JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Ascorbyl Palmitate, γ-Tocopherol, and EDTA Affect Lipid Oxidation in Fish Oil Enriched Salad Dressing Differently

METTE B. LET,† CHARLOTTE JACOBSEN,*,† AND ANNE S. MEYER§

Department of Seafood Research, Danish Institute for Fisheries Research, and Bioprocess Engineering, Department of Chemical Engineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

The aim of the study was to investigate the ability of γ-tocopherol, ethylenediaminetetraacetate (EDTA), and ascorbyl palmitate to protect fish oil enriched salad dressing against oxidation during a 6 week storage period at room temperature. The lipid-soluble γ-tocopherol (220 and 880 μg g^{-1} of fish oil) reduced lipid oxidation during storage by partly retarding the formation of lipid hydroperoxides (PV) and by decreasing the concentrations of individual volatile oxidation products by 34−39 and 42−66%, respectively. EDTA (10 and 50 μg g^{-1} of dressing) was the most efficient single antioxidant, and overall peroxide values and volatiles were reduced by approximately 70 and 77−86%, respectively. Conversely, prooxidant effects were observed with a high concentration of ascorbyl palmitate (300 μg g^{-1} of fish oil), whereas a low concentration was slightly antioxidative (50 μg/g of fish oil). Finally, a combination of all three antioxidants completely inhibited oxidation during storage, indicating that the prooxidant effects of ascorbyl palmitate were reverted or overshadowed by EDTA and γ-tocopherol.

KEYWORDS: Fish oil; lipid oxidation; antioxidants; omega-3 PUFA; ascorbyl palmitate; tocopherol; EDTA; salad dressing

INTRODUCTION

Marine oils are rich sources of the nutritionally important omega-3 long-chain polyunsaturated fatty acids (PUFA). Notably, the intake of eicosapentaeneoic acid [EPA, 20:5(n-3)] and docosahexaeneoic acid [DHA, 22:6(n-3)] appears to correlate with important health effects. For this reason considerable efforts have been made to incorporate marine oils into various food products. However, due to the high degree of unsaturation in their fatty acid profile, fish oils undergo rapid oxidation, leading to the formation of volatile oxidation products, which result in undesirable fishy off-flavors. Significant amounts of work on protection against lipid autoxidation through the addition of antioxidants have been reported in model systems, whereas relatively few studies on the antioxidant mechanisms and efficacy in real omega-3 enriched food systems have been reported. However, the problems of oxidation appear to be particularly prominent in emulsions and other complex food systems, in which transition metals, a large interfacial area, and harsh processing conditions can facilitate oxidation. Factors such as pH, temperature, oxygen availability, type of emulsifier, and droplet size can also affect lipid autoxidation (*1*, *2*). The main antioxidative mechanisms necessary to protect food systems from oxidation are radical scavenging, metal chelation, and oxygen scavenging. Some antioxidants are able to contribute more than one possible mode of action. Furthermore, the polarity and solubility of an antioxidant determine the actual location of the antioxidant in a given food matrix, which again influences the antioxidative efficacy of the antioxidant (*2*). Overall, many factors complicate the prediction of antioxidant efficacy in real food systems.

Our investigations on the oxidative stability of fish oil enriched foods have included low-fat products such as pasteurized milk, flavored milk drinks, and yogurt, as well as high-fat products such as mayonnaise. The efficacies of different types of antioxidants have been thoroughly evaluated in these food systems. The majority of these studies concern (1) tocopherols, which are lipid-soluble antioxidants, recognized as efficient chain-breaking antioxidants, on the basis of their radical scavenging potential; (2) metal chelators, mainly ethylenediaminetetraacetate (EDTA), which efficiently chelates transition metals present in food matrices; and (3) ascorbic acid or ascorbyl palmitate, for which several antioxidant mechanisms have been proposed. These include singlet oxygen quenching, metal chelation, and also free radical scavenging potential, which furthermore enables regeneration of oxidized tocopherols.

In fish oil enriched mayonnaise oxidative stability and acceptable sensory characteristics were achieved only by the addition of EDTA (*3*). Tocopherols were less efficient in mayonnaise and were tested only in mixtures of α-, β-, γ-, and δ-tocopherol. On the other hand, EDTA did not reduce oxidation in fish oil enriched milk significantly, whereas γ-tocopherol alone was more efficient (*4*). Overall, ascorbic palmitate was the most efficient antioxidant in this milk system and reduced

* Corresponding author (telephone +45 4525 2559; fax +45 4588 4774; e-mail cja@difres.dk).
† Danish Institute for Fisheries Research.
§ Department of Chemical Engineering.

