Lipid Oxidation in Fish Oil Enriched Mayonnaise: Calcium Disodium Ethylenediaminetetraacetate, but Not Gallic Acid, Strongly Inhibited Oxidative Deterioration

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The antioxidative effects of gallic acid, EDTA, and extra emulsifier Panodan DATEM TR in mayonnaise enriched with 16% fish oil were investigated. EDTA reduced the formation of free radicals, lipid hydroperoxides, volatiles, and fishy and rancid off-flavors. The antioxidative effect of EDTA was attributed to its ability to chelate free metal ions and iron from egg yolk located at the oil−water interface. Gallic acid reduced the levels of both free radicals and lipid hydroperoxides but promoted slightly the oxidative flavor deterioration in mayonnaise and influenced the profile of volatiles. Gallic acid may therefore promote the decomposition of lipid hydroperoxides to volatile oxidation products. Addition of extra emulsifier reduced the lipid hydroperoxide levels but did not influence the level of free radicals or the oxidative flavor deterioration in mayonnaisse; however, it appeared to alter the profile of volatiles. The effect of the emulsifier on the physical structure and rheological properties depended on the presence of antioxidants.

INTRODUCTION

There has been considerable interest during the past three decades in the highly polyunsaturated marine *n*−3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), due to their reported positive effects on cardiovascular diseases and visual function in infants (*1*, *2*). The incorporation of EPA- and DHA-rich oils into foods requires efficient antioxidants to retain these unsaturated lipids and protect against oxidative flavor deterioration. However, at present the comprehension of oxidation and antioxidation mechanisms in complex food products is inadequate. We are currently investigating oxidation and antioxidation mechanisms in fish oil enriched mayonnaise to improve oxidative protection of such real food emulsions (*3*−*7*).

The polarity and thereby the partitioning properties of an antioxidant appear to influence its activity in oil/water (o/w) emulsions. In heterophasic food systems, such as mayonnaise, antioxidants may partition into at least three different phases, the oil phase, the aqueous phase, and the oil−water interface (*8*, *9*). Thus, in mayonnaise, we previously found that tocopherols were located in the oil phase, whereas the more polar antioxidants were concentrated in the aqueous phase, for example, 80% of gallic acid, but that a significant proportion was distributed into the interface, for example, ∼20% of the gallic acid (*9*). When the lipophilic antioxidants tocopherol and methyl carnosate were added to model emulsions containing 10% corn oil emulsified with 1% Tween 20 (percentages refer to by weight of emulsion), these antioxidants partitioned into the oil phase (*10*). These antioxidants were more efficient in o/w emulsions than their hydrophilic counterparts Trolox and rosmarinic acid, which in partition studies were shown to be located in the aqueous phase or retained in the Tween 20 phase of 10% corn oil−1% Tween 20 mixtures (*10*−*12*). These results confirm that a so-called "polar paradox" exists with regard to efficacy of antioxidants in emulsions (*13*).

Mayonnaise has a much higher oil content than the model emulsions usually employed in antioxidant studies (80 versus 10%) (*10*−*12*). Furthermore, in mayonnaise, egg yolk, which is a complex mixture of lipids and proteins, is used as emulsifier in contrast to the more well-defined and simpler emulsifier, Tween 20, used in the model emulsions (*10*−*12*). The relationship between the polarity of antioxidants and their antioxidative efficacy in mayonnaise may therefore be different from the observed inverse correlation between polarity of

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antioxidants and their efficacy in model emulsions. To investigate this hypothesis, gallic acid was included in the present study, as 80% gallic acid partitioned into the aqueous phase of mayonnaise (*9*).

Egg yolk has a high content of iron (40 μg/g). It is well-known that transition metals accelerate oxidation reactions by hydrogen abstraction and peroxide decomposition, which results in the formation of free radicals (*14*). Moreover, our previous investigations indicated that iron ions, originating from egg yolk, participate in the initiation of the oxidation processes in fish oil enriched mayonnaise (*15, 16*) and that EDTA can inhibit the formation of free radicals in this system (*16*). Hence, we wished to investigate whether EDTA was also able to inhibit the oxidative flavor deterioration in mayonnaise. EDTA is allowed as an additive in mayonnaise and other emulsified sauces in the European Union (*17*).

Our previous results indicated that the oil droplet size influenced the oxidation rate in fish oil enriched mayonnaise (*6*). The effect of adding extra emulsifier (Panodan DATEM TR; *18*) to the mayonnaise was therefore included in this study in order to manipulate the physical structure of the mayonnaise and in turn perhaps affect the antioxidant activity. Oxidation was monitored by sensory evaluation and determination of free radicals, lipid hydroperoxides, and secondary volatile oxidation products. Oil droplet sizes and rheological properties of the mayonnaises were also measured.

MATERIALS AND METHODS

Materials. Refined rapeseed oil was obtained from Aarhus Olie A/S, Aarhus, Denmark [composition of unsaturated fatty acids: 18:1, 60.3%; 18:2, 20.6%; 18:3, 9.0%; 20:1, 1.5%; peroxide value (PV), 0.3 mequiv/kg; anisidine value (AV), 1.7; free fatty acids (FFA), 0.10%; iron, <0.1 μg/g; copper, <0.05 μg/g]. Raw fish oil from sand eel was obtained from Esbjerg Fiskeindustri, Esbjerg, Denmark. The fish oil was refined and deodorized at the pilot plant of the Department of Biotechnology, Technical University of Denmark (composition of unsaturated fatty acids, 16:1, 11.7%; 18:1, 7.7%; 18:2, 3.4%; 18:3, 1.8%; 18:4, 4.6%; 20:1, 8.4%; 20:5, 7.9%; 22:1, 11.2%; 22:6, 7.8%; PV, <0.3 mequiv/kg; AV, 3.8; FFA, 0.06%; iron, <0.1 μg/g; copper, <0.05 μg/g; no additions were made to stabilize the oil). Egg yolk with 3% NaCl was from Sanovo Foods, Odense, Denmark (PV, 0.8 mequiv/kg; iron, 41 μg/g; copper, 1.2 μg/g), tarragon vinegar (7%) was from Lagerberg (Hamburg, Germany) (iron, 0.8 μg/g; copper, <0.05 μg/g), and potassium sorbate (food grade) was purchased from Merck (Darmstadt, Germany). Grindsted FF DC stabilizer (guar gum and sodium alginate) and Panodan DATEM TR emulsifier (containing diacetyltartaric acid ester of mono- and diglycerides of fatty acids) were donated by Danisco Ingredients (Brabrand, Denmark) (*18*). Gallic acid and EDTA (calcium disodium ethylenediaminetetraacetate) were from Sigma (Steinheim, Germany).

