained from the laser diffraction measurements. Thus, the values for all four microscopy variables were lower for FE3 than for FE1.

Typical CLSM photos of R1 and mayonnaise with fish oil and emulsifier (FE1) are shown in Fig. 1. Thus, in general R1 was relatively homogeneous and had few very small droplets. In contrast, the samples with fish oil seemed to be less homogenous with respect to droplet-size distribution. In mayonnaise with fish oil and emulsifier (FE1, FE2 and FE3) a higher number of very big droplets were thus observed compared to samples without extra emulsifier (F1, F2 and F3).

The production method used was the same for all mayonnaises, no matter whether fish oil was added or not. The result of the rheological measurements and the droplet size measurements therefore indicated that addition of fish oil gave rise to smaller droplets and to a mayonnaise with a higher gel strength, viscosity and S0. The changes in the rheological properties caused by the addition of fish oil may have been due to the reduced droplet size. The decreased droplet size may have been due to fish oil being more surface active than rapeseed oil, but this hypothesis needs to be investigated further.

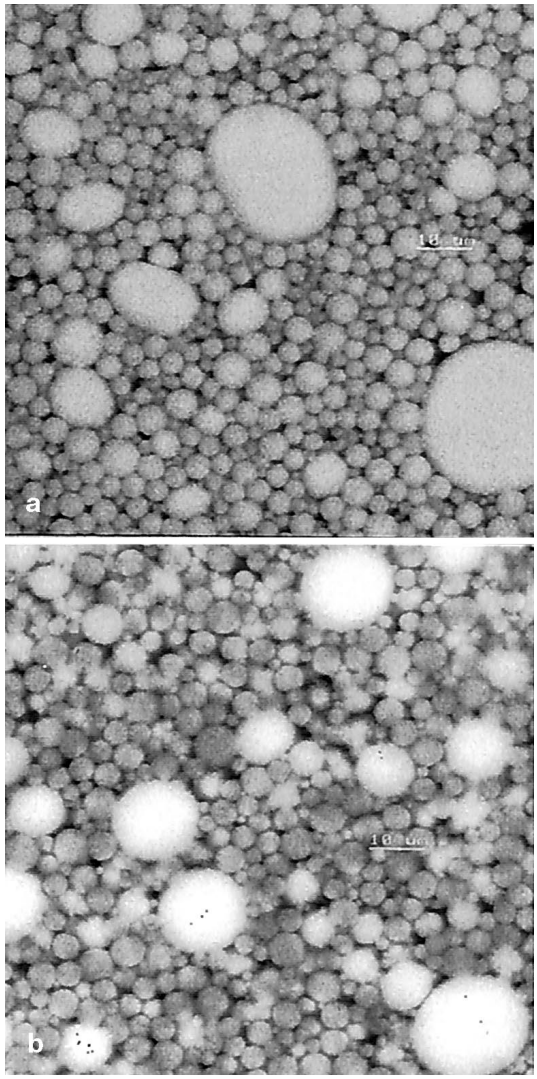

Fig. 1 Confocal laser scanning microscopy photos of **a** mayonnaise with fish oil and extra emulsifier (FE1) and **b** mayonnaise without fish oil (R1)

Results of multivariate data analysis on oxidation data

As described, two different DPLSR analyses were performed on the data. The reason why all the data were not included in one analysis was that it would have been difficult to interpret the loadings plots due to the high number of variables (>200). The results of the two DPLSR analyses are shown in Figs. 2–11. In both analyses only data from the mayonnaises with fish oil were included, as R1 was basically a reference sample used to validate the different analytical data. Inclusion of R1 in the DPLSR analysis only interfered with the comparisons of the fish-oil-enriched mayonnaises, as the lack of fish oil was the overriding difference shielding the effect of other parameters between the fish-oil-containing mayonnaises.

The DPLSR method was chosen to analyse the data because our primary aim was to investigate the effect of emulsifier and antioxidant addition. This meant that the variation caused by the addition of antioxidant and emulsifier was used to compute the model. Thus, we did not try to make direct correlations between sensory and, for example, GC-MS data. This could have been done by using GC-MS data as the x-variables and sensory data as the y-variables, or vice versa.

DPLSR analysis on oxidation data (excluding GC-MS data)

In the DPLSR analysis, which included all variables except data from dynamic headspace GC-MS measurements, six principal components (PCs) were validated, and 64% of the variance in x (sensory and instrumental

variables) and 59% in y (design variables) were explained by these six components.

PC 1 and PC 2

Antioxidant differences. The first two PCs, PC1 and PC2, described 28% of the variance in x and 15% of the variance in y. The scores plot of PC1 and PC2 (Fig. 2) showed that samples without antioxidant (FE1 and F1) had negative values for PC1, whereas samples with antioxidants had positive values for PC1. This meant that PC1 described differences between samples with and without antioxidant. Samples with propyl gallate added in the water phase, i.e. with Grindox 413 (FE3 and F3), had negative values for PC2, whereas samples with propyl gallate added in the oil phase, i.e. Grindox 370 (FE2 and F2), had positive values for PC2 (Fig. 2). Thus, PC2 mainly described differences between samples with propyl gallate added in the water or oil phase. It should be noted that the effect of the addition of extra emulsifier (Panodan TR) could not be interpreted from the plots of PC1 vs PC2. This meant that antioxidant addition affected the sensory, droplet and rheological variables more than the addition of emulsifier.

The loadings plots contained more than 100 variables (not shown), which made it very difficult to interpret. Therefore, the loadings plot was split into four plots showing the flavour and aroma variables (Fig. 3a), the PV plus the rancid and fishy flavour variables (Fig. 3b), the rheology, appearance and "mouthfeel" variables (Fig. 3c), the droplet size variables, plus the replicate variables (Fig. 3d). The y-variables, F1-F3 and FE1-FE3, are not shown in these plots as their location can be seen from the scores plot.

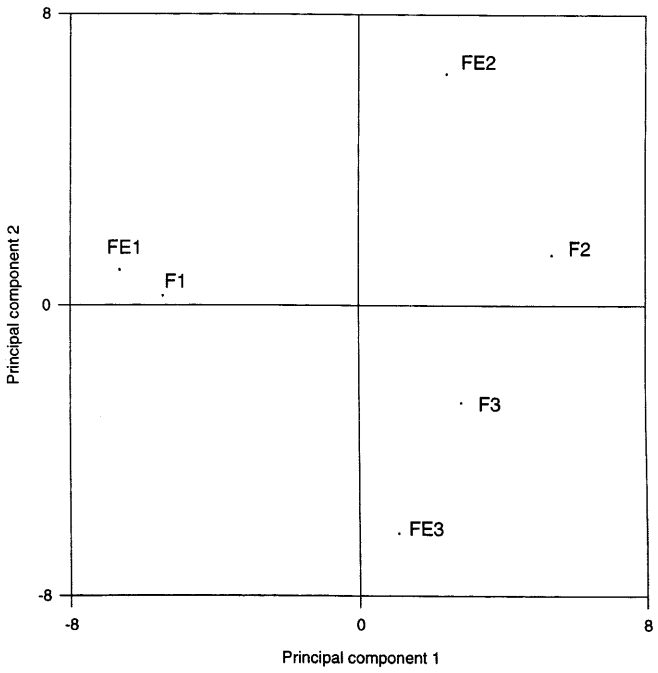

Fig. 2 Scores plot of principal component (PC)1 versus PC2 from discriminant partial least squares regression (DPLSR) analysis of sensory, rheological, droplet size and microscopy data and peroxide values (PV). *FE2* Mayonnaise with Grindox 370 and Panodan TR, *FE3* mayonnaise with Grindox 413 and Panodan TR, *F1* mayonnaise without antioxidant and emulsifier, *F2* mayonnaise with Grindox 370, *F3* mayonnaise with Grindox 413; for other abbreviations, see Fig. 1