10.1021/jf062675c CCC: $37.00
Published on Web 02/24/2007

Table 1. Fatty Acid Composition (Percent), Free Fatty Acids (FFA), Anisidine Value (AV), Peroxide Value (PV), and Content of Tocopherols in Fish Oil and Rapeseed Oil

fatty acid[a]	fish oil	rapeseed oil
14:0	3.9	
16:0	10.2	4.5
18:0	2.2	1.8
20:0	1.3	
SAT	**17.6**	**6.3**
16:1(n-7)	6.8	0.2
18:1(n-9)	23.2	60.4
18:1(n-7)	4.1	3.2
20:1(n-9)	10.8	1.5
22:1(n-9)	7.1	
MUFA	**52.0**	**65.3**
18:2(n-6)	4.3	19.2
18:3(n-3)	2.2	8.6
18:4(n-3)	2.5	
19:2	0.1	0.6
20:4(n-3)	0.8	
20:5(n-3)	8.0	
22:5(n-3)	1.1	
22:6(n-3)	11.5	
PUFA	**30.4**	**28.4**
total	100	100
FFA (%)	**0.01 ± 0.01**	**0.02 ± 0.01**
AV	**1.7 ± 0.1**	**0.7 ± 0.0**
PV (mequiv kg^{-1})	**0.11 ± 0.01**	**0.16 ± 0.01**
tocopherols (mg kg^{-1})		
α	**200 ± 3**	**220 ± 3**
γ	**36 ± 4**	**365 ± 5**

[a] Fatty acids present in concentrations greater than 0.5% in at least one of the oils were summed to 100%.

oxidation almost completely (*4*). Hence, the currently available data confirm that different mechanisms of oxidation dominate in different food emulsion systems and, in turn, that the efficacy of different antioxidants and antioxidant mechanisms varies significantly in different types of emulsions.

The present work concerns fish oil enriched salad dressing, which may be a potential vehicle for fish oil supplementation. Similarly to mayonnaise, salad dressing is an oil-in-water emulsion with a low pH, but in order to avoid the iron-containing egg yolk, which is the emulsifier usually applied in mayonnaise, the salad dressing was emulsified by a denatured whey protein preparation. Oil droplets in the salad dressing are larger than those in mayonnaise, which is why dressings are often stabilized by additional substances such as gums or starches. In this study we investigated the oxidative stability of fish oil enriched salad dressing during storage at ambient temperature. On the basis of the experiments on milk and mayonnaise, the influence on oxidation of the antioxidants γ-tocopherol, EDTA, and ascorbyl palmitate, alone or in combination, was assessed. Each antioxidant was evaluated in two concentrations selected on the basis of previous investigations on milk.

MATERIALS AND METHODS

Materials. Refined cod liver oil and a refined rapeseed oil, both without added antioxidants, was provided by Maritex A/S, Sortland, Norway. Fatty acid composition, peroxide value (PV), anisidine value (*5*), and contents of free fatty acids (*6*) and tocopherols of each oil are given in **Table 1**. The fatty acid composition was determined by preparation of methyl esters (*7*) that were in turn analyzed by gas chromatography (*8*). The levels of tocopherols were determined by HPLC (*9*). Vinegar (5% acetic acid) was purchased locally (De Jyske Eddikebryggerier A/S, Randers, Denmark). Denatured whey protein emulsifier (Nutrilac DR8080) was provided by Arla Foods, a.m.b.a., Aarhus, Denmark. A mixture of guar gum, xanthan gum, and acetylated distarch adipate (Grindsted FF2110) was used as stabilizing agent together with ascorbyl palmitate obtained from Danisco Ingredients, Brabrand, Denmark. EDTA and γ-tocopherol were purchased from Sigma Aldrich, Steinheim, Germany, and Merck, Darmstadt, Germany, respectively, and all chemicals and external standards for identification of volatile oxidation products were also from either Sigma Aldrich or Merck. All solvents were of HPLC grade from Lab-Scan, Dublin, Ireland.

Table 2. Addition of Ascorbyl Palmitate (AP), γ-Tocopherol, EDTA, and Combinations of Antioxidants to the Salad Dressings

	concentration (mg kg^{-1})	
antioxidant	in final product	in phase added
none		
AP low	5	50 in fish oil
AP high	30	300 in fish oil
γ-toc low	22	220 in fish oil
γ-toc high	88	880 in fish oil
EDTA low	10	14.8 in aqueous phase
EDTA high	50	73.9 in aqueous phase
EDTA + γ-toc low	22 + 10	
EDTA + γ-toc high	88 + 50	
all low	5 + 22 + 10	
all high	30 + 88 + 50	

Production of Dressings and Preparation of Samples for Analyses. Dressing emulsions were produced in 600 g batches [15% w/w rapeseed oil, 10% w/w fish oil, 6% w/w vinegar (here of 5% acetic acid), 1.2% w/w stabilizer, 0.1% w/w potassium sorbate, 0.08% denatured whey protein emulsifier, 67.62% deionized water]. The denatured whey protein emulsifier and potassium sorbate were dissolved in deionized water. The stabilizer dispersed in rapeseed oil (24 g) was mixed into the water phase in a Stephan Universal Mixer (Stephan, Hameln, Germany). Fish oil, remaining rapeseed oil, and vinegar were added slowly during mixing (3 min, 1200 rpm). The dressings were mixed for an additional 4 min at reduced pressure (0.4 bar). Dressings were cooled with circulating water at 0 °C throughout processing. The pH of the salad dressing was measured to be pH 4.0. Antioxidants (**Table 2**) were added as follows: EDTA (calcium disodium ethylenediaminetetraacetate) was dissolved in water and added after the denatured whey protein and sorbate. Ascorbyl palmitate and γ-tocopherol were dissolved in ethanol, and 30 or 120 μL of antioxidant solution was added to the fish oil before emulsification to reach the low and high levels of antioxidants, respectively. Dressings (100 mL) were stored in closed 100 mL Pyrex bottles (one bottle per sample for each sampling week) in the dark at room temperature (21−22.5 °C). Oil droplet sizes of the emulsions were measured after 1 week and remained similar throughout the storage period (Mastersizer 2000, Malvern Instruments, Worcestershire, U.K.). The average surface mean diameter of the emulsions was $D[3,2] = 22 \pm 2$ μm, whereas the average volume mean diameter was $D[4,3] = 32 \pm 1$ μm. Samples for chemical analysis were taken after 0, 1, 2, 4, and 6 weeks of storage and stored in separate, brown glass bottles, which were immediately flushed with nitrogen and kept at −80 °C until analyses of peroxide value (PV) and secondary volatile oxidation products.