Production of Mayonnaises and Sampling. Mayonnaise batches of 25 kg were produced in a continuous process on a Schröder Combinator pilot plant (Schröder and Co., Lübeck, Germany). Each batch contained by weight 16.0% fish oil, 64.0% rapeseed oil, 10.4% water, 4.0% vinegar, 0.3% salt (NaCl), 1.0% sugar, 0.1% potassium sorbate, 4.0% egg yolk, and 0.15% Grindsted FF DC. Gallic acid, EDTA, and Panodan DATEM TR, all evaluated at an addition level of 200 ppm, were mixed into the water phase before mayonnaise production (Table 1). Mayonnaises were stored at 20 °C for 4 weeks in brown glass jars. At each of the storage time points a new jar was opened for each different type of sampling, that is, for GC-MS, PV, ESR, and sensory analysis. The jars for lipid hydroperoxide and GC-MS measurements were frozen at the set sampling time and kept at −80 °C until analysis, whereas all other analyses were made directly after sampling. Except for GC-MS samples that were taken directly in the frozen state

Table 1. Experimental Design

code name[a]	addition of antioxidant (200 ppm[b])	addition of Panodan DATEM TR (200 ppm[b])
Ref	−	−
E_Ref	−	+
gallic	gallic acid	−
E_Gallic	gallic acid	+
EDTA	EDTA	−
E_EDTA	EDTA	+

[a] Abbreviations: Ref, reference mayonnaise; Gallic, gallic acid addition; EDTA, EDTA addition; E_, addition of extra emulsifier. [b] Concentrations are gross basis in whole mayonnaise.

(see below), mayonnaises were gently mixed prior to sampling to avoid gradient effects.

Sensory Analysis. Descriptive sensory analysis was used to evaluate mayonnaises (*4*). The following attributes were evaluated: *aroma* (vinegar/acidic, fishy/train oil, rancid, oily, dusty, miscellaneous); *texture* (appearance and mouthfeel); *flavor* (vinegar/acidic, fishy/train oil, rancid, oily, dusty/dry, synthetic, metallic, nutty, egg yolk, and miscellaneous). A scale from 0 to 9 was employed, where 0 indicated no intensity or thin mayonnaise and 9 high intensity or thick (viscous) mayonnaise.

Determination of the Tendency of Formation of Radicals. After addition of the spin trap *N-tert*-butyl-α-phenyl-nitrone, mayonnaise was incubated at 37 °C for 24 h and the formation of radicals was measured by electron spin resonance spectroscopy (ESR) as described by Thomsen et al. (*19*), employing 12-doxylstearic acid as an external standard in mayonnaise.

Determination of Lipid Hydroperoxides. Mayonnaises were gently thawed (2 h at 5 °C) and separated by centrifugation at 2500*g* for 10 min at 4 °C. Hydroperoxy triacylglycerols (TAGOOH) and hydroperoxy cholesterol esters (CEOOH) in the isolated, clear oil phase were quantified by an HPLC method based on size exclusion separation and fluorescence detection of diphenyl-1-pyrenylphosphine (DPPP) oxides formed from the reaction between DPPP and lipid hydroperoxides. Monohydroperoxy trilinolein was employed as an external standard (*20*).

Determination of Secondary Volatile Oxidation Products. Mayonnaise (4 g) and the internal standard, *n*-dodecane, were weighed into a pear-shaped glass flask followed by collection of volatiles. Volatile acids, notably acetic acid, were trapped and removed by potassium hydroxide during dynamic headspace sampling, and the collected volatiles were separated and quantified by gas chromatography−mass spectrometry (GC-MS) as previously described (*21*).

Particle Size Measurements by Laser Diffraction Analysis. The size of the oil droplets was determined by laser diffraction measurements. Mayonnaise samples (5 g) were solubilized in 45 g of SDS buffer (10 mM NaH$_2$PO$_4$, 5 mM SDS, pH 7) and measured as previously described (*6*). Particle sizes were reported as volume mean diameters *D*[4,3], surface mean diameters *D*[3,2], and the 10th, 50th, and 90th percentiles *D*[v0.1], *D*[v0.5], and *D*[v0.9], respectively, of the droplet distribution.

Rheological Measurements. Measurements of stress sweep, yield stress, and viscosity at 1.3 s^{-1} were carried out as previously described (*4*). From the stress sweep measurements, values for the gel strength (*G**), the phase angle, and the critical stress were obtained.

Data Analysis. To correlate the different analytical data, discriminant partial least-squares regression (DPLSR) was employed (*4*). The software program Unscrambler version 7.51 (CAMO, Oslo, Norway) was used as an aid for this analysis.

ANOVA PLSR on Sensory Data. Prior to the main data analysis differences in the sensory score levels of the assessors were projected away by a preliminary ANOVA partial least-squares regression (APLSR) (*4*). Differences in how assessors scale sensory scores is a recognized problem in sensory assessement, and the phenomenon occurs despite intensive

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training of panelists (*22*). The means of the residuals obtained after the optimal number of principal components were then used for the subsequent DPLSR analysis because only the APLSR residuals will be unaffected by differences in the assessors' use of the sensory scale; that is, differences in residuals will be caused only by differences in samples (treatments) and not be impacted by differences in the assessors' sensory score levels.

DPLSR Analyses. Two different DPLSR analyses were performed. In both DPLSR analyses addition of antioxidants (gallic acid, EDTA, and no antioxidant = Ref) and emulsifier addition (emulsifier) were used as design variables. In the first analysis, sensory data (residuals from the preliminary APLSR), data from free radical and lipid hydroperoxide determination, rheology, and particle size measurements were used as X variables, and design variables were used as Y variables. In the second analysis, sensory and GC-MS data were used as X variables and design variables were used as Y variables. Full cross-validation on all replicates and mean values were used. The validity of these models was examined by calculating a model based on the replicates only. The jack-knifing principle was used to identify variables with significant regression coefficients ($p < 0.1$) for each of the design variables (*23*). For instance, if a variable (e.g., concentration of a given volatile) has a significant negative regression coefficient for a given design variable (e.g., EDTA addition), then EDTA addition significantly reduced the concentration of that volatile. Correlation loadings plots were obtained from the DPLSR analysis. In this plot the inner and outer ellipses indicate 50 and 100% explained variance, respectively. Variables located near each other and close to the outer ellipse are thus positively correlated with a correlation coefficient of ~1.0.

RESULTS

The original measured data will be discussed in connection with the interpretation of the models obtained from the two DPLSR analyses.

DPLSR Analysis on All Data Except GC-MS Data. Three principal components (PCs) were validated, explaining 57% of the variance in the X variables and 53% of the variance in the Y variables.

