Oxidation in Fish Oil Enriched Mayonnaise: Ascorbic Acid and Low pH Increase Oxidative Deterioration

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The effect of ascorbic acid (0−4000 ppm) and pH (3.8−6.2) on oxidation and levels of iron and copper in various fractions of mayonnaise enriched with 16% fish oil was investigated. Ascorbic acid induced release of iron from the assumed oil−water interface into the aqueous phase at all pH levels, but this effect of ascorbic acid was strongest at low pH (pH 3.8−4.2). Ascorbic acid generally promoted formation of volatile oxidation compounds and reduced the peroxide value in mayonnaises. Peroxide values and total volatiles generally increased with decreasing pH values, suggesting that low pH promoted oxidation. It is proposed that iron bridges between the egg yolk proteins low-density lipoproteins, lipovitellin, and phosvitin at the oil−water interface are broken at low pH values, whereby iron ions become accessible as oxidation initiators. In the presence of ascorbic acid, oxidation is further enhanced due to the reduction of Fe^{3+} to Fe^{2+} that rapidly catalyzes lipid oxidation via lipid hydroperoxide decomposition at the oil−water interface in mayonnaise.

Keywords: *Mayonnaise; pH; ascorbic acid; iron release; oxidation*

INTRODUCTION

Foods containing *n*−3 polyunsaturated fatty acids are highly susceptible to oxidation, which causes undesirable flavors and loss of those *n*−3 fatty acids that may be physiologically beneficial. To minimize oxidation, antioxidants, including aqueous compounds such as ascorbic acid, are often added to protect such food products from oxidative deterioration (*1*). Ascorbic acid mainly exerts its antioxidative effect by radical scavenging via direct reaction with hydrophilic free radicals (*2*) but may also act as an O_2 scavenger (*3*). Ascorbic acid has, however, also been shown to act as a pro-oxidant due to its ability to reduce Fe^{3+} to Fe^{2+}, which can catalyze lipid oxidation via the breakdown of already existing lipid hydroperoxides (LOOH) (*4*).

In our previous study we observed that the addition of ascorbic acid in concentrations between 40 and 800 ppm (0.23 and 4.45 mM) to fish oil enriched mayonnaises immediately promoted the formation of strong fishy, rancid, and metallic off-flavors. The intensity of these off-flavors increased with increasing levels of ascorbic acid. Moreover, ascorbic acid appeared to induce release of iron ions from the egg yolk protein phosvitin located at the oil/water (o/w) interface into the aqueous phase of mayonnaise (*5*). This iron-releasing effect of ascorbic acid was, however, partly confounded with the decreasing effect of ascorbic acid addition on pH, and we did not assess the combined effect of ascorbic acid and low pH on oxidation during storage (*5*).

In a recent study on the tendency of formation of free radicals in fish oil enriched mayonnaise with or without vinegar, it was observed that free radicals could be detected only when vinegar was present (*6*). The same phenomenon was observed when vinegar was substituted with iron- and copper-free acetic acid (<50 ng/g). In mayonnaises with vinegar or acetic acid, the pH was ∼4.0, whereas the pH was 6.0 in mayonnaise without these ingredients. It was therefore proposed that a low pH catalyzed oxidation in fish oil enriched mayonnaise.

The objectives of the present study were therefore (1) to investigate the effect of ascorbic acid at different pH levels on both iron release from the oil−water interface and oxidation and (2) to determine the effect of pH on iron release and oxidation during the storage of mayonnaise.

Mayonnaises at six different pH levels were produced by varying the addition level of acetic acid. At each pH level, mayonnaises were prepared with two to four different concentrations of ascorbic acid (Table 1). The selection of ascorbic acid concentrations was to a certain extent limited by the fact that the addition of ascorbic acid to the mayonnaise reduces pH, especially at high pH levels. Thus, it was not possible to produce mayonnaises with high ascorbic acid concentrations and high pH values. Moreover, we did not test low ascorbic acid concentrations at low pH values, as such experiments had already been carried out in our previous study (*5*). To quantify iron and copper ions in the different phases, mayonnaises were separated into different fractions by ultracentrifugation (Figure 1) (*7*). To establish the effect of pH and ascorbic acid on oxidation, mayonnaises were stored at 20 °C for 4 weeks and the formation of lipid hydroperoxides and selected volatile oxidation products was determined after 0, 2, and 4 weeks.

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Table 1. Iron Concentrations and Recoveries in Aqueous Phase 2, Precipitate, and Emulsion Phase