Analyses of Primary Oxidation Products and Tocopherols. Lipids from the dressings were extracted by chloroform/methanol (1:1 w/w), using a reduced amount of solvent (*10*, *11*). PV and tocopherols were measured directly on the oils or on the lipid extract from the dressings by colorimetric determination of iron thiocyanate (*12*) and HPLC (*9*), respectively. The PV and tocopherol data reported are averages of measurements made on duplicate lipid extractions.

Dynamic Headspace Analysis of Volatile Secondary Oxidation Products. Dressing (10 g, triplicate measurement) was diluted with 10 g of deionized water, and volatile secondary oxidation products were

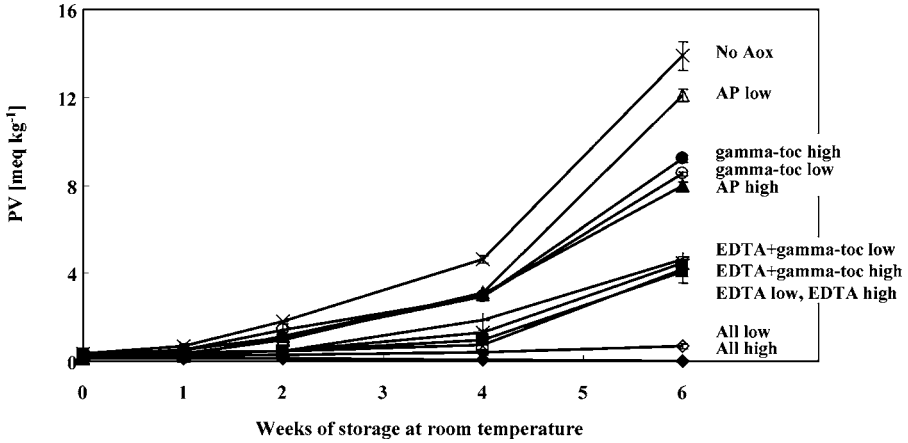

Figure 1. Peroxide values of dressings during storage at room temperature (mequiv kg^{-1}): (×) no antioxidant; (△, ▲) ascorbyl palmitate; (○, ●) γ-tocopherol; (□, ■) EDTA; (+, ∗) EDTA and γ-tocopherol; (◇, ◆) all three antioxidants. Open symbols refer to low concentrations; solid symbols refer to high concentrations. Data points are averages of duplicate measurements ± 1 standard deviation. Sample names refer to **Table 2**.