Flavour and aroma differences. The loadings plot (Fig. 3a,b) corresponding to the scores plot of PC2 vs PC1 (Fig. 2) showed that fishy off-flavour ("Ffish") always had a negative PC2 value, and moved from left to right over time, i.e. during storage from week 0 to week 14. This implied that samples with propyl gallate developed more of a fishy flavour during storage than samples without propyl gallate. The loadings values for rancid flavour ("Franc") were in close proximity for week 0 and week 14, and were located to the right on the plot (Fig. 3a,b).

The variables describing fishy and rancid aroma ("Afish" and "Aranc") as well as the variables describing rancid and vinegar flavour ("Franc" and "Fvine") all had positive values for PC1. This implied that all four mayonnaises with propyl gallate received higher scores for "Afish", "Aranc", "Franc" and "Fvine" than did the mayonnaises without propyl gallate. The only exceptions were "Franc-11" and "Aranc-11", that had negative values for PC1. This may have been due to interference between the "Franc" and "Ffish" variables in week 11. Thus, "Ffish-11" was located far to the right, indicating that the fishy off-flavour was judged as being particularly strong in week 11. The strong fishy off-flavour may have shielded the rancid off-flavour. Nonetheless, the results showed that propyl gallate apparently promoted the development of fishy and rancid off-flavours, and that the fishy off-flavour was particularly pronounced in mayonnaise with propyl gallate added in the water phase. The addition of extra emulsifier, however, did not seem to influence the development of fishy and rancid off-flavours.

Oily flavour ("Foily") moved from left to right and from negative PC2 values to positive PC2 values with time of storage (Fig. 3a). Thus, the development of an oily flavour was apparently enhanced by the addition of the oil-soluble propyl gallate (G370), as PC2 discriminated between the type of antioxidant used (Fig. 2). However, the analysis did not permit any conclusion to be drawn about the possible chemical properties causing this effect.

PC2 values for oily aroma ("Aoily") were all positive, except for the point after 5 weeks ("Aoily-5"). The positive PC2 values obtained for "Aoily" in the loadings plot therefore indicated that mayonnaises with propyl gallate in the oil phase had a more oily aroma than samples with propyl gallate added in the water phase. This finding was in accordance with the data for oily flavour (Fig. 3a). Synthetic flavour ("Fsynt") exhibited no trend with respect to storage time, but, except

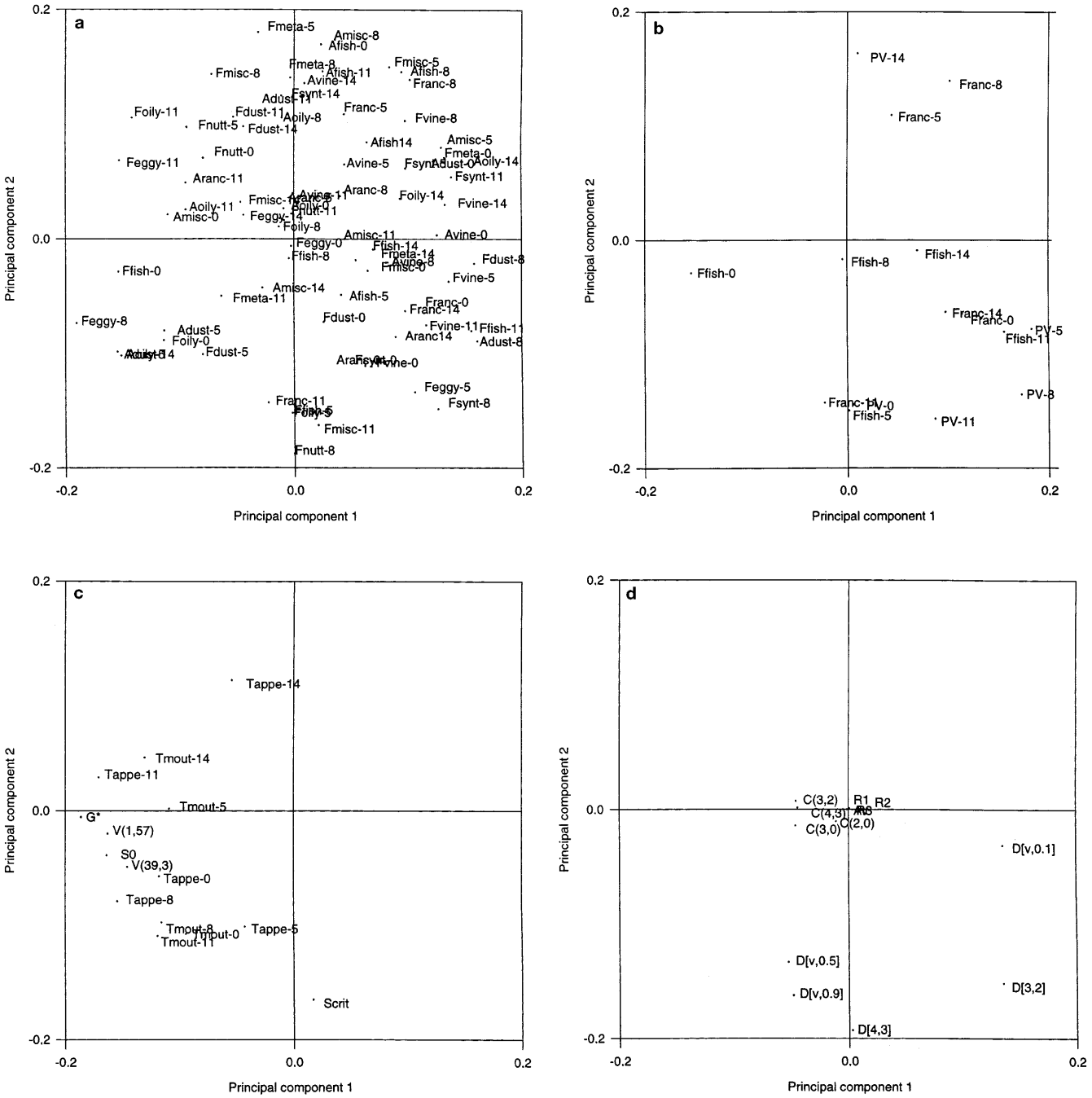

Fig. 3 Loadings plot of PC1 vs PC2 for sensory data (**a**), selected sensory data and PV (**b**), selected sensory data and rheological measurements (**c**), droplet size and microscopy data (**d**); all corresponding to scores plot in Figs. 2. *A* Aroma, *F* flavour, *T* texture, *vine* vinegary/acidic, *fish* fishy/train oil, *ranc* rancid, *meta* metallic, *synt* synthetic, *dust* dusty, *eggy* egg yolk, *nutt* nutty, *misc* miscellaneous, *appe* appearance, *mout* mouthfeel, *G** complex modulus (gel strength), *Scrit* critical stress, *S0* yield stress, *V* viscosity, *D* mean diameter calculated from droplet size measurements, *C* mean diameter calculated from microscopy measurements, *R* replicate, *av* mean value; the *numbers* after each hyphen indicate the storage time (weeks); for other abbreviations, see Fig. 2