PCs 1 and 2. PC1 and PC2 explained 29 and 15% of the variance in the X variables and 21 and 19% of the variance in the Y variables, respectively. The total variance explained by PC1 and PC2 is relatively low. However, it is important to note from the plots (Figure 1) that the design variables EDTA, Ref, and Gallic as well as most of the important experimental variables (Ffish, Franc, ESR, TAGOOH, the rheology variables, and most of the droplet size variables) are all located outside the inner ellipse and some are even located near the outer ellipse. Because these ellipse borders indicate the loci for 50 and 100% explained variance of the individual input variables, the loading structure signifies that 50–100% of the variance is explained for these variables by PC1 and PC2. In the scores plot mayonnaises with extra emulsifier were located close to the corresponding mayonnaises without emulsifier. The location of the mayonnaises without emulsifier in the scores plot corresponded to the location of the corresponding design variables; that is, reference is made to point locations of variables Ref, EDTA, and Gallic, in the correlation loadings plot, and therefore only the latter are shown (Figure 1). The correlation loadings plot was split into three plots showing the flavor and aroma variables (Figure 1a), the lipid hydroperoxide (TAGOOH and CEOOH) and the free radical variables (ESR) (Figure 1b), and the droplet size, rheology, and appearance and mouthfeel variables (Figure 1c), respectively. EDTA was located to the far right close to the PC1 axis, whereas Ref and Gallic were located to the left in

the second and third quadrants, respectively (Figure 1). Hence, PC1 explained differences between the EDTA-containing mayonnaises and the other mayonnaises (Ref and Gallic), and PC2 explained differences between the reference mayonnaises and the mayonnaises with gallic acid. The emulsifier design variable was located close to the origin, which indicates that this variable was not explained by PC1 and PC2.
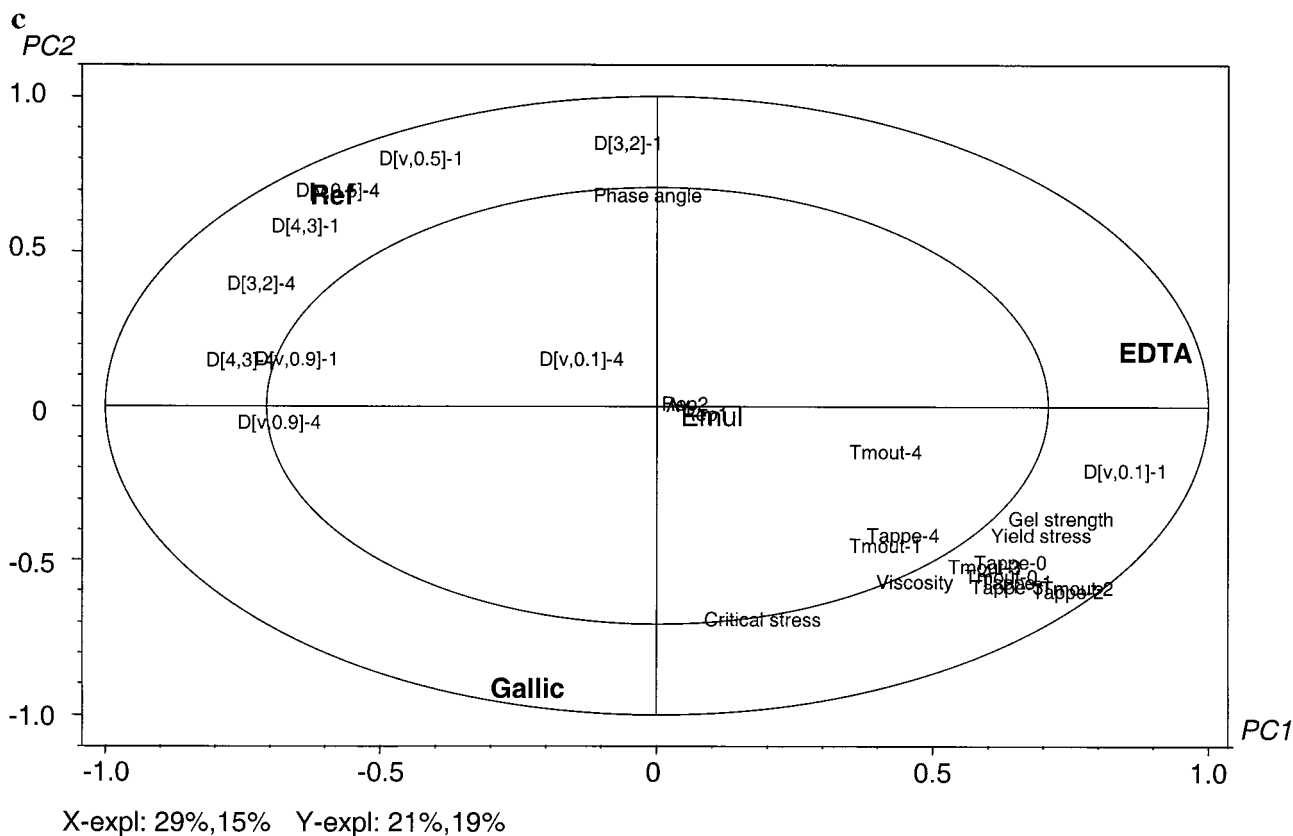
Effect of Antioxidants on Aroma and Flavor Variables. All fishy and rancid sensory variables in week 0 had PC1 values close to 0, which indicated that the freshly produced mayonnaises had similar intensities of these off-flavors (Figure 1a). However, in the stored mayonnaises, most of these variables as well as the metallic variables were located in the third quadrant, near the borderline of the ellipse indicating 100% explained variance (see Materials and Methods). Thus, the intensity of fishy, rancid, and metallic off-flavors was lower in mayonnaises with EDTA than in the other mayonnaises, and mayonnaises with gallic acid, furthermore, had higher intensities of these off-flavors than the reference mayonnaises (Ref and E_Ref). The sensory data supported the finding that the intensity scores for fishy flavor were significantly lower ($p < 0.05$) in mayonnaises with EDTA than in the other mayonnaises after 1–4 weeks (Table 2). Moreover, the scores for both fishy aroma and flavor remained <0.5 in mayonnaises with EDTA throughout the whole storage period, which indicated that no fishy off-flavor was detected (Table 2). Mayonnaises with gallic acid appeared to have slightly more fishy aroma and flavor than mayonnaises without antioxidant during most of the storage period, but the difference was not significant ($p > 0.05$) (Table 2).

The original sensory data also indicated that mayonnaises with EDTA tended to have less rancid aroma and flavor than the other mayonnaises and that mayonnaises with gallic acid appeared to have slightly more rancid flavor than mayonnaises without (data not shown). However, the rancid aroma scores increased only slightly during storage in all mayonnaises.

All variables describing the egg yolk, oily, and miscellaneous flavors moved toward the EDTA design variable with time (Figure 1a). The locations of these variables in week 0 varied between slightly negative to slightly positive PC1 values. Furthermore, the nutty variables always had positive PC1 values. Thus, mayonnaises with EDTA had more egg yolk, oily, nutty, and miscellaneous flavors than the other mayonnaises during the later part of the storage period. The descriptors used by the assessors to describe the miscellaneous flavor included cream-like and salty. The objectionable fishy and rancid off-flavors increased with time in mayonnaises without EDTA, and these off-flavors may shield the relatively pleasant flavors (oily, nutty, egg yolk, and miscellaneous) in mayonnaises without EDTA. Thus, the movement of variables describing the pleasant flavors toward the EDTA design variable with time could be due to the pleasant flavors being more and more shielded by the fishy off-flavors in mayonnaises without EDTA.