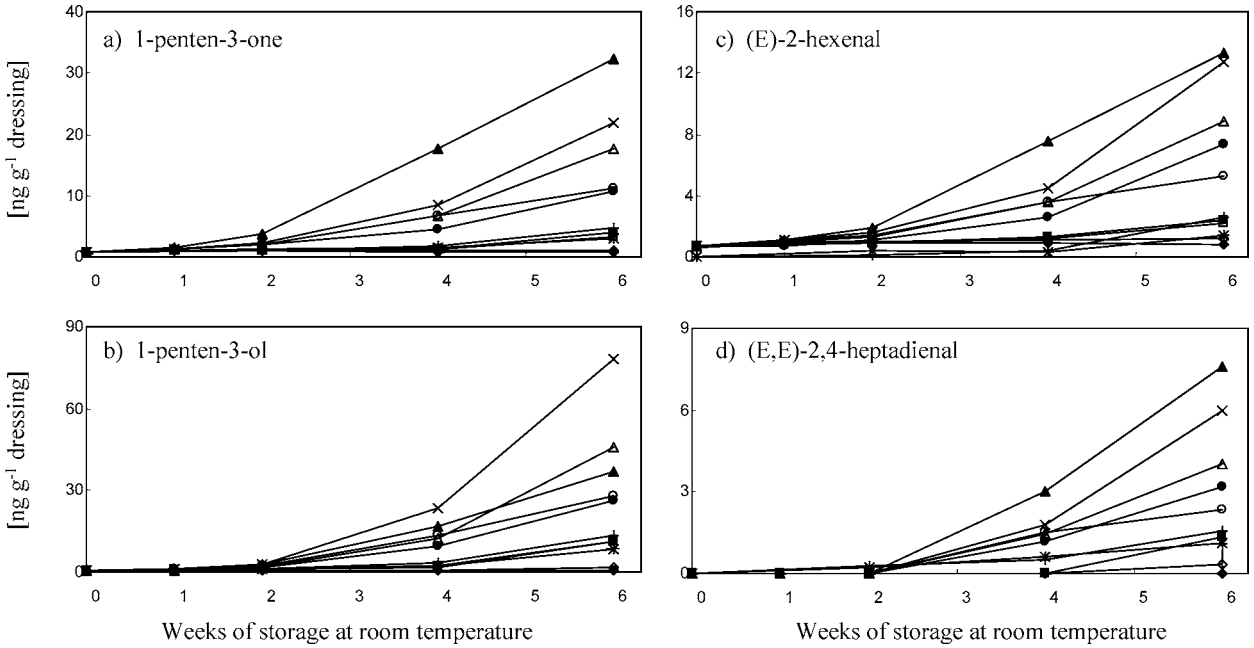

Figure 2. Development in volatile oxidation products as exemplified by 1-penten-3-one, 1-penten-3-ol, (*E*)-2-hexenal, and (*E*,*E*)-2,4-heptadienal during storage at room temperature (ng g^{-1} of emulsion): (×) no antioxidant; (△, ▲) ascorbyl palmitate; (○, ●) γ-tocopherol; (□, ■) EDTA; (+, ∗) EDTA and γ-tocopherol; (◇, ◆) all three antioxidants. Open symbols refer to low concentrations; solid symbols refer to high concentrations. Average relative standard deviation was 6%.

purged through dry, powdered potassium hydroxide (200 mg) to remove acetic acid (*13*) and trapped on Tenax GR tubes with nitrogen (150 mL/min) for 30 min at 45 °C using 4-methyl-1-pentanol as internal standard. The volatiles were desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin-Elmer, Norwalk, CT) and cryofocused on a Tenax GR cold trap. Volatiles were analyzed by gas chromatography with mass spectrometrical detection (GC-MS) as follows: volatiles were separated by gas chromatography (HP 5890 IIA, Hewlett-Packard, Palo Alto, CA; column DB-1701) as described previously (*14*) and analyzed by mass spectrometry (HP 5972 mass-selective detector). The oven temperature program was as follows: 45 °C held for 5 min, raised at 1.5 °C min^{-1} to 55 °C, raised at 2.5 °C min^{-1} to 90 °C, raised at 12 °C min^{-1} to 220 °C, and finally held at 220 °C for 4 min. The individual compounds were identified by both MS library searches (Wiley138K, John Wiley and Sons, Hewlett-Packard) and authentic external standards. The compounds were quantified through individual calibration curves in the range of 2−1500 ng g^{-1} of dressing. The compounds were dissolved in ethanol and added to a dressing based on only rapeseed oil, and headspace analysis was performed in triplicate as described above.

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

RESULTS AND DISCUSSION

The oxidation of the different emulsions was assessed by the peroxide values (**Figure 1**) and by the development in concentrations of selected, individual volatile oxidation products, as analyzed by GC-MS (**Figure 2**). The compounds quantified included 1-penten-3-ol, 1-penten-3-one, (*E*)-2-pentenal, (*E*)-2-hexenal, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal as these compounds were shown previously to be sensitive off-flavor indicators of the oxidative deterioration of fish oil omega-3 fatty acids in fish oil enriched milk during storage (*15*). The development of (*Z*)-4-heptenal in the salad dressing during storage was similar to the development of the other five

Table 3. Inhibition of Peroxide Value (PV) and Volatiles after 6 Weeks of Storage at Room Temperature

	% inhibition[a] of PV and individual volatiles					
antioxidant	PV	1-penten-3-one	1-penten-3-ol	2-pentenal	2-hexenal	2,4-heptadienal
none	0	0	0	0	0	0
AP low	13	20	42	33	31	32
AP high	43	–48	53	–16	–5	–27
γ-toc low	39	49	64	62	59	60
γ-toc high	34	52	66	60	42	47
EDTA low	70	86	86	84	83	77
EDTA high	71	82	86	82	82	78
EDTA + γ-toc low	66	84	85	82	77	83
EDTA + γ-toc high	68	90	91	89	87	88
all low	95	96	98	93	91	94
all high	100	97	99	93	93	100

[a] Inhibition (percent) was calculated as 100 × (control − sample)/control. Sample names refer to **Table 2**.

mentioned compounds, but the levels were very small (0.4−1.0 ng g^{-1} of dressing), which is why we decided not to include the quantitative data for this compound in the oxidation assessment of the salad dressing. The longer chain alkadienals, especially 2,4- and 2,6-nonadienal, were also detected, but the amounts were too low for quantification. Although the contents of α- and γ-tocopherol tended to decrease during the storage of emulsions with a high concentration of ascorbyl palmitate, no significant changes in the tocopherol levels were observed in any of the dressings at the end of the storage period (data not shown). Overall, the three antioxidants, EDTA, γ-tocopherol, and ascorbyl palmitate, had significantly different impacts on the oxidative stability of the salad dressings during storage.