for the point after 14 weeks, had positive values for PC1 (Fig. 3a). The overall positive PC1 values indicated that samples with propyl gallate (both with G370 and with G413) had a stronger synthetic off-flavour than samples without, as mayonnaises with propyl gallate added had positive values for PC1 (Fig. 2). Egg yolk off-flavour ("Feggy") had negative values for PC1 after 8, 11, and 14 weeks of storage (Fig. 3a). Thus, samples without antioxidant had more egg yolk off-flavour after 8–14 weeks of storage as compared to mayonnaises with propyl gallate added. This may have been because the development of fishy off-flavour masked the egg yolk flavour in samples with antioxidant.

The variations in rancid and miscellaneous aroma values, as well as the scattered dusty, metallic, nutty and miscellaneous flavour loadings (Fig. 3a), seemed to be accidental and thus not attributable to differences in the composition of the mayonnaise.

Peroxide values. All PV had positive values for PC1 (Fig. 3b). Thus, samples with propyl gallate generally had higher PV than samples without, which is also obvious from Table 3. PV correlated positively with the sensory variables describing fishy and rancid off-flavours (Fig. 2, 3b). Thus, after storage of 5, 8 and 11 weeks, mayonnaises with antioxidant (FE2, FE3 F2 and F3) had higher PV than mayonnaises without antioxidant (F1 and FE1). After 14 weeks, PV had a high value for PC2. This was due to the fact that FE2 had the highest PV of all the samples after 14 weeks. It should, however, be stressed that differences in PV between the samples were very small even at the end of the storage period (ranging from 0.32 to 0.43 μmol/g; Table 3). Nonetheless, the results indicated that propyl gallate was a pro-oxidant and not an antioxidant. In a study by Jafar et al. 1994 [9], propyl gallate added at a concentration of 200 μg/g inhibited the development of fishy off-odours in Menhaden oil based mayonnaise. The propyl gallate system used in our study contained only 20% propyl gallate, which meant that the effective concentration of propyl gallate was only 40 μg/g. It is therefore unlikely that the pro-oxidative effect of propyl gallate found in our study was due to an excessively high concentration of propyl gallate. Since the results showed that the addition of the propyl gallate systems affected the structure of the mayonnaise (Table 5), it may be speculated that the propyl gallate systems interfered with the interface and affected the egg yolk in such a way that the iron bound by the egg proteins [30] became more accessible. Thereby, iron from egg yolk may have promoted oxidation. This conclusion needs to be substantiated by further experiments, and this issue is currently being studied further in our laboratory.

Rheology, appearance, and mouthfeel. The rheology variables G*, S0, V(1.57) and V(39.3) were located far to the left in the loadings plot (Fig. 3c). The appearance and mouthfeel variables, "Tappe" and "Tmouth", respectively, also had negative values for PC1, and exhibited a trend of increasing PC2 values with time. The proximity of the loadings values for the rheology, appearance and mouthfeel parameters indicated that there was a positive correlation between mouthfeel plus appearance and G*, S0, V(1.57) and V(39.3). As the scores plot showed that negative PC1 values were associated with mayonnaises without propyl gallate added (Fig. 2), the loadings obtained in Fig. 3c showed that antioxidant addition apparently resulted in mayonnaises which were perceived as thinner and which had a "weaker" structure. Scrit was located close to the PC2 axis, but had a negative PC2 value (Fig. 3c). Thus, the variation in Scrit was mainly described by PC2, indicating that the composition of the antioxidant system, i.e. inclusion of different carriers, affected this parameter more than the addition of propyl gallate.

Droplet size. All variables describing droplet size had negative PC2 values (Fig. 3d). Thus, in accordance with the direct observations of the droplet parameters (Table 5), samples with propyl gallate added in the water phase (FE3 and F3) had bigger droplets than samples with propyl gallate added in the oil phase (FE2 and F2). This effect on droplet size may be ascribed to the different carriers employed in the two propyl gallate preparations. Thus, the carrier used in the water-dispersible propyl gallate seemed to interfere with egg yolk at the emulsion interface to a higher degree than the carrier used in the oil-soluble propyl gallate. D[v,0.9], D[v,0.5] and D[4,3] were very close to the PC2 axis. This meant that these variables were not described by PC1 and they therefore neither correlated with rancid, fishy and vinegar flavours nor with the "Tappe", "Tmout" and rheological variables. Contrary to this, D[v,0.1] and D[3,2] had positive values for PC1, and thus seemed to correlate positively with the "Franc" and "Ffish" variables, and negatively with the "Tappe", "Tmout" and rheological variables. The reason for this discrepancy may have been that the mayonnaise was not diluted in buffer before droplet size measurements, but in water. As dilution in water may not separate agglomerates of droplets formed by flocculation, it is likely that D[4,3] expressed the diameter of these agglomerates, whereas D[3,2] did not. Therefore, D[3,2] may be a more reliable diameter to use for the interpretation of the DPLSR. In this case, addition of propyl gallate, i.e. the oil-dispersible as well as the water-dispersible form, would have given rise to mayonnaise with bigger droplets (Fig. 3d), but with a tendency for the water-dispersible preparation to have increased the droplet size more than the oil-dispersible form. This interpretation signifies that the carriers used in the two propyl gallate systems employed in this experiment may have interfered with egg yolk in such a way that its emulsifying properties were reduced. The higher G* and higher S0 observed for the samples without propyl gallate may thus be correlated with the finding that these samples also had smaller droplets. The results obtained therefore indicated that a big droplet size and a "weaker" structure were associated with the development of fishy and rancid off-flavours. However, as antioxidant addition and droplet size did not vary independently of each other, the data did not allow us to draw any clear conclusion about which of the two parameters, antioxidants or droplet size, was the most significant. It has previously been reported that the release of flavour compounds from emulsions is influenced by the structure and rheological properties of the emulsion [31]. However, the data obtained in the present study demonstrated for the first time that droplet size is affected by the type of antioxidant mixture

employed in mayonnaise. There are obviously several confounding factors influencing both flavour release and rheology in food emulsions. The determination of the most important parameters responsible for the perception of off-flavours in food emulsion systems deserves further study.

The droplet size data obtained from microscopy only partly correlated with the data obtained from the laser diffraction measurements (Fig. 3d). Thus, all the microscopy variables, C(2,0), C(3,0), C(4,3) and C(3,2), were located very close to the origin and were thus not very well explained by PC1 or PC2. This was probably due to the finding that FE2 had higher values than FE3 for all the microscopy variables, whereas the opposite was the case for the laser diffraction data. FE2 also had higher values for the microscopy variables than F1, FE1, F2 and F3, with the exception of C(2,0). In accordance with the laser diffraction data, the microscopy data indicated that addition of propyl gallate in the water phase resulted in bigger droplets when no extra emulsifier was added. By visual inspection, it was observed that mayonnaises with extra emulsifier had an increased number of very big droplets and were therefore more heterogenous. This meant that the results of the microscopy became more sensitive to the number of big droplets present within the area viewed in the microscope. Very large droplets make up a relatively large proportion of the area viewed in the microscope. Thus, if by coincidence a relatively high number of large droplets was present in the area viewed in the microscope, this would significantly have increased the mean droplet diameter calculated from the microscopy data to a value that may have been higher than the "real" mean diameter of the droplets. Thus, the discrepancy between particle sizes obtained by laser diffraction and CLSM may have been due to the low sampling sensitivity of CLSM with respect to mayonnaises with extra emulsifier.