The variables describing the dusty aroma and flavor, the oily and miscellaneous aroma, the vinegar aroma and flavor, and synthetic flavor were either scattered around the origin or there was no trend in their location pattern (data not shown). These observations indicated that these variables were not well explained by PC1 and

a

PC2

1.0

0.5

Ref

Fmisc-0

Fnutt-2

Fnutt-3

Fmisc-4

Fmisc-1   Fnutt-1   Fmisc-2

Fmisc-3

EDTA

Feggy-4

0

Fmeta-3   Foily-1   Foily-0   Foily-2   Rep2   Emul   Fnutt-4   Feggy-2   Foily-4   Feggy-3

Afish-0   Franc-0

Ffish-3   Fmeta-4   Feggy-0

Afish-4

Afish-3   Franc-4   Aranc-0   Feggy-1   Aranc-4

Franc-3   Fmeta-1   Foily-2   Fmeta-0

Ffish-2   Fmeta-2

Afish-1   Ffish-0

Franc-2   Aranc-3

Franc-1   Aranc-2

−0.5   Aranc-1

Gallic

−1.0

−1.0   −0.5   0   0.5   1.0   *PC1*

X-expl: 29%,15%   Y-expl: 21%,19%

b

PC2

1.0

Ref   CEOOH-3

TAGOOH-3

0.5   TAGOOH-2 / TAGOOH-0   CEOOH-2

ESR-2   TAGOOH-1   CEOOH-1

ESR-3 / ESR-4

ESR-1   CEOOH-4   **EDTA**

0   TAGOOH-4   Rep2   Emul

CEOOH-0

ESR-0

−0.5

Gallic

−1.0

−1.0   −0.5   0   0.5   1.0   *PC1*

X-expl: 29%,15%   Y-expl: 21%,19%

c

X-expl: 29%,15%   Y-expl: 21%,19%

Figure 1. Correlation loadings plot from DPLSR analysis for (a) sensory data, (b) lipid hydroperoxide (TAGOOH, CEOOH) and free radical (ESR) data, and (c) laser diffraction data and selected sensory and rheological data. Particle sizes measured by laser diffraction analysis are reported as volume and surface mean diameters $D[4,3]$ and $D[3,2]$, respectively, and as 10th, 50th, and 90th percentiles of the droplet distribution $D[v,0.1]$, $D[v,0.5]$, and $D[v,0.9]$, respectively. The first letter in the variable name for sensory scores refers to either aroma (A), flavor (F), or texture (T). Abbreviations after the first letter refer to the following: fish, fishy/train oil; ranc, rancid; meta, metallic; oily, oily; eggy, egg yolk; nutt, nutty; misc, miscellaneous; appe, appearance; mout, mouthfeel. The number after the hyphen in the variable name refers to the storage time in weeks. Rep refers to the different replicates and Av to the mean value. Ref, reference mayonnaise without antioxidant; Gallic, addition of gallic acid; EDTA, addition of EDTA; Emul, addition of extra emulsifier

Table 2. Sensory Scores for Fishy Aroma and Flavor for Mayonnaises during Storage at 20 °C (Sensory Scale from 0 to 9; Mean Score $\pm$ SD, $n = 9-12$)[a]

	fishy aroma					fishy flavor				
code name	0 weeks	1 week	2 weeks	3 weeks	4 weeks	0 weeks	1 week	2 weeks	3 weeks	4 weeks
Ref	0.3 ± 0.4^a	1.3 ± 1.6^{ab}	1.5 ± 1.7^{ab}	2.0 ± 1.1^b	1.6 ± 1.4^{ab}	0.2 ± 0.4^a	2.1 ± 1.5^b	2.4 ± 1.6^b	2.8 ± 1.3^b	3.4 ± 1.8^b
E_Ref	0.2 ± 0.4^a	1.0 ± 0.9^{ab}	1.5 ± 1.5^{ab}	1.7 ± 2.2^{ab}	1.5 ± 1.4^{ab}	0.2 ± 0.5^a	1.0 ± 0.8^{ab}	2.2 ± 1.5^b	3.0 ± 2.3^b	3.0 ± 1.6^b
Gallic	0.3 ± 0.6^a	1.7 ± 1.8^b	2.0 ± 2.0^b	2.5 ± 1.9^b	2.1 ± 2.3^b	0.3 ± 0.4^a	2.1 ± 1.5^b	3.1 ± 2.0^b	3.4 ± 1.6^b	3.4 ± 2.4^b
E_Gallic	0.1 ± 0.2^a	1.4 ± 1.2^{ab}	2.7 ± 2.1^b	2.1 ± 1.6^b	1.4 ± 1.6^{ab}	0.6 ± 0.6^a	2.1 ± 1.0^b	3.6 ± 1.9^b	3.0 ± 1.6^b	3.3 ± 1.1^b
EDTA	0.1 ± 0.2^a	0.4 ± 0.9^a	0.1 ± 0.2^a	0.3 ± 0.6^a	0.1 ± 0.3^a	0.2 ± 0.3^a	0.2 ± 0.4^a	0.1 ± 0.3^a	0.3 ± 0.5^a	0.2 ± 0.3^a
E_EDTA	0.3 ± 0.4^a	0.3 ± 0.6^a	0.2 ± 0.2^a	0.2 ± 0.5^a	0.3 ± 0.6^a	0.2 ± 0.4^a	0.4 ± 0.5^a	0.3 ± 0.6^a	0.1 ± 0.2^a	0.1 ± 0.2^a

[a] For interpretation of code names please refer to Table 1. Values followed by the same letter within the same column are not significantly different ($p < 0.05$)

PC2 and thus apparently not affected by different additions of antioxidants to the mayonnaises.

Effect of Antioxidants on Lipid Hydroperoxides and Free Radical Formation. All free radical variables (ESR) were located to the far left in the plot near the 100% explained variance borderline, and all ESR variables had slightly positive PC2 values, except for ESR-0 (Figure 1b). Thus, as also obvious from the original measured data (Table 3), EDTA addition reduced the tendency of free radical formation and mayonnaises with gallic acid had lower concentrations of trapped free radicals after 1−4 weeks of storage than the reference mayonnaise. Thus, in mayonnaises with EDTA almost no free radicals were detected during storage ($<0.5\ \mu$M). In contrast, the tendency of free radical formation increased rapidly after 1 week of storage in the other

Table 3. Concentration of Free Radicals Trapped by PBN during Incubation (37 °C, 24 h) Following Storage at 20 °C (Micromolar; Mean Value $\pm$ SD, $n = 3$)[a]

code name	0 weeks	1 week	2 weeks	3 weeks	4 weeks
Ref	5.5 ± 0.9	17.2 ± 10.3	21.0 ± 3.5	26.1 ± 3.0	17.2 ± 0.9
E_Ref	4.0 ± 0.1	27.2 ± 0.9	29.6 ± 3.6	25.3 ± 3.5	24.2 ± 7.5
Gallic	5.6 ± 0.9	16.5 ± 1.1	13.8 ± 0.7	13.6 ± 0.4	11.7 ± 0.1
E_Gallic	7.6 ± 3.5	14.9 ± 0.1	15.9 ± 1.7	15.6 ± 1.6	13.2 ± 2.8
EDTA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3
E_EDTA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1

[a] For interpretation of code names please refer to Table 1. n refers to number of replicate samples analyzed in duplicate.

mayonnaises. In mayonnaises without antioxidant supplementation, the free radical concentration continued to increase until 3 weeks of storage, and then the

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Table 4. Concentration of Lipid Hydroperoxides during Storage at 20 °C (Milliequivalents per Kilogram; Mean Value ± SD, n = 3)[a]