Antioxidative Effects of EDTA. Irrespective of the concentration, both dressings containing EDTA had low and stable PVs in the beginning of the storage period (**Figure 1**). After 4 weeks of storage, a small increase in PVs was observed, and after 6 weeks, PVs had increased significantly (**Figure 1**). However, compared to the dressing without added antioxidant, EDTA reduced PV about 70% after 6 weeks of storage (**Table 3**). Similar results were obtained regarding the volatile oxidation products. No development in volatiles was observed for up to 4 weeks of storage, but after 6 weeks, significant increases were observed for all five volatiles, 1-penten-3-ol, 1-penten-3-one, (*E*)-2-pentenal, (*E*)-2-hexenal, and (*E*,*E*)-2,4-heptadienal (**Figure 2**). Overall, EDTA inhibited the development of volatiles by 77−86% at the dosages added (**Table 3**). Dressings with high and low EDTA concentrations were nearly identical regarding both PV and volatiles throughout the entire storage period, indicating that there was no concentration-dependent effect of EDTA.

EDTA is an efficient metal chelating compound. The significant antioxidative effect of EDTA therefore indicated that transition metals promoted oxidation significantly in the dressing emulsions. The inhibition of the volatiles was more pronounced than the inhibition of the PV. This observation supports the hypothesis that the degradation of lipid hydroperoxides is mediated or catalyzed by transition metals and thus can be prevented by chelation of these by EDTA.

Antioxidative Effects of γ-Tocopherol. The dressings containing γ-tocopherol had significantly increased PVs after only 2 weeks of storage, but the PVs were lower than those of the control without antioxidant (**Figure 1**). After 2 weeks, the PVs of the dressings supplemented with γ-tocopherol were also significantly higher than those in dressings containing EDTA or all three antioxidants. However, γ-tocopherol still inhibited PV by 34−39% after 4 and 6 weeks compared to dressings with no antioxidant addition. Similarly to EDTA, γ-tocopherol inhibited the volatiles more than PV. Concentrations of volatiles were reduced by 42−66% for the five compounds listed in **Table 3**.

Up to 4 weeks of storage, no difference in oxidation was observed between the two γ-tocopherol concentrations (220 and 880 mg kg^{-1} in the fish oil). After 6 weeks of storage, the dressing with high tocopherol concentration had significantly higher PV and also significantly higher levels of both (*E*)-2-hexenal and (*E*,*E*)-2,4-heptadienal. Thus, increasing the concentration of γ-tocopherol did not improve the stability further. No difference was observed regarding the volatiles 1-penten-3-ol, 1-penten-3-one, and (*E*)-2-pentenal. It is not unusual that volatile oxidation products are affected differently by antioxidants, and also the profile of volatiles formed depends on the conditions during oxidation (*16*, *17*). The antioxidant mechanism of tocopherols is hydrogen donation to lipid and/or to peroxide radicals during autoxidation; thus, they are chain-breaking antioxidants. However, it is well-known that high concentrations of especially α-tocopherol can promote the formation of lipid hydroperoxides in both bulk oils and emulsions (*16*, *18*). This is proposed to be due to the potential of tocopheroxyl radicals, formed during oxidation, to abstract hydrogen from lipid hydroperoxides and in this way fuel the oxidation by regenerating reactive peroxide radicals rather than inhibiting their formation. Whether a similar mechanism is responsible for the reduced antioxidative capacity of high concentrations of γ-tocopherol in emulsions needs further investigation.

The present salad dressing emulsion was based on 10% fish oil and 15% rapeseed oil, which both contained a significant amount of naturally occurring tocopherols. Fish oil had 200 mg kg^{-1} α- and 36 mg kg^{-1} γ-tocopherol, whereas rapeseed oil had 220 mg kg^{-1} α- and 365 mg kg^{-1} γ-tocopherol (**Table 1**). Despite these relatively high levels of tocopherols, it seemed that supplementation of only an additional 220 mg kg^{-1} γ-tocopherol was able reduce oxidation significantly. Differences in inhibition of PV and volatiles could indicate that the added γ-tocopherol was able to prevent the hydroperoxides from further degradation to secondary oxidation products or rather that γ-tocopherol scavenged the peroxyl radicals and thereby retarded formation of volatiles.

Pro- and Antioxidative Effects of Ascorbyl Palmitate. In the present experiment different effects were observed for ascorbyl palmitate. After 2 weeks and throughout the storage experiment, both high and low concentrations of ascorbyl palmitate inhibited the PV of the emulsions compared to the dressing without antioxidants (**Figure 1**). After 6 weeks, the high concentration of ascorbyl palmitate was significantly more efficient in reducing PV than the low concentration of ascorbyl palmitate (43 vs 13% inhibition). However, these results were not reflected in the development of volatiles. For 1-penten-3-one, (*E*)-2-pentenal, (*E*)-2-hexenal, and (*E*,*E*)-2,4-heptadienal significant prooxidant effects of the high concentration of ascorbyl palmitate were observed. Concentrations of (*E*)-2-hexenal and (*E*,*E*)-2,4-heptadienal were low in all samples at the beginning of the storage, but after 4 weeks, concentrations were significantly elevated in dressing with the high concentration of ascorbyl palmitate. For (*E*)-2-pentenal and 1-penten-3-one a prooxidant effect was already evident after 1 and 2 weeks of storage, respectively. In contrast, 1-penten-3-ol was the only compound analyzed that did not show increased concentrations

at high ascorbyl palmitate addition. Although it has been reported that ascorbyl palmitate has no effect on the formation of 1-penten-3-ol in bulk cod liver oil, we propose that the reduced levels of 1-penten-3-ol in dressings with ascorbyl palmitate may be due to further oxidation of 1-penten-3-ol to 1-penten-3-one. During autoxidation of bulk cod liver oil (*17*) the additions of tocopherols with or without ascorbyl palmitate were equally antioxidative, and the development of 1-penten-3-ol was similar to the development in PV, which therefore corresponds to our observations in the dressing.