Replicates. All replicates (rep 1, rep 2, rep 3 and av) were located very near to the origin (Fig. 3d). This finding showed that variations between replicates were small and that PC1 and PC2 did not describe differences between replicates.

PC 3 and PC 4

PC3 and PC4 described 24% of the variance in x and 19% of the variance in y. In the scores plot (Fig. 4), FE1 (without propyl gallate but with extra emulsifier) and F2 [with propyl gallate (Grindox 370) but without extra emulsifier] were located together in the first quadrant, FE3 [containing propyl gallate (Grindox 413) and extra emulsifier] was located together with FE2 [with emulsifier and propyl gallate (Grindox 370)] in the second quadrant, F1 (without antioxidant and without emulsifier) was located in the third quadrant and F3 [with propyl gallate (Grindox 413) and without emulsifier] was located alone in the fourth quadrant (Fig. 4). This meant that no general trend with respect to the effect of Grindox 370, Grindox 413 or Panodan TR could be deduced from this regression plot. However, it is worth noting that mayonnaises with the same antioxidant, with and without emulsifier, never occurred in the same quadrant. This could indicate that the addition of extra emulsifier may have been a latent factor, which does not seem to correlate with principal component 3 or 4, but which anyhow in part governed the location of the different mayonnaises in the scores plot.

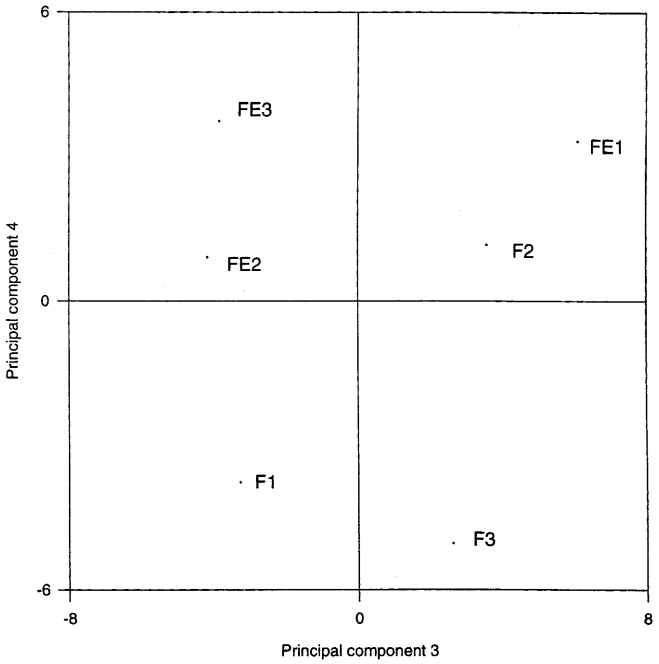

Fig. 4 Scores plot of PC3 vs PC4 from DPLSR analysis of sensory, PV, rheological, droplet size and microscopy data. For abbreviations, see Figs. 1 and 2

Aroma and flavour differences. The loadings for aroma and flavour were widely spread and covered most of the plot (Fig. 5a). There was no clear trend in the location of most of the sensory variables as judged from the loadings plots (Fig. 5a,b). For example "Ffish" moved from the first quadrant ("Ffish-0" and "Ffish-5") to the second quadrant ("Ffish-8") then to the fourth quadrant ("Ffish-11") and finally to the third quadrant ("Ffish-14"; Fig. 5a,b). However, metallic flavour after 14 weeks of storage ("Fmeta-14") was located far to the left of the plot, indicating that PC3 was mainly explained by this variable (Fig. 5a).

PV differences. PV moved from the right to the left over time and had positive PC4 values throughout the storage period, with the exception of PV-8 (the value after 8 weeks), which was slightly negative (Fig. 5b). The locations of PV in the plot were apparently gov-