	TAGOOH					CEOOH				
	week 0	week 1	week 2	week 3	week 4	week 0	week 1	week 2	week 3	week 4
Ref	2.21 ± 0.14	3.73 ± 0.12	2.33 ± 0.07	7.86 ± 0.52	3.23 ± 0.25	0.16 ± 0.00[b]	0.19 ± 0.03	0.14 ± 0.02	0.34 ± 0.00[b]	0.22 ± 0.00[b]
E_Ref	1.91 ± 0.02	2.45 ± 0.16	4.88 ± 0.13	6.87 ± 0.06	3.51 ± 0.21	0.16 ± 0.01	0.19 ± 0.03	0.22 ± 0.01	0.26 ± 0.01	0.15 ± 0.02
Gallic	1.98 ± 0.15	2.54 ± 0.05	2.99 ± 0.22	3.13 ± 0.18	3.46 ± 0.18	0.22 ± 0.04	0.19 ± 0.02	0.19 ± 0.01	0.19 ± 0.02	0.19 ± 0.01
E_Gallic	1.37 ± 0.11	1.99 ± 0.11	2.30 ± 0.16	2.49 ± 0.14	2.93 ± 0.12	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.0l	0.13 ± 0.01	0.15 ± 0.01
EDTA	1.66 ± 0.02	1.92 ± 0.05	1.96 ± 0.03	1.86 ± 0.03	2.02 ± 0.04	0.18 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.18 ± 0.03	0.17 ± 0.01
E_EDTA	1.36 ± 0.05	1.37 ± 0.08	1.56 ± 0.09	1.60 ± 0.07	1.57 ± 0.03	0.13 ± 0.02	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01

[a] Adapted after Hartvigsen et al. (*20*). For interpretation of code names please refer to Table 1. *n* refers to number of replicate samples each analyzed in triplicate. [b] Only one replicate.

Table 5. Oil Droplet Diameters Measured by Laser Diffraction after 1 and 4 Weeks of Storage at 20 °C (Micromoles; Mean Value ± SD, n = 2)[a]

	1 week					4 weeks				
code name	$D[3,2]$	$D[4,3]$	$D[v0.1]$	$D[v0.5]$	$D[v0.9]$	$D[3,2]$	$D[4,3]$	$D[v0.1]$	$D[v0.5]$	$D[v0.9]$
Ref	2.7 ± 0.1	5.4 ± 0.0	1.1 ± 0.0	4.8 ± 0.1	9.0 ± 0.3	2.7 ± 0.2	5.4 ± 0.2	1.0 ± 0.1	5.2 ± 0.1	9.7 ± 0.3
E_Ref	2.8 ± 0.0	5.2 ± 0.0	1.0 ± 0.0	5.6 ± 0.0	8.2 ± 0.1	2.9 ± 0.1	5.5 ± 0.1	1.0 ± 0.0	5.8 ± 0.0	9.0 ± 0.3
Gallic	2.5 ± 0.1	4.6 ± 0.0	1.1 ± 0.0	4.2 ± 0.1	8.6 ± 0.0	2.6 ± 0.0	5.7 ± 0.1	1.1 ± 0.0	4.3 ± 0.0	10.3 ± 0.2
E_Gallic	2.6 ± 0.2	4.3 ± 0.1	1.1 ± 0.1	4.2 ± 0.1	7.6 ± 0.1	2.4 ± 0.0	4.3 ± 0.0	1.0 ± 0.0	4.3 ± 0.0	7.8 ± 0.0
EDTA	2.7 ± 0.0	4.4 ± 0.0	1.1 ± 0.0	4.3 ± 0.0	7.6 ± 0.0	2.4 ± 0.1	4.2 ± 0.1	1.0 ± 0.0	4.4 ± 0.1	7.4 ± 0.1
E_EDTA	2.6 ± 0.1	4.2 ± 0.1	1.1 ± 0.1	4.3 ± 0.0	7.3 ± 0.2	2.3 ± 0.3	4.1 ± 0.2	0.9 ± 0.1	4.0 ± 0.4	7.3 ± 0.1

[a] For interpretation of code names please refer to Table 1. *n* refers to number of replicate samples, each analyzed once. Surface mean diameter: $D[3,2] = \sum d^3/\sum d^2$. Volume mean diameter: $D[4,3] = \sum d^4/\sum d^3$, where *d* is the diameter of the droplet (*32*). $D[v0.1]$, $D[v0.5]$, and $D[v0.9]$ refer to the 10th, 50th, and 90th percentiles of the droplet size distributions, respectively.

concentration seemed to decrease or level off (Table 3). However, in mayonnaises with gallic acid, the free radical concentration did not increase further after 1 week of storage (Table 3). No general trend on the formation of free radicals of the addition of extra emulsifier was observed.

Most of the TAGOOH variables had positive PC2 values, and they were all located to the left in the correlation loadings plot between the 50 and 100% explained variance borderlines (Figure 1b). Hence, in accordance with the original measured data (Table 4) mayonnaises without antioxidants developed higher levels of TAGOOH than mayonnaises with gallic acid or EDTA. In particular, TAGOOH concentrations in mayonnaises with EDTA increased to a maximum value of only 2 mequiv/kg, whereas the maximum values were 3.5 and 7.9 mequiv/kg in the gallic acid and reference mayonnaises, respectively. The maximum TAGOOH level in the two reference mayonnaises was obtained after 3 weeks of storage and decreased after 4 weeks, whereas the TAGOOH concentration in the other mayonnaises was highest after 4 weeks of storage.

The CEOOH variables from the early storage period were located close to the origin, whereas CEOOH-3 and -4 were located around PC1 ∼ −0.5 in the second quadrant. Thus, CEOOH concentrations apparently developed to higher levels in the reference mayonnaise after 3 and 4 weeks than in the other mayonnaises. This interpretation of Figure 1b was also in accordance with the original measured data, which showed that CEOOH concentrations were almost constant in mayonnaises with EDTA and gallic acid but increased slightly in the reference mayonnaise at the end of the storage period (Table 4). CEOOH concentrations were >10 times lower than TAGOOH concentrations.

The original measured data showed a significant reducing effect of the addition of extra emulsifier on both TAGOOH and CEOOH concentrations (Table 4) (*20*). This finding was also evident in a loadings plot of PC3 versus PC1 (not shown).

Effect of Antioxidant Addition on Droplet Size. Most of the droplet size variables were located in the second

quadrant near Ref and between the 50 and 100% explained variance borderlines (Figure 1c). This localization of the variables indicated that mayonnaises without antioxidants had larger droplets than mayonnaises with either EDTA or gallic acid. This interpretation of the DPLSR model was in agreement with the original measured data with a few exceptions. $D[3,2]$ was always highest in the reference mayonnaises and was lowest in EDTA mayonnaises after 4 weeks. $D[4,3]$ after both 1 and 4 weeks were smaller in EDTA mayonnaises than in the other mayonnaises, and mayonnaise with gallic acid had smaller $D[4,3]$ than the reference mayonnaise after 1 week; however, after 4 weeks, the opposite behavior was observed (Table 5). The different results obtained for the surface and volume mean diameters may be attributed to different droplet distributions for the mayonnaise as a high value for the 90th percentile will affect $D[4,3]$ more than it will affect $D[3,2]$. Thus, although the values for the 10th percentile ($D[v0.1]$) were similar for all mayonnaises, the values for the 50th and 90th percentiles were generally lowest for mayonnaise with EDTA, which indicated that this mayonnaise had a narrower droplet size distribution than the other mayonnaises.