			Fe concn (μM)			Fe recovery (%)		
group	pH in mayonnaise[a]	ascorbic acid added (ppm)	in aqueous phase 2	in precipitate	in emulsion phase[b]	in aqueous phase 2[b,c]	in precipitate[b,c]	in emulsion phase[b,c]
A1	6.0	0	13.1 ± 0.2	486 ± 16	237	24.2	58.4	18.7
A2	6.2	400 (2.27)[d]	16.8 ± 0.5	475 ± 18	190	32.4	45.4	20.7
B1	5.1	0	2.7 ± 0.7	625 ± 54	371	5.2	72.2	31.4
B2	5.4	400 (2.27)	10.6 ± 0.7	541 ± 41	229	20.4	51.4	27.6
B3	5.4	1200 (6.81)	12.5 ± 0.5	491 ± 23	258	25.5	43.2	30.0
B4	5.3	1300 (7.38)	15.8 ± 2.2	432 ± 32	229	29.4	44.1	25.1
C1	4.8	0	1.4 ± 0.2	529 ± 13	281	2.9	67.0	35.2
C2	5.1	800 (4.54)	12.5 ± 0.2	428 ± 7	253	22.9	44.2	26.5
D1	4.4	0	4.3 ± 2.9	667 ± 39	111	6.4	68.5	8.0
D2	4.4	800 (4.54)	36.4 ± 1.4	204 ± 2	73	48.0	16.1	5.9
D3	4.6	1200 (6.81)	35.3 ± 3.9	260 ± 16	131	56.6	22.1	12.8
E1	4.2	0	2.3 ± 0.7	724 ± 32	122	4.3	95.1	12.9
E2	4.2	2000 (11.35)	48.7 ± 0.4	131 ± 2	34	93.8	15.4	3.2
F1	3.8	0	nd[e]	823 ± 90	145	0.0	102.6	13.2
F2	3.8	2000 (11.35)	51.4 ± 2.3	91 ± 0	30	104.4	10.9	2.2
F3	3.8	4000 (22.70)	50.9 ± 0.7	90 ± 7	27	105.8	11.1	3.0

[a] Mayonnaises within the same group were intended to have exactly the same pH value, but it was difficult to obtain the desired pH values when the pH was >~4.5. Mayonnaise 4 in group B originally was intended to belong to group C, but because its actual pH value was closer to the pH values in group B, it was moved to this group. [b] Only one replicate. [c] Recoveries were calculated relative to the total amount of iron in mayonnaises, which was the same in all mayonnaises (25−34 μM). [d] Values in parentheses are concentrations in mM. [e] nd, not detectable.

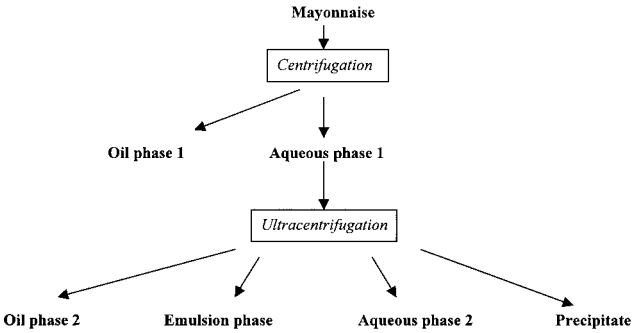


Figure 1. Flow chart of the separation procedure.

MATERIALS AND METHODS

Materials. Refined, unhydrogenated rapeseed oil was obtained from Aarhus Olie A/S, Aarhus, Denmark [peroxide value (PV), <0.3 mequiv/kg; anisidine value, 1.8; iron content, < 0.1 ppm (<1.8 μM); and copper content, <0.05 ppm (<0.8 μM), as analyzed by atomic absorption spectrophotometry (*8*)]. 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 [PV, <0.3 mequiv/kg; anisidine value, 4.9; iron content, <0.1 ppm (<1.8 μM); and copper content, <0.05 ppm (<0.8 μM)]. Egg yolk with 3% salt (NaCl) was from Danæg, Copenhagen, Denmark [iron content, 41 ppm (=734 μM); copper content, 1.1 ppm (=17.3 μM)]. Potassium sorbate and ascorbic acid [Fe < 0.0002% (<3.6 μM)] were purchased from Merck (Darmstadt, Germany), and ultrapure acetic acid (Cu and Fe < 50 ng/g) was from Romil, Cambridge, U.K. Grindsted FF 5105 stabilizer was donated by Danisco Cultor (Brabrand, Denmark).

Mayonnaise Production. Mayonnaises were produced in 1 kg batches composed of 640.0 g of rapeseed oil (64% w/w), 160.0 g of fish oil (16% w/w), 40.0 g of egg yolk (4.0% w/w), 3.0 g of NaCl (0.3% w/w), 10.0 g of sugar (1.0% w/w), 1.0 g of potassium sorbate (0.1% w/w), and 2.0 g of Grindsted FF 5105 (0.2% w/w). Depending on the desired concentration, 0.4, 0.8, 1.2, 1.3, 2.0, or 4.0 g of ascorbic acid (weighed to an accuracy of ±0.0001 g) was mixed with water before mayonnaise production. The amount of water was adjusted so that the total amount of ingredients was 1000.0 g in all experiments. Depending on the desired pH, between 0 and 2.0 mL of acetic acid was mixed with 270 g of oil before mayonnaise production to imitate the conditions of industrial mayonnaise production. Mayonnaises were produced as previously described (*9*) except that vinegar was substituted with acetic acid.