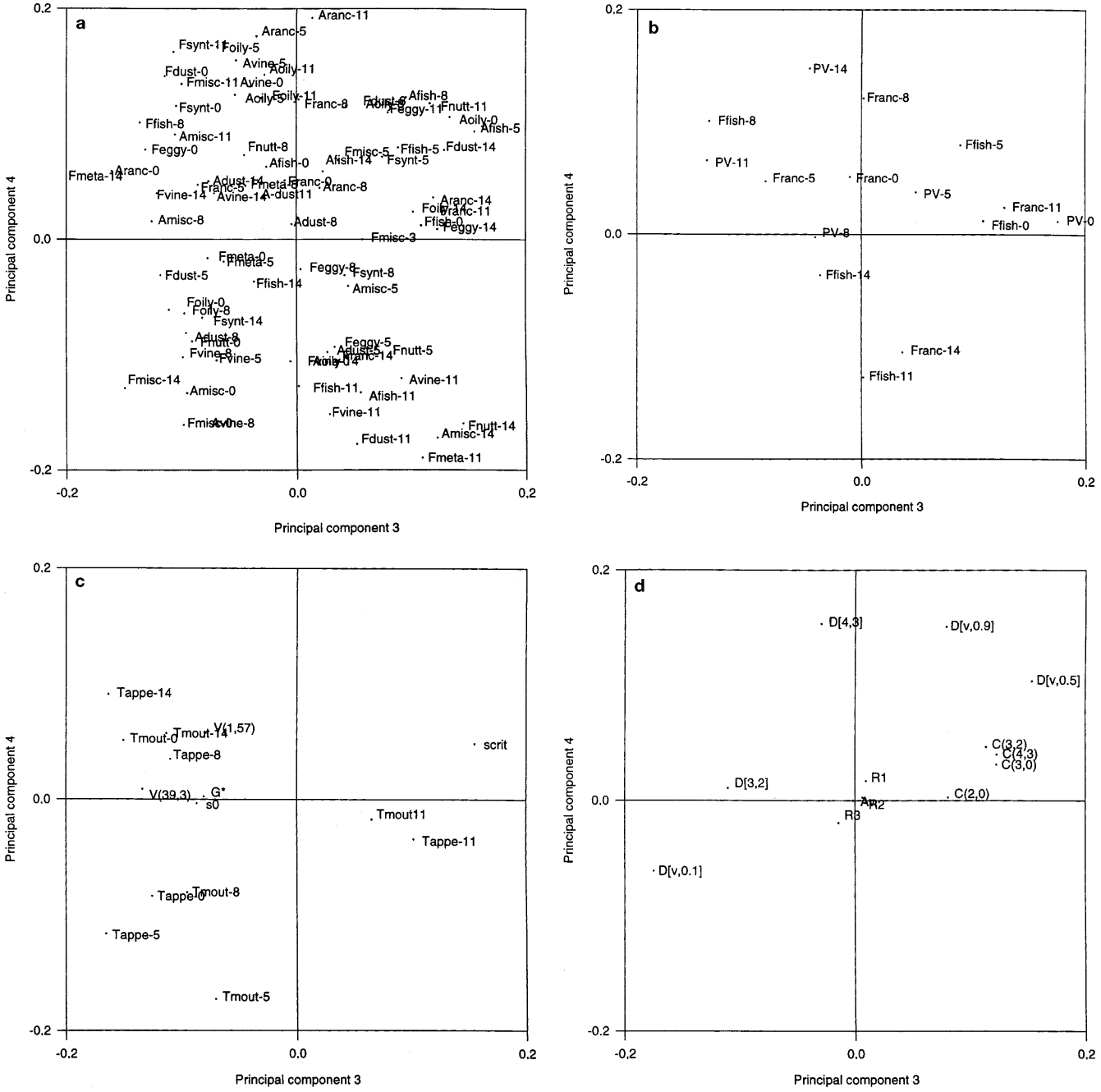

Fig. 5 Loadings plot of PC3 vs PC4 for sensory data (**a**), loadings plot of PC3 vs PC4 for selected sensory data and PV (**b**), loadings plot of PC3 vs PC4 for selected sensory data and rheological measurements (**c**), loadings plot of PC3 vs PC4 for droplet size and microscopy data (**d**); all corresponding to scores plot in Fig. 4. For abbreviations, see Figs. 2 and 3

erned by the development of PV in FE3, as FE3 had high PV after 5, 8, 11 and 14 weeks.

Differences in rheological properties and droplet size. All variables describing mouthfeel and appearance, except for "Tmouth-11" and "Tappear-11", had negative values for PC3 (Fig. 5c). It was also observed that G*, S0, V(1.57) and V(39.3), and D[v,0.1], D[3,2] and D[4,3] had negative values for PC3 (Fig. 5c,d). Thus, these plots confirmed the positive correlation between the viscosity variables and the "Tmout" and "Tappe" variables observed previously (Fig. 3c). Furthermore, the location to the left in the plot of the rheological variables G*, S0, V(1.57) and V(39.3), as well as the droplet size variables D[3,2] and D[v,0.1], helped clarify the effect of the addition of extra emulsifier on these variables, as this effect obviously depended on whether propyl gallate was added or not. As F1, FE3 and FE2 were also located to the left in the plot (Fig. 4), Fig. 5c,d shows that the addition of emulsifier gave rise to bigger droplets (D[3,2], D[v,0.1]), a

"stronger" structure (G*, S0) and a higher viscosity (V1.57 and V39.3) in mayonnaise with antioxidants (compare F2, and F3 with FE2, and FE3). Contrary to this, emulsifier addition also gave rise to smaller droplets (D[3,2] and D[v,0.1]), a "weaker" structure and a lower viscosity in mayonnaise with no antioxidant (compare FE1 with F1). These conclusions could also be deduced from Table 4. The only exception was that D[3,2] was higher for F2 than for FE2 (Table 5).

The data therefore indicated that Panodan TR and the carriers used in Grindox 413 and Grindox 370 interfered with each other. As a consequence of this interference, the carriers in Grindox 413 and Grindox 370 may at least in part have compensated for the effect on the droplet size and rheological properties caused by the addition of Panodan TR alone. If this was the case, such interference may also have compensated for the effect of Panodan TR on the sensory properties.

The variables measured by CLSM were all located together in the second quadrant (Fig. 5d). This could probably be ascribed to the fact that FE2, which had very high values for all CLSM variables, was located in the second quadrant in the scores plot (Fig. 4).

PC 5 and PC 6

PC5 and PC6 described 12% of the variation in x and 25% of the variation in y. In the scores plot, F1 and F2 both had positive values for PC5, whereas all the remaining mayonnaises had negative values for PC5 (Fig. 6). In the loadings plot, "Feggy-14", "Afish-14",

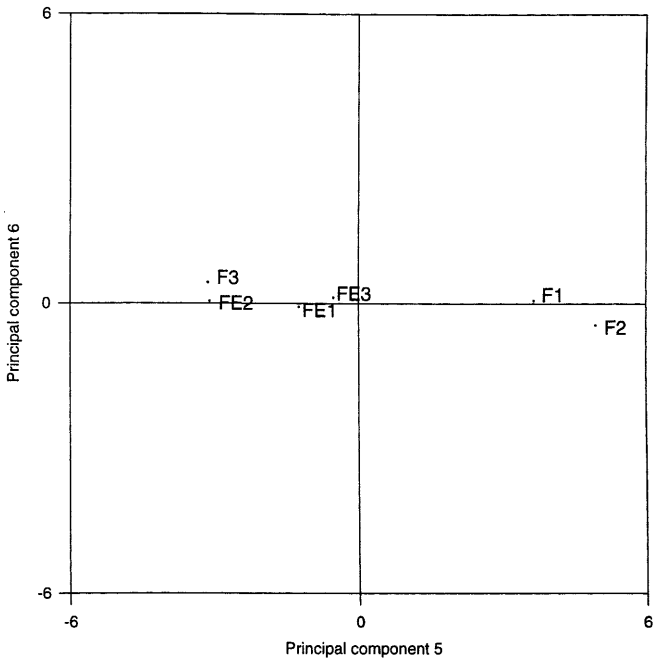

Fig. 6 Scores plot of PC5 vs PC6 from DPLSR analysis of sensory, PV, rheological, droplet size and microscopy data. For abbreviations, see Fig. 2

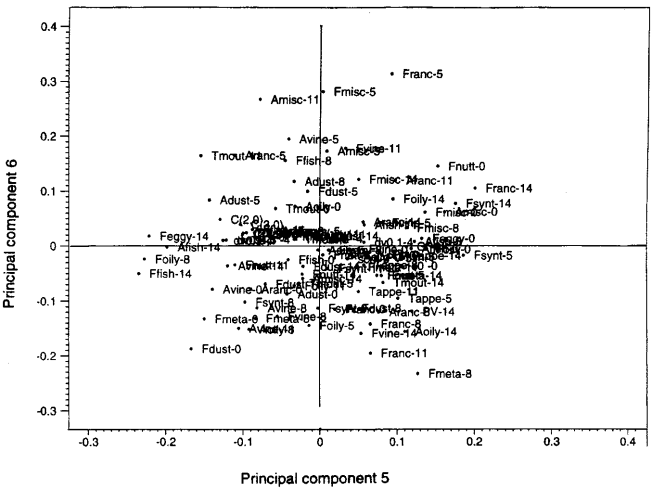

Fig. 7 Loadings plot of PC5 and PC6 of all variables corresponding to scores plot in Fig. 6. For abbreviations, see Figs. 2 and 3

"Foily-8" and "Ffish-14" were located far to the left, whereas "Franc-14", "Fsynt-14" and "Fsynt-5" were located far to the right (Fig. 7). This meant that PC5 mainly described differences between samples with respect to rancid, synthetic, fishy and egg-yolk-like flavours after 14 weeks of storage. After 14 weeks of storage, "Afish" and "Ffish" were located opposite to "Franc". This indicated that rancid flavour and fishy flavour were negatively correlated. Thus, samples with a high rancid flavour score had a low fishy flavour score, and vice versa. However, as this correlation was only observed after 14 weeks of storage, it is difficult to conclude that this trend was present throughout the storage period. PC5 and PC6 did not provide any clear information on the effect of antioxidants nor emulsifier addition. These effects were described mainly by PC1, PC2, PC3 and PC4, as discussed above. PC5 and PC6 rather described variation between replicates and differences among flavour and aroma descriptors at the end of the storage period.