Effect of Antioxidants on Rheological Properties. All variables from the rheological measurements of the mayonnaises were located in the fourth quadrant, except for the phase angle, which was located in the second quadrant almost diagonally to the other rheological variables (Figure 1c). These observations indicated a negative correlation between the reference mayonnaises and all of the rheological variables, except the phase angle. The original measured data showed that in mayonnaises without emulsifier, addition of gallic acid or EDTA generally had little effect on the rheological properties (Table 6). However, slight changes in the gel strength upon addition of gallic acid (decrements) and EDTA (increments) were observed (Table 6). The critical stress of the mayonnaise without extra emulsifier was not affected by gallic acid addition and was only slightly decreased by addition of EDTA. The yield stress tended to decrease and the phase angle to

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Table 6. Results of Rheological Measurements after 1 Week of Storage (Mean Value ± SD, *n* = 2)[a]

code name	gel strength (Pa)	phase angle (deg)	critical stress (Pa)	yield stress (Pa)	viscosity (Pa s)
Ref	855.5 ± 36.1	6.0 ± 0.7	26.6 ± 0.0	300.7 ± 26.3	140.0 ± 12.7
E_Ref	586.5 ± 3.5	6.9 ± 0.4	12.7 ± 0.0	168.8 ± 11.2	82.2 ± 3.3
Gallic	810.5 ± 74.2	6.1 ± 0.5	26.6 ± 0.0	287.8 ± 7.5	144.0 ± 1.4
E_Gallic	791.0 ± 26.9	5.5 ± 0.3	26.6 ± 0.0	266.1 ± 4.3	130.5 ± 2.1
EDTA	892.5 ± 29.0	6.4 ± 0.2	23.7 ± 4.1	297.8 ± 1.0	139.0 ± 7.1
E_EDTA	862.5 ± 12.0	5.8 ± 0.2	20.8 ± 0.0	318.4 ± 1.3	132.5 ± 2.1

[a] For interpretation of code names please refer to Table 1. *n* refers to number of replicate samples, each analyzed only once.

increase upon addition of gallic acid or EDTA. Mayonnaises without extra emulsifier generally had higher gel strength, critical stress, yield stress, and viscosity than the corresponding mayonnaise with emulsifier. The decreasing effect of the emulsifier on these variables was much more pronounced for the reference mayonnaise than for the other two mayonnaises, and this is probably the main reason a negative correlation between the reference mayonnaise and the rheological variables was observed (Figure 1c). In turn, this interpretation implies that there seemed to be interactions between the DATEM emulsifier and gallic acid and EDTA, respectively, and that these interactions affected the rheological properties of mayonnaises.

All variables describing the mouthfeel and appearance of the mayonnaises (Tmout and Tappe) were located in the fourth quadrant near all of the rheological variables, except the phase angle, indicating a positive correlation between these sensory variables and the rheological properties. Moreover, Figure 1c indicated a negative correlation between the droplet size and the rheological properties, which is in agreement with our previous results (*4, 6*) and with generally accepted theory (*24*). The original measured data in Tables 5 and 6 showed that when the DATEM emulsifier was added to the reference mayonnaise, the droplet size increased and the values for all of the rheological variables, except the phase angle, decreased significantly. However, when the DATEM emulsifier was added to mayonnaise with either EDTA or gallic acid, both the droplet size and the values for most of the rheological variables decreased (Tables 5 and 6). Hence, a positive correlation between the droplet size and the rheological variables seemed to exist when the droplet size was manipulated by addition of extra emulsifier in mayonnaises with EDTA or gallic acid. Thus, the negative correlation between droplet size and most of the rheological variables observed in the correlation loadings plot in Figure 1c is mainly due to the emulsifier's ability to increase the droplet size when added to the reference mayonnaise. This increase in turn affected the rheological properties.

The design variables describing the replicates were located near the origin and were thus not explained by PC1 and PC2 (Figure 1).

DPLSR Analysis on GC-MS and Sensory Data. In total 148 volatile compounds were identified and quantified by dynamic headspace GC-MS. These compounds included 16 alcohols, 39 aldehydes, 7 furans, 20 ketones, 40 noncyclic and 15 cyclic hydrocarbons, and 11 so-called miscellaneous compounds. A complete list of the compounds has previously been reported by Hartvigsen et al. (*21*). In the DPLSR analysis on the GC-MS and sensory data, three PCs were validated explaining 74 and 47% of the variance in the *X* and *Y* variables, respectively. Only PC1 and PC2 will be discussed.

Correlation Loadings Plot. In the correlation loadings

plot (Figure 2) the location of the design variables corresponded to the location of the corresponding mayonnaise codes in the scores plot, and therefore only the loadings plot is shown. The design variables Ref and Gallic were located to the right in the plot, in the fourth and first quadrants, respectively (Figure 2). The EDTA design variable was located to the far left close to the PC1 axis. PC1 thus mainly explained differences between mayonnaises with EDTA and the other mayonnaises, whereas PC2 explained differences between the reference mayonnaise and mayonnaise with gallic acid. The Emulsifier design variable was located in the second quadrant close to the origin. Hence, PC1 and PC2 did not explain the variation caused by emulsifier addition.

The variables describing fishy, rancid, and metallic off-flavor were in agreement with Figure 1a located near Gallic in the first quadrant. Most of the volatile compounds were located to the far right between Ref and Gallic, whereas only a few compounds were located near EDTA. Hence, addition of EDTA reduced the formation of secondary volatile oxidation compounds. This finding was confirmed by the fact that most of the variables for the volatiles had significant negative regression coefficients ($p < 0.1$) for the EDTA design variable. A closer examination of the regression coefficients for the volatiles located near Gallic or Ref revealed that the volatiles could be categorized in two groups: group A, consisting of 48 volatiles that were formed in significantly higher concentrations in the reference mayonnaises, and group B, consisting of 51 volatiles that were formed in significantly higher concentrations in mayonnaises with gallic acid ($p < 0.1$) (Table 7). Hydrocarbons and alkadienals were much more prevalent in group A, whereas more than twice as many alcohols, alkanals, alkenals, and alkenones occurred in group B as compared to group A. Hence, supplementation with gallic acid apparently influenced which secondary oxidation products were formed. Similar observations have been made for tocopherol addition in other products and in mayonnaise (*5, 7, 25*). Compounds that previously were found to be important oxidation compounds in fish oil enriched mayonnaise are marked in Table 7. The data in this table thus suggest that 11 new compounds may be added to the list of important oxidation products, which perhaps may influence oxidative flavor deterioration in fish oil enriched mayonnaise (compounds **15**, **60**, **61**, **67**, **84**, **118**, **132**, **142**, **143**, **145**, and **148**). The odors of the most important volatile oxidation products have previously been reported by Hartvigsen et al. (*21*).