The low addition level of ascorbyl palmitate was not prooxidant, and concentrations of the volatiles were reduced 20−42% after 6 weeks (**Table 3**). Ascorbyl palmitate holds a hydrogen-donating potential, which allows it to function as a chain-breaking antioxidant (*19*). It can act as an independent free radical scavenging antioxidant, but it can also regenerate tocopherols from their oxidized tocopheroxyl radicals formed during oxidation (*20*, *21*). As described above, the salad dressing emulsion naturally contained a significant amount of tocopherols through the fish and rapeseed oils (**Table 1**). In theory, ascorbyl palmitate should therefore be able to work by both the proposed mechanisms. Still, the antioxidant efficacy of the low concentration of ascorbyl palmitate (50 mg kg^{-1} in oil) was limited and less efficient in reducing PV and volatiles than that of γ-tocopherol (**Table 3**).

Additive Effects of Antioxidants. The simultaneous addition of EDTA and γ-tocopherol did not improve the oxidative stability beyond the effect of EDTA alone. However, addition of all three antioxidants together practically inhibited oxidation completely (**Table 3**). No significant development in PV or volatiles was observed in the emulsions containing all three antioxidants during the entire storage period. At all sampling points, the dressing with low concentrations of all antioxidants had higher PV than the dressing with high concentrations of all antioxidants. For the volatiles 1-penten-3-one, 2-hexenal, and 2,4-heptadienal higher levels were also observed in the dressing with low concentrations of antioxidants. Especially, 2,4-heptadienal could not be detected in the dressing with high concentrations of antioxidants. Only for 2-pentenal was no difference observed between the two emulsions. The inhibition of the oxidation thus generally tended to be higher at the highest concentrations of all three antioxidants (**Figures 1** and **2**), but the differences in the extent of inhibition between the high and low antioxidant addition levels of all three antioxidants were not statistically significant. Neither could any synergistic effects be identified, mainly because EDTA alone was very efficient in retarding oxidation.

It is well-known that transition metals, and especially ferrous ions, possess significant prooxidant potential, presumably by mediating the degradation of lipid hydroperoxides. Propagation of lipid autoxidation reactions through degradation of hydroperoxides further results in increased formation of secondary volatile oxidation products (*2*, *22*). Generally, the solubility of transition metal ions increases with decreasing pH. In the salad dressing of pH 4.0, we therefore assume that these metal ions are soluble in the water phase and that it is very likely that transition metal ions contribute to the significant progress in oxidation observed during storage. This assumption is supported by the fact that we did see a significant antioxidative effect of the metal chelator EDTA. Second, studies in fish oil enriched mayonnaise, which is an oil-in-water emulsion system of low pH similar to our salad dressing, have shown that decreasing pH resulted in increased oxidation during storage and that the effect of low pH was circumvented by EDTA (*23*, *24*). Also, in other studies on mayonnaise or oil-in-water model emulsions, EDTA has been shown to protect against oxidation during storage (*3*, *25*−*27*). Overall, these results show that metal ions are very important in promoting oxidation in fish oil enriched mayonnaise. We therefore suggest that also in low-pH salad dressing emulsion, the transition metal mediated oxidation is a very important factor.

We also suggest that the prooxidant effects observed for high concentrations of ascorbyl palmitate were related to these metal ions. Ascorbic acid is able to reduce transition metals, that is, Fe^{3+} to Fe^{2+}, and, as mentioned, Fe^{2+} is a very potent prooxidant (*22*). At high concentration of ascorbyl palmitate the regeneration of metal ions might override the antioxidative properties of ascorbyl palmitate and lead to the observed prooxidant effects. The fact that a high concentration of ascorbyl palmitate still reduced PV, and only increased the concentration of volatiles, supports this hypothesis.

The prooxidative effect of ascorbyl palmitate was not observed in the dressing with high concentrations of all antioxidants; thus, EDTA and γ-tocopherol were able to override this effect of high ascorbyl palmitate concentration. Surprisingly, the addition of EDTA and γ-tocopherol together was less efficient than the addition of all three antioxidants (**Table 3**). Thus, the presence of both γ-tocopherol and ascorbyl palmitate seemed to be necessary to significantly improve the stability beyond that provided by EDTA alone, even though ascorbyl palmitate itself was only slightly antioxidative. If the prooxidant nature of ascorbyl palmitate was based on the reduction or regeneration of prooxidant transition metals, as suggested above, it then seemed that the metal chelator EDTA was able to bind these metal ions sufficiently.