DPLSR analysis on sensory and GC-MS data

In the DPLSR analysis of GC-MS and sensory data, six PCs were validated and the results are shown in Figs. 8-11. These six components explained 63% of the variation in the x-data and 59% of the variation in the y-data.

Antioxidant and emulsifier differences. The scores plot of PC 1 and PC 2 (Fig. 8) showed that they explained 31% of the variation in the x-data and 17% of the variation in the y-data. All mayonnaises without extra emulsifier (F1, F2 and F3) were grouped together in the third quadrant, and mayonnaises with propyl gallate and with emulsifier (FE2 and FE3) were located in the first quadrant. Finally, mayonnaise without propyl gallate and with extra emulsifier (FE1) was located alone

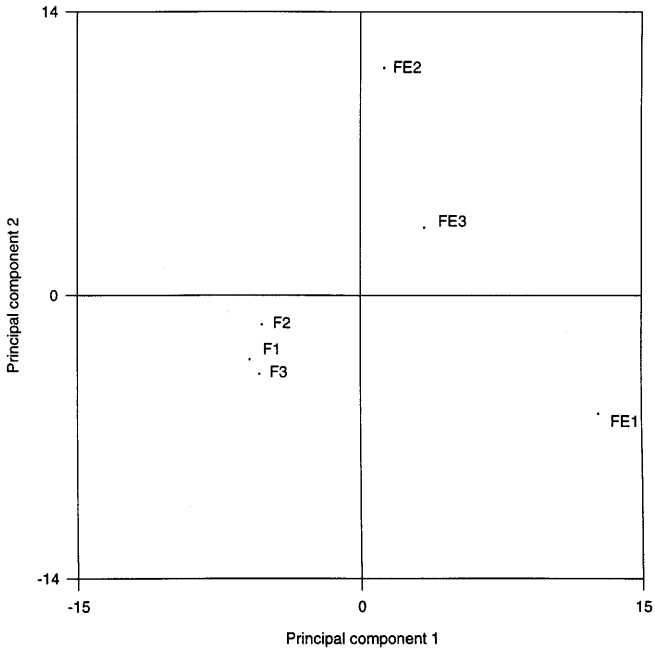

Fig. 8 Scores plot of PC1 vs PC2 for DPLSR analysis of sensory and GC-MS data. For abbreviations, see Figs. 1 and 2

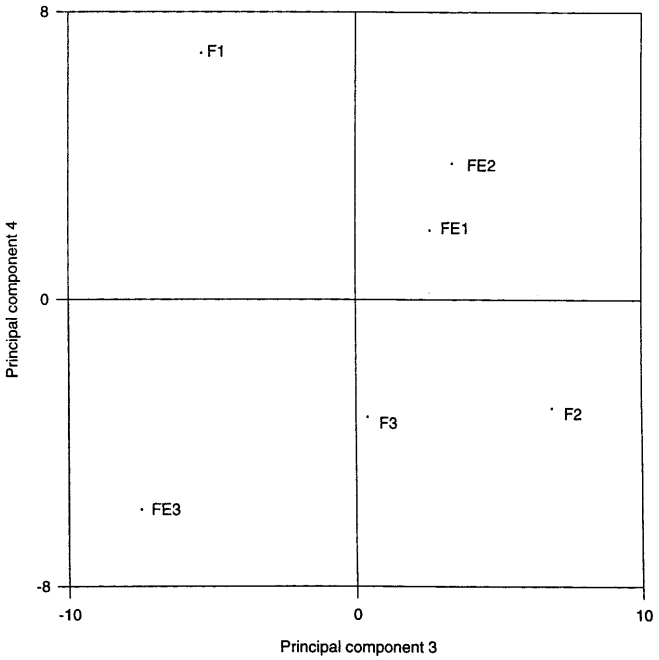

Fig. 9 Scores plot of PC3 vs PC4 for DPLSR analysis of sensory and GC-MS data. For abbreviations, see Figs. 1 and 2

in the fourth quadrant. Thus, this plot of PC1 vs PC2 did not show the same structure in the data as was observed in plots of PC1 and PC2 from the DPLSR analysis of the sensory data and the data from the other analyses (Fig. 2). The PC1 vs PC2 plot (Fig. 8) from the sensory/GC-MS data analysis mainly described differences between mayonnaises with and without extra emulsifier, whereas the PC1 vs PC2 plot of the sensory data and the other data (Figs. 2, 3a–d) mainly described differences between mayonnaises caused by the antioxidant addition. Thus, surprisingly, emulsifier addition apparently caused greater variation in the composition of volatile compounds than did addition of propyl gallate. However, the scores plot of PC3 and PC4 (Fig. 9) showed similarities to the data structure in Fig. 2. Thus, in both plots, FE3, F3 and F2 were located on the opposite side of the axes to FE1 and F1. However, in Fig. 9, FE2 was located together with FE1, whereas this was not the case in Fig. 2. Nevertheless, the plots of PC3 of PC4, to a certain extent, also described the variation in the variables caused by the addition of antioxidants, as did Fig. 2. Furthermore, differences between the three mayonnaises with emulsifiers (FE1, FE2 and FE3) could also be interpreted from the plot of PC1 vs PC2 (Fig. 8) with FE1 data located in the fourth quadrant and FE2 and FE3 separated into two distinct clusters in the first quadrant. Plots of PC5 vs PC6 are not shown, as these PCs mainly described differences between replicates.

Loadings plots. In the loadings plots of PC1 vs PC2 (Fig. 10) and PC3 vs PC4 (Fig. 11) only the legends of the fishy plus rancid flavours and aromas are included. Likewise, only the numbers of peaks that are discussed in the interpretation of the plots are shown in the figures. This was done in order to make the plots more legible. Another reason was that the GC-MS data were only used to interpret differences between samples with respect to the fishy and rancid off-flavours. The location of the remaining sensory variables and peaks are therefore only indicated by dots.

In the loadings plot of PC1 vs PC2 (Fig. 10), most of the peaks obtained in the fresh samples were located to the right in the plot. PC1 thus explained differences in peak areas for several compounds in the fresh samples. "Ffish" moved from negative PC2 values to positive PC2 values over time. However, there did not seem to be any clear trend for "Ffish" with respect to PC1 values. PC2 values for "Afish" were all positive except for "Afish-5" (Fig. 10). As FE1 had negative PC1 values (Fig. 8), the PC1 vs PC2 loadings plot (Fig. 10) confirmed the previous observation that propyl gallate promoted the development of fishy off-flavours in mayonnaise with extra emulsifier. However, the effect of propyl gallate in mayonnaise without extra emulsifier could not be deduced from this plot. Interestingly, "Franc" moved in the opposite direction as compared to "Ffish". Thus, both "Franc-11" and "Franc-14" had negative PC2 values whereas "Franc-0", "Franc-5" and "Franc-8" had positive PC2 values. The same trend was observed for rancid aroma ("Aranc"). The reason behind this finding may be that the pronounced fishy off-flavours in FE2 and FE3 shielded the rancid off-flavours in these samples.