Examination of the regression coefficients of the various volatiles for the Emulsifier design variable showed that addition of extra emulsifier increased the concentration of some volatiles while it reduced the concentration of other volatiles (data not shown). The effect of the emulsifier was strongest in the reference mayonnaise. However, the emulsifier addition did not seem to influence the intensity of fishy and rancid off-flavors. Therefore, the observed effect of the emulsifier

Figure 2. Correlation loadings plot from DPLSR analysis of sensory and GC-MS data. "o" refers to volatile compounds. Refer to Figure 1 for interpretation of names of design and sensory variables.

addition on the profile of volatiles may be due to its ability to interfere with or trap some of the compounds, thereby reducing the release of volatiles from the mayonnaise during the dynamic headspace sampling procedure (*26*).

DISCUSSION

The results obtained in the present study showed the following: (1) EDTA was an efficient antioxidant in fish oil enriched mayonnaise as it strongly inhibited the tendency to formation of free radicals, development of CEOOH, TAGOOH, volatile oxidation compounds, and fishy, rancid, and metallic off-flavors. (2) Gallic acid addition decreased the levels of free radicals and lipid hydroperoxides, influenced the profile of volatiles formed as a result of oxidation, and slightly promoted the oxidative flavor deterioration in mayonnaises. (3) Both EDTA and gallic acid addition slightly decreased the droplet size in mayonnaise. (4) Addition of emulsifier did not affect the formation of free radicals or the oxidative flavor deterioration in mayonnaises, but it significantly reduced the formation of lipid hydroperoxides and also affected the profile of volatiles. (5) The effect of the emulsifier on the droplet size and rheological properties depended on the presence of antioxidants.

In a recent study on the effect on free radical formation of different concentrations of EDTA (0, 50, 75, 125, and 200 ppm) in fish oil enriched mayonnaise, it was observed that EDTA inhibited the tendency of formation of free radicals irrespective of the concentration of EDTA (*16*). Real mayonnaise, as used in our studies, is made up of several ingredients that may impact the oxidative stability, that is, egg yolk, NaCl, sugar, and vinegar. Vinegar, such as tarragon vinegar, is added to decrease the pH to <4.2 to ensure microbial stability. In previous

studies we showed that this lowering of pH increased oxidation presumably via inducing release of iron from egg yolk via disruption of phosvitin−iron bonds in egg yolk granules at the oil−water interface, in effect making vinegar a pro-oxidant in mayonnaise (*15, 16*). The results of the present investigation are in accordance with the conclusions made in a previous report (*16*), in which we suggested that the antioxidative effect of EDTA appears to be due to its ability to chelate free iron as well as phosvitin-bound iron in egg yolk at the oil−water interface. Thereby, the iron ions are inactivated and rendered unable to catalyze lipid hydroperoxide decomposition to radicals that may promote oxidation or may decompose further to off-flavor volatiles. The proposition that EDTA was also able to chelate phosvitin-bound iron is supported by the previous finding of Galdi and Valencia (*27*) that the apparent association constant, K', for the EDTA−Fe(III) complex was larger ($10^{7.1}$) than K' for egg yolk−Fe(III) ($10^{5.3}$) and for phosphoserine−Fe(III) ($10^{5.2}$) at pH 4. Iron is bound by strong ion bonds to the phosphoserine residues in phosvitin (*27*).

The observed antioxidative effect of EDTA in mayonnaise is also in accordance with the results obtained by Warner et al. (*28*) in egg yolk based salad dressings and with the results obtained by Jafar et al. (*29*) in mayonnaise based on 100% fish oil. These authors also suggested that the antioxidative effect of EDTA was due to its metal chelating ability, but they did not provide any explanation with respect to the exact mechanism of how metals may catalyze oxidation in emulsions.

The finding that addition of the emulsifier resulted in an increased droplet size is in accordance with our previous findings that demonstrated that addition of extra emulsifier, Panodan TR, can increase droplet size

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Table 7. Important Volatiles in Fish Oil Enriched Mayonnaise

group A: compounds occurring in highest concentration in reference mayonnaises (Ref and E_Ref) ($p < 0.10$)			group B: compounds occurring in highest concentration in Gallic acid mayonnaises (Gallic and E_Gallic) ($p < 0.10$)		
peak	name	ID[a]	peak	name	ID[a]
5	1,*trans*-4-hexadiene[c]		14	3-methylbutanal[b,c]	+
6	1,*cis*-4-hexadiene[c]	+	15	2-methyl-1-propanol	+
7	2-methylpropanal[c]	+	17	2-*trans*-butenal[b,c]	+
8	2-methylfuran[b,c]	+	18	1-penten-3-one[b,c]	+
16	2-ethylfuran[b,c]	+	19	pentanal[b,c]	+
21	2-methyl-2-butenal[c]	+	22	1-penten-3-ol[b,c]	+
25	dimethyl disulfide[c]	+	23	1-chloropentane[c]	
26	1,6-octadiene[c]		24	octane[c]	+
28	4-*trans*-octene[c]		36	3-*trans*-penten-2-one[c,d]	+
30	5,5-dimethyl-1,3-cyclopentadiene[c]		37	2-*cis*-pentenal[c]	
31	2-methylthiophene[c]	+	40	2-*trans*-pentenal[b,c]	+
32	2-*trans*-octene[c]		41	3-methylfuran[b,c]	
33	2,4-*trans*,*trans*-octadiene[b,c]		44	hexanal[b,c]	+
35	3-methyl-1,*trans*-4-heptadiene[c]		45	1-hexen-3-one[b,c]	
36	3-*trans*-penten-2-one[c,d]	+	46	2-*trans*-penten-1-ol[b,c]	
43	2-hexanone[c]	+	48	2-*cis*-penten-1-ol[c]	+
55	1,3,6-*trans*,*trans*-octatriene[c]		49	2,3-dihydro-4-methylfuran[c]	
61	3-methyl-1-butanol acetate		51	1-methoxy-3-methylene-2-pentanone[c]	
71	3-heptanone[c]	+	58	2-butylfuran[b,c]	
73	1,2-dimethyl-1,4-cyclohexadiene[c]		60	1-ethenyl-3-methylenecyclopentene	
76	heptanal[b,c,d]	+	63	4-methyl-3-pentenal[b,c]	
81	2-decyne[c]		66	2-*trans*-hexenal[b,c]	+
83	1-decyne[c]		67	3,3-diethylpentane	
84	2,6-dimethyl-3-octene		69	2-furaldehyde[b]	
85	1,9-decadiene[c]	+	70	5-methylhexanal[c]	
87	2,8-*trans*,*trans*-decadiene[c]	+	72	4-ethylphenol[c]	
91	2-pentylfuran[b,c]	+	75	2-heptanone[b,c]	+
96	1-cyclohexyl-2-buten-1-ol[c]		76	heptanal[b,c,d]	+
107	3-ethenylcyclooctene[c]		77	1-methoxy-3-methylene-2-pentanone[b,c]	
109	2,4-diethenyl-1- methylcyclohexane[c]		79	4-*cis*-heptenal[b,c]	+
110	3-(2-propenyl)-cyclooctene[c]		82	cyclohexanone[b,c]	+
111	2,4-*trans*,*cis*-heptadienal[b,c]		88	*cis*-2-heptenal[c]	
112	2,4-*trans*,*trans*-heptadienal[b,c]	+	90	2,4-*trans*,*trans*-hexadienal[c]	+
113	3-*trans*-undecen-5-yne[b,c]		92	4,5-dimethyl-2,6-octadiene[c]	
115	*cis*-3-undecen-5-yne[c]		97	1-ethyl-3-methylcyclopentane[c]	
127	2-nonanone[b,c]	+	98	2-*trans*-heptenal[b,c]	
130	3,5-*trans*,*cis*-octadien-2-one[c]		99	1-octen-3-one[b,c]	+
132	2-butyl-1-octanol[d]		100	benzaldehyde[c]	+
134	3,5-*trans*,*trans*-octadien-2-one[c]		101	2,3-octanedione[c]	
135	2,4- *trans*,*cis*-octadienal[c]		103	1,*cis*-5-octadien-3-one[c]	
136	3,7-dimethyl-1,3,6-octatriene[c]		104	1-octen-3-ol[b,c]	+
137	2,4- *trans*,*trans*-octadienal[b,c]	+	105	octanal[c]	+
139	2-*trans*-nonenal[c,d]	+	106	1,5-*cis*-octadien-3-ol[c]	+
141	decanal[c]	+	117	1-methylcyclooctene[b,c]	
142	2,4-*trans*,*trans*-nonadienal	+	118	2-*cis*-octenal	
143	2-*trans*-decenal	+	123	2-*trans*-octenal[b,c]	+
145	undecanal		125	benzeneacetaldehyde[c]	+
148	2-*trans*-undecenal	+	129	nonanal[b,c]	
			132	2-butyl-1-octanol[d]	
			139	2-*trans*-nonenal[c,d]	+
			140	2,6-*trans*,*cis*-nonadienal[b,c]	+