In fish oil enriched mayonnaise tocopherols have previously been shown to exert only limited efficiency as antioxidants (*28*, *29*). However, these investigations were performed using tocopherol mixtures of α-, β-, γ-, and δ-tocopherol isomers. Maximum inhibition was observed with a water-dispersible tocopherol mixture in the lowest dosage applied (16 mg kg^{-1} in the final product), whereas the oil-soluble mixture even gave significantly prooxidative effects regarding PV and volatiles (*28*, *29*). In emulsions, tocopherol isomers have, to our knowledge, been tested only individually in model systems based on vegetable oil or in fish oil enriched milk (*4*, *18*, *30*). δ- and γ-tocopherols have been found to be more effective than α-tocopherol in inhibiting the autoxidation of 10% rapeseed oil emulsions (*30*), but in neither of these investigations did the presence of γ-tocopherol reduce oxidation completely.

In the present experiment, EDTA was significantly more efficient than γ-tocopherol (34−66% inhibition by γ-tocopherol vs 77−86% by EDTA; **Table 3**). Apparently, it is very important to inhibit the metal-catalyzed degradation of lipid hydroperoxides, and it seemed that if this degradation was already initiated, the presence of 220 or 880 mg kg^{-1} γ-tocopherol was not capable of inhibiting oxidation alone. Overall, this and previous works indicate that neither individual tocopherol isomers nor tocopherol mixtures are capable of inhibiting the oxidation of fish oil enriched oil-in-water emulsions completely.

In milk enriched with fish oil (PV 0.7 mequiv kg^{-1}) we have previously investigated the effects of the same three antioxidants (*4*). It was observed that neither EDTA nor a combination of α- and γ-tocopherol protected this emulsion from oxidation (pH 6.7). As mentioned, γ-tocopherol alone reduced oxidation during storage. Contrary to the present findings in salad dressing, ascorbyl palmitate was a very efficient antioxidant in the milk emulsion system. All milk emulsions containing ascorbyl

palmitate were stable against oxidation throughout the 11 day storage period regarding both PV and volatiles and also sensory off-flavor. In mayonnaise enriched with fish oil a broad range of antioxidants have been tested. EDTA was the only efficient antioxidant applied (*3*). The remaining antioxidants tested, including gallic acid, propyl gallate, ascorbic acid, ascorbyl palmitate, and different forms of tocopherol mixtures, showed activities ranging from weak antioxidative to highly prooxidative (*3*, *23*, *29*). Taken together these data first of all demonstrated that antioxidants of various solubilities and modes of action work differently in different emulsion systems. Also, the data do not fully comply with the so-called polar paradox, which suggests that nonpolar antioxidants work better than polar antioxidants in emulsion systems, because they are located in the oil droplets and thus close to the interface, where oxidation is presumed to be initiated (*20*). The fact that the water-soluble EDTA was significantly more efficient than tocopherol and ascorbyl palmitate in both salad dressing and mayonnaise indicates that other factors, apart from solubility, are important determinants for antioxidant efficacy in emulsion systems. It seems to be evident that the oxidation mechanisms, and subsequently the antioxidant efficacies, in each system are affected by the particular composition including emulsifier, metal ions, and pH.

This experiment and comparison with the knowledge on the subject available in the literature clearly signify that antioxidants can affect lipid oxidation very differently in food systems. In the acid environment of salad dressing, EDTA apparently inhibited lipid oxidation efficiently, presumably by chelating transition metals. In the salad dressing, ascorbyl palmitate in high concentration (300 mg kg^{-1}) had prooxidant effects on the formation of volatiles, whereas low concentration (50 mg kg^{-1}) gave slight antioxidant effects on both PV and volatiles. The chain-breaking antioxidant, γ-tocopherol, also had antioxidative effects. γ-Tocopherol was more efficient in retarding oxidation than ascorbyl palmitate, but still less efficient than EDTA. Finally, a combination of all three antioxidants inhibited oxidation in the salad dressing almost completely, and these emulsions were stable during 6 weeks of storage at room temperature.

ACKNOWLEDGMENT

We thank Lis Berner for excellent work in the laboratory. We also thank Maritex AS, Norway, for providing the oils.

LITERATURE CITED

(1) McClements, D. J.; Decker, E. A. Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **2000**, *65* (8), 1270−1282.

(2) Frankel, E. N. *Lipid Oxidation*, 2nd ed.; P. J. Barnes and Associates: Bridgewater, U.K., 2005.

(3) Jacobsen, C.; Hartvigsen, K.; Thomsen, M. K.; Hansen, L. F.; Lund, P.; Skibsted, L. H.; Holmer, G.; Adler-Nissen, J.; Meyer, A. S. Lipid oxidation in fish oil enriched mayonnaise: calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *J. Agric. Food Chem.* **2001**, *49*, 1009−1019.

(4) Let, M. B.; Jacobsen, C.; Pham, K. A.; Meyer, A. S. Protection against oxidation of fish-oil-enriched milk emulsions through addition of rapeseed oil or antioxidants. *J. Agric. Food Chem.* **2005**, *53*, 5429−5437.

(5) AOCS official method Cd 18-90, *p*-anisidine value; AOCS: Champaign, IL, 1994.

(6) AOCS official method Ca 5a-40, free fatty acids; AOCS: Champaign, IL, 1998.