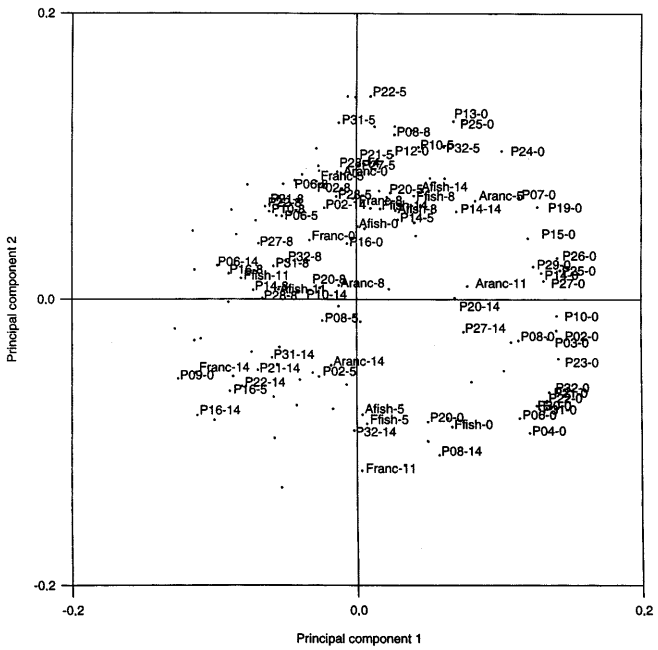

Fig. 10 Loadings plot of PC1 vs PC2 for selected sensory variables and selected volatile compounds corresponding to scores plot in Fig. 8. *Px-y* Peak no. x, y weeks of storage; for other abbreviations, see Figs. 2 and 3

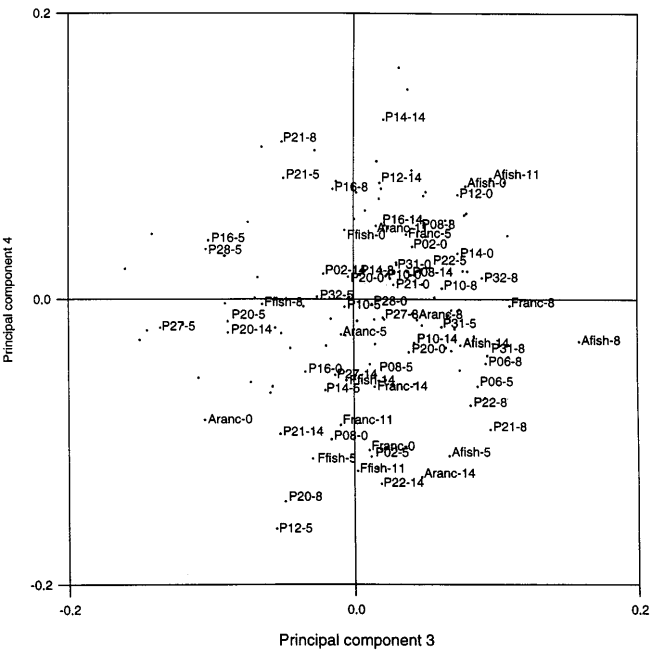

Fig. 11 Loadings plot of PC3 vs PC4 for selected sensory variables and selected volatile compounds corresponding to scores plot in Fig. 10. For abbreviations, see Figs. 2, 3 and 10

In the loadings plot of PC3 vs PC4 (Fig. 11) "Ffish" moved from slightly positive PC4 values to negative PC4 values, whereas PC3 values were close to zero for all five "Ffish" variables. FE2 did not have a negative PC4 value. Nevertheless, this observation once more confirmed the promoting effect of propyl gallate on the development of a fishy off-flavour.

Volatile compounds responsible for rancid and fishy off-flavour. Fishy and rancid flavours were pronounced in mayonnaises containing propyl gallate (Figs. 3a,b, 10, 11). To investigate which of the identified volatile compounds were mainly responsible for a rancid and fishy off-flavour, peaks located in the vicinity of "Franc", "Aranc", "Ffish" and "Afish" were compared for the different weeks (Figs. 10, 11). "Vicinity" was defined as points located within approximately 0.03 units from "Ffish", "Afish", "Franc" and "Aranc" on the axes of PC1, PC2, PC3 and PC4. Plots of PC1 vs PC2 and PC3 vs PC4 were used for this comparison (Figs. 10, 11). Subsequently, the peak areas of compounds detected by GC-MS that appeared to correlate well with these sensory variables were analysed further. The criterion for including compounds in the further data analysis was that each compound was observed in the vicinity of the above-mentioned sensory variables more than once. The following compounds were eventually included for further analysis: ethyl benzene (peak 2), 3-furaldehyde (peak 6), cyclohexanone (peak 8), 2-pentyl furan (peak 10), D-limonene (peak 12), *t*-2-heptenal (peak 14), 1-methyl-4-(1-methylethyl)-benzene (peak 16), 1-octen-3-ol (peak 20), *t*,*c*-2,4-heptadienal (peak 21), *t*,*t*-heptadienal (peak 22), *t*,*c*-2,6-nonadienal (peak 27), *t*-2-nonenal (peak 28), *t*,*t*-2,4-decadienal (peak 31), and 2-undecenal (peak 32) (Figs. 10, 11, Table 4).

FE1 and F1 (both without propyl gallate) developed a less distinct fishy flavour than mayonnaises with propyl gallate (FE2, FE3, F2 and F3; Figs. 2, 3a,b). This observation was used to identify those compounds whose peak areas increased more in FE2, FE3, F2 and F3 than in FE1 and F1. The peak areas of the following compounds increased over time, and their peak areas for mayonnaises FE1 and F1 after 5–14 weeks of storage generally were lower than for the other mayonnaises: ethylbenzene (peak 2), 3-furaldehyde (peak 6), *t*,*t*-2,4-heptadienal (peak 22) and *t*,*t*-2,4-decadienal (peak 31). Thus, the peak areas of these four compounds correlated with the fishy off-flavour which developed in samples with propyl gallate, in particular. Table 6 shows the peak areas for these compounds. It is worth noting that the peak areas for all of these four compounds in R1 and FE1 decreased between week 0 and week 5 for unknown reasons. In accordance with lipid autocatalytic oxidation theory, the peak areas for all these compounds were almost constant between week 5 and 8, but subsequently increased steeply between week 8 and 14 (Table 6). In our previous studies [26] we have shown that 2-pentenal, octanal and, to a certain extent, 2,4-heptadienal partitioned into the water phase of mayonnaise, where they were more volatile than in the oil phase. As the chain-lengths of these compounds were within the same range as those of 3-

Table 6 Normalized peak areas for potentially interesting compounds after 0, 5,8 and 14 weeks of storage. Peak areas were normalized against an internal standard. For abbreviations, see Table 1