[a] All compounds were tentatively identified by MS library (NIST). Compounds marked with "+" have been further identified by retention time and spectra of authentic reference compounds. [b,c] Compounds found to be important oxidation products in previous investigations on fish oil enriched mayonnaise (refs *6* and *21*, respectively). [d] No significant difference in peak areas over time between gallic acid and reference mayonnaise.

and that the emulsifiers' influence on droplet diameters interacts significantly, but differently, with different antioxidant systems in mayonnaise (*5*). We recently suggested that a small oil droplet size may increase the oxidation rate in metal-catalyzed oxidation in mayonnaise (*6*). EDTA addition decreased the droplet size in the present study, but nevertheless oxidation was strongly inhibited in mayonnaises with EDTA. This result supports our previously stated hypothesis that the droplet size is important only when oxidation is catalyzed by iron stemming from phosvitin, where phosvitin is a part of the egg yolk emulsifier (*6*, *16*).

The observations that gallic acid mayonnaises had lower levels of lipid hydroperoxides but a slightly increased intensity of fishy, rancid, and metallic off-flavors, as compared to the reference mayonnaises, suggested a faster decomposition of lipid hydroperoxides caused by gallic acid, that is, an increased formation of secondary oxidation products. However, although small differences in total peak areas were recorded, the present GC-MS data do not allow us to conclude whether larger total amounts of volatiles were formed in the gallic acid mayonnaise than in the reference mayonnaise, because we did not determine exact concentration levels but only relative peak areas. Neither does our present experimental setup allow us to conclude whether gallic acid could change the partitioning of volatiles between the different phases in mayonnaise

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and thus impact the volatiles profile. Further knowledge on the possible influence of gallic acid and other widely used antioxidants on volatile oxidation compounds in food emulsion systems is highly warranted. Gallic acid may catalyze lipid hydroperoxide decomposition via its ability to reduce metal ions (*30*). In our previous study we found that 20% of the gallic acid was located at the oil–water interface (*9*). Therefore, it seems plausible that a fraction of the gallic acid molecules may interact with metal ions from phosvitin at the oil–water interface. The proposed faster decomposition of lipid hydroperoxides in gallic acid mayonnaises may also be explained by the observed decreasing effect of this antioxidant on the droplet size, as a reduced droplet size previously was found to increase the rate of lipid peroxide decomposition in mayonnaise (*6*). Finally, it should be emphasized that the observed effect of gallic acid—a polar antioxidant—was in accordance with the generally accepted hypothesis that the efficacy of polar antioxidants in o/w emulsions is poor (*11*–*13*).

We previously observed that a high concentration of extra emulsifier (2000 ppm) did not affect the oxidative flavor deterioration or the levels of lipid hydroperoxides in fish oil enriched mayonnaise (*4, 5*). In the present study, the concentration of the emulsifier was lower (200 ppm). Also at this concentration level, the emulsifier did not have any effect on the oxidative flavor deterioration, but the peroxide levels were significantly reduced by the emulsifier and the volatile profile was also affected. As our study was not designed to give an explanation on the effect of the various emulsifier concentrations, more experiments need to be conducted to clarify these observations.

Moreover, it was observed that the effect of the emulsifier on the droplet size and rheological properties varied in mayonnaises with and without antioxidants. Thus, in the reference mayonnaise emulsifier addition increased the droplet size and decreased the values of most of the rheological parameters. However, in mayonnaise with gallic acid or EDTA addition of extra emulsifier decreased the droplet size and also decreased the values of the rheological parameters. This latter result is in conflict with the relationship between droplet size and viscosity generally observed in mayonnaise, namely, that a small droplet size gives rise to a firm, viscous mayonnaise (*29*). However, the viscosity in mayonnaise is governed by not only the droplet size but also the protein network between oil droplets in mayonnaise formed by fragments of egg yolk granules (*31*). Our results therefore suggest that the extra emulsifier in combination with either EDTA or gallic acid may interact with this protein network and thereby affect the rheological properties independently of the droplet size and without influencing the ability of iron from egg yolk compounds at the oil–water interface to catalyze lipid oxidation. As mentioned above, the assumed interaction between the extra emulsifier and antioxidant was also observed in our recent investigations on the antioxidative effect of propyl gallate and tocopherol (*4, 5*).

In conclusion, EDTA is an efficient antioxidant in fish oil enriched mayonnaise due to its metal chelating abilities. A primary conclusion is also that when iron stemming from egg yolk compounds located at the oil–water interface is inactivated by EDTA, a decrease in the oil droplet size in mayonnaise no longer accelerates the oxidation rate.

Egg yolk is frequently used as an emulsifier in food emulsions such as salad dressing, sauces, and mayonnaise-based salads. Therefore, it is most likely that EDTA will be an efficient antioxidant in these food products. However, it needs to be investigated whether EDTA or other metal chelators in combination with primary antioxidants such as tocopherol would be even more efficient in protecting food products against oxidation than EDTA alone.

ABBREVIATIONS USED

AV, anisidine value; APLSR, ANOVA partial least-squares regression; CEOOH, hydroperoxy cholesterol esters; DPLSR, discriminant partial least-squares regression; EDTA, calcium disodium ethylenediamine tetraacetate; ESR, electron spin resonance spectroscopy; FFA, free fatty acids; GC-MS, gas chromatography–mass spectrometry; HPLC, high-pressure liquid chromatography; PC, principal component; PV, peroxide value; TAGOOH, hydroperoxy triacylglycerols.

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