(7) AOCS official method Ce 2-66, preparation of methyl esters of long-chain fatty acids; AOCS: Champaign, IL, 1992.

(8) AOCS official method Ce 1b-89, fatty acid composition by GLC, marine oils; AOCS: Champaign, IL, 1992.

(9) AOCS official method Ce 8-89, determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC; AOCS: Champaign, IL, 1992.

(10) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 900−917.

(11) Iverson, S. J.; Lang, S. L. C.; Cooper, M. H. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* **2001**, *36* (11), 1283−1287.

(12) IDF Standard 103A:1986, milk and milk products, determination of the iron content; International Dairy Federation: Brussels, Belgium, 1986.

(13) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Holmer, G. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *J. Agric. Food Chem.* **2000**, *48*, 4858−4867.

(14) Timm-Heinrich, M.; Xuebing, X.; Nielsen, N. S.; Jacobsen, C. Oxidative stability of milk drinks containing structured lipids produced from sunflower oil and caprylic acid. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 459−470.

(15) Venkateshwarlu, G.; Let, M. B.; Meyer, A. S.; Jacobsen, C. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *J. Agric. Food Chem.* **2004**, *52*, 311−317.

(16) Karahadian, C.; Lindsay, R. C. Action of tocopherol-type compounds in directing reactions forming flavor compounds in autoxidizing fish oil. *J. Am. Oil Chem. Soc.* **1989**, *66* (9), 1302−1308.

(17) Olsen, E.; Vogt, G.; Saarem, K.; Greibrokk, T.; Nilsson, A. Autoxidation of cod liver oil with tocopherol and ascorbyl palmitate. *J. Am. Oil Chem. Soc.* **2005**, *82* (2), 97−103.

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

(19) Perricone, N.; Nagy, K.; Horváth, F.; Dajko, G.; Uray, I.; Zs-Nagy, I. The hydroxyl free radical reactions of ascorbyl palmitate as measured in various *in vitro* models. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 661−665.

(20) Frankel, E. N.; Huang, S. W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants—bulk oils vs emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.

(21) Marinova, E. M.; Yanishlieva, N. V. Inhibited oxidation of lipids—III. On the activity of ascorbyl palmitate during the autoxidation of 2 types of lipid systems in the presence of α-tocopherol. *Fett Wiss. Technol. (Fat Sci. Technol.)* **1992**, *94* (12), 448−452.

(22) Halliwell, B.; Gutteridge, J. *Free Radicals in Biology and Medicine*, 2nd ed.; Clarendon Press: Oxford, U.K., 1989.

(23) Jacobsen, C.; Timm, M.; Meyer, A. S. Oxidation in fish oil enriched mayonnaise: ascorbic acid and low pH increase oxidative deterioration. *J. Agric. Food Chem.* **2001**, *49*, 3947−3956.

(24) Thomsen, M. K.; Jacobsen, C.; Skibsted, L. H. Mechanism of initiation of oxidation in mayonnaise enriched with fish oil as studied by electron spin resonance spectroscopy. *Eur. Food Res. Technol.* **2000**, *211* (6), 381−386.

(25) Cuvelier, M. E.; Lagunes-Galvez, L.; Berset, C. Do antioxidants improve the oxidative stability of oil-in-water emulsions? *J. Am. Oil Chem. Soc.* **2003**, *80* (11), 1101−1105.

(26) Mancuso, J. R.; McClements, D. J.; Decker, E. A. The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. *J. Agric. Food Chem.* **1999**, *47*, 4112−4116.

(27) Djordjevic, D.; McClements, D. J.; Decker, E. A. Oxidative stability of whey protein-stabilized oil-in-water emulsions at pH

3: potential omega-3 fatty acid delivery systems (part B). *J. Food Sci.* **2004**, *69* (5), C356−C362.

(28) Jacobsen, C.; Hartvigsen, K.; Lund, P.; Thomsen, M. K.; Skibsted, L. H.; Holmer, G.; Adler-Nissen, J.; Meyer, A. S. Oxidation in fish oil-enriched mayonnaise: 4. Effect of tocopherol concentration on oxidative deterioration. *Eur. Food Res. Technol.* **2001**, *212* (3), 308−318.

(29) Jacobsen, C.; Hartvigsen, K.; Lund, P.; Adler-Nissen, J.; Holmer, G.; Meyer, A. S. Oxidation in fish-oil-enriched mayonnaise 2. Assessment of the efficacy of different tocopherol antioxidant systems by discriminant partial least squares regression analysis. *Eur. Food Res. Technol.* **2000**, *210* (4), 242−257.

(30) Wagner, K. H.; Isnardy, B.; Elmadfa, I. γ- and δ-tocopherols are more effective than α-tocopherol on the autoxidation of a 10% rapeseed oil triacylglycerol-in-water emulsion with and without a radical initiator. *Eur. J. Lipid Sci. Technol.* **2004**, *106* (1), 44−51.

Received for review September 19, 2006. Revised manuscript received January 11, 2007. Accepted January 25, 2007. This study was financed by a grant from the Danish Føtek III program, Tine BA, Norway, and Arla Foods a.m.b.a, Denmark.

JF062675C