Code	Peak 2 (ethyl benzene)				Peak 6 (3-furaldehyde)				Peak 22 (*t,t*-2,4-heptadienal)				Peak 31 (*t,t*-2,4-decadienal)			
	0 (weeks)	5	8	14	0 (weeks)	5	8	14	0 (weeks)	5	8	14	0 (weeks)	5	8	14
R1	158±1	40±1	36±5	111±18	13±2	3±0	2±1	7±1	11±1	3±1	3±1	17±3	27±7	4±1	2±1	11±4
FE1	175±11	34±2	29±2	88±6	24±1	5±0	6±1	12±2	13±0	2±0	3±0	18±2	33±2	2±0	1±0	5±0
FE2	92±17	33±2	38±13	112±35	10±3	6±0	9±3	25±16	2±0	5±0	5±1	16±1	3±2	5±1	3±2	4±3
FE3	77±2	34±3	32±3	101±2	11±1	6±1	7±1	21±3	4±1	3±1	4±1	24±7	4±3	3±1	2±1	9±5
F1	49±8	33±1	32±2	96±10	8±1	5±0	6±2	22±10	1±0	2±0	3±0	21±2	1±1	1±0	2±0	7±2
F2	59±12	36±2	33±2	90±4	13±2	7±0	10±0	30±2	2±1	3±1	5±2	30±3	1±2	3±2	5±4	14±0
F3	40±1	36±1	30±8	106±10	11±0	5±0	7±0	28±2	1±0	2±1	4±0	34±3	2±1	2±1	2±1	7±0

furaldehyde, *t,t*-2,4-heptadienal and *t,t*-2,4-decadienal, it is therefore likely that these latter three compounds also partitioned into the water phase. Thus, even a very low concentration of these compounds may have influenced negatively the sensory qualities of mayonnaise as perceived by the assessors. Although ethyl benzene has not previously been identified as an oxidation compound, our data suggested that this compound may stem from the oxidation of rapeseed oil and not from fish oil. In weeks 5 and 8 differences in the concentrations of ethyl benzene between the different mayonnaises were small, whereas the peak area of this compound after week 14 was lowest for FE1, followed by F2 and F1.

The peak areas of the second compound, 3-furaldehyde, for the sample without fish oil (R1) was lower throughout the whole storage period than for mayonnaise with fish oil, except in the fresh mayonnaises. Furthermore, mayonnaise with propyl gallate developed higher concentrations of 3-furaldehyde than samples without propyl gallate. As previously mentioned, furan derivatives have been reported as oxidation products [32]. The peak areas of the third compound, *t,t*-2,4-heptadienal, also increased in R1 over time. It is generally recognised [33] that this compound may be generated in oxidised soybean oil, and has also been found in oxidised fish oil [28]. In the latter study it was suggested that 2,4-heptadienal originated from n-3 fatty acids. Since rapeseed oil also contains a n-3 fatty acid, namely linolenic acid, this may explain why the peak areas for 2,4-heptadienal also increased in the mayonnaise without fish oil. 2,4-Heptadienal is associated with a glue-like odour [28]. In addition, it has been reported that 2,4-heptadienal has a very low odour threshold in oil (0.04 μg/g) [32]. The fourth compound, *t,t*-2,4-decadienal, has been reported as an oxidation product in fish oil originating from n-3 fatty acids [28, 34]. It has a relatively low flavour threshold in oil (0.10 μg/g) [32]. Thus, our data were generally in agreement with those of other reports available on the subject.

Conclusions

As expected, and in accordance with our previous observations [25], addition of fish oil to mayonnaise caused the development of unpleasant, fishy, off-flavour compounds. However, the chemical oxidation data did not show that the mayonnaises with fish oil were more oxidised than the mayonnaise without fish oil. We therefore propose that the fishy off-flavour in mayonnaise with fish oil may be caused by small amounts of specific volatile off-flavour compounds, with low sensory threshold values, present in the water phase of mayonnaise. These off-flavour compounds apparently stem from the oxidation of EPA and DHA.

It was found that the addition of two different propyl gallate systems to mayonnaise with fish oil not only influenced negatively its sensory quality, but also affected the structure and rheological properties of the mayonnaise. Firstly, propyl gallate caused a faster development of fishy and rancid off-flavours, and this was most marked for the water-soluble preparation (Grindox 413). Secondly, PV were also slightly increased in mayonnaise with propyl gallate. Thus, the two propyl gallate systems employed (Grindox 413 and Grindox 370) proved to be pro-oxidants and not antioxidants in mayonnaise with fish oil. Thirdly, the propyl gallate systems employed also gave rise to thinner, less viscous mayonnaises with bigger droplets and a lower gel strength. It is likely that the change in structural and rheological properties caused by the addition of propyl gallate influenced the release of flavour compounds and thereby the sensory qualitites of the mayonnaise as perceived by the assessors. Alternatively, the propyl gallate mixtures were able to promote the partitioning of pro-oxidant metal ions and/or off-flavours into the water phase. However, the data did not allow us to draw any conclusions about the mechanistic details, nor whether the change in structure was caused by the propyl gallate itself or the carriers employed. However, it was apparent that the structural changes influenced the oxidation processes.

The addition of extra emulsifier (Panodan TR) apparently did not affect the activity of propyl gallate.

Neither did it affect the development of fishy and rancid off-flavours in mayonnaise without antioxidant. However, a very interesting observation was that the effect of Panodan TR on the droplet size and rheological properties depended on whether the propyl gallate systems were present or not. Thus, if no propyl gallate was present Panodan TR gave rise to bigger droplets and weaker rheological properties. The opposite was observed when propyl gallate was present. Thus, a primary conclusion of this study was that the emulsifier employed (Panodan TR) interacted with the commercial propyl gallate systems and that this interaction affected the physical composition and functional properties of the mayonnaise system.

The oxidation compounds, 3-furaldehyde, *t*,*t*-2,4-heptadienal and *t*,*t*-2,4-decadienal, and perhaps also ethyl benzene, seemed to correlate with the fishy and rancid off-flavours that developed in mayonnaise with propyl gallate, in particular. The compounds *t*,*t*-2,4-heptadienal and *t*,*t*-2,4-decadienal have been identified as off-flavours from pure fish oil, and they apparently play a significant role in the development of fishy and rancid off-flavours in fish-oil-enriched mayonnaise, even when the mayonnaise is not significantly oxidised as judged from chemical analyses.

The present study thus demonstrated that in food emulsions such as mayonnaise, oxidation and antioxidation are very complex processes not only involving chemical but also physical processes. Therefore, a multivariate approach appears useful or even necessary in order to enhance our knowledge about these processes in the future. Furthermore, a better understanding of interactions among functional ingredients, i.e. antioxidants, stabilisers and emulsifiers, appear necessary to intelligently develop food emulsions of a high nutritional and sensory quality.

Acknowledgements The authors wish to thank Harald Martens (Institute for Biotechnology, Technical University of Denmark) for advice on the data analysis. We also wish to thank Trang Thi Vu, Suvra Datta, Kirsten Brandt, Birgitte Vesterlund Pedersen, Charles Johansen, and Emil Thorsteinsson for their excellent technical assistance. Furthermore, we are grateful to Else Green, Suzie Reitz and the members of the sensory panel for their skilful sensory work, and to Grethe Hyldig for performing the microscopy. This study was financed by the Danish Association of Fish Meal and Fish Oil Manufacturers, Danisco Ingredients, Denmark, and the Danish Food Research Programme, FØTEK II.

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