The amounts of acetic acid required to obtain a certain pH value in mayonnaises with different ascorbic acid levels were determined in preliminary experiments using only the aqueous phase of mayonnaise. Apart from water this aqueous phase also contained egg yolk, salt, sugar, potassium sorbate, and ascorbic acid. Acetic acid was added dropwise to the aqueous phase of mayonnaise until the desired pH was obtained. However, when producing the real mayonnaises with pH values above 4.5, we did not obtain the same pH value as in the preliminary experiments on the aqueous phase, even though the same amounts of acetic acid were used. Hence, pH values were slightly higher in some of the mayonnaises, which is why we included mayonnaises with slightly different pH values in the same experimental groups (see Table 1). pH values in the real mayonnaises was measured by a pH-electrode immediately after production.

Separation of Mayonnaises for Determination of Iron and Copper. Aliquots of 350 g of freshly produced mayonnaise were packed in aluminum bags from Danisco Flexible (Lyngby, Denmark). Then mayonnaises were frozen at −40 °C, thawed at room temperature, and separated by centrifugation at 25400*g* for 10 min (*7*). After this centrifugation, an oil phase 1 and an aqueous phase 1 were obtained (Figure 1). The latter was diluted 1:2 with distilled water and was subsequently ultracentrifuged at 85800*g* for 15 h at 15 °C (*10*). Thereby, four phases were obtained: a precipitate, aqueous phase 2, an emulsion phase, and oil phase 2 (Figure 1).

Determination of Iron and Copper. The samples (whole mayonnaise, oil phase 1, aqueous phase 2, emulsion phase, and precipitate) were incinerated and dissolved in acid, and iron and copper were determined by atomic absorption spectrophotometry (*8*). The analysis did not differentiate between Fe^{2+} and Fe^{3+} ions. Analyses were made in duplicates. Oil phase 2 was not analyzed as preliminary experiments had shown that the composition of this phase was the same as that of oil phase 1.

Storage Experiment. Mayonnaises were stored in 50 mL screw-capped, brown glass jars in the dark at 20 °C for 4 weeks. Samples were taken after 0, 2, and 4 weeks and frozen at −40 °C until determination of peroxide values and selected volatile oxidation products.

Determination of PV. Mayonnaises were thawed and centrifuged at 25400*g* for 10 min. PV was determined in the separated oil phase by spectrophotometric determination of free iodine formed by the reaction of lipid peroxides with

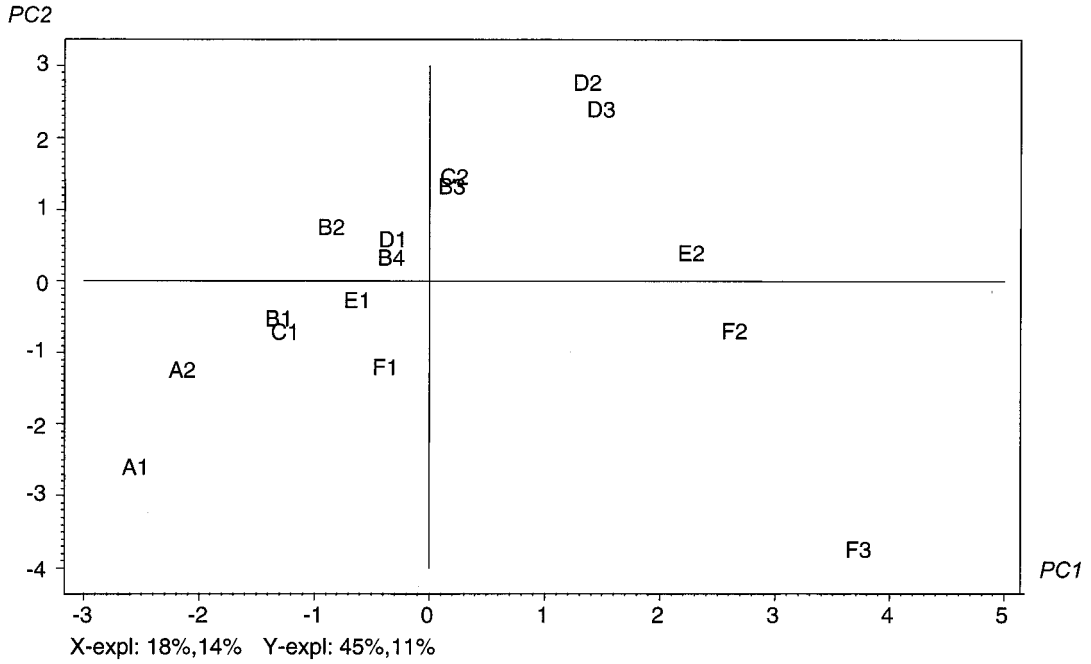


Figure 2. Scores plot from the APLSR analysis on all variables. For interpretation of object names, refer to group names in Table 1, which also shows the actual levels of pH and ascorbic acid.

potassium iodine with $AlCl_3$ as a catalyst (*11*). Analyses were made in duplicate.

Determination of Volatile Oxidation Products. Frozen mayonnaise (4 g) was weighed into a pear-shaped glass flask together with *n*-dodecane as an internal standard, and headspace volatiles were collected on Tenax tubes by purging the mayonnaise with nitrogen as previously described (*12*). Volatile acids, notably acetic acid, were removed by neutralization with potassium hydroxide during the dynamic headspace collection (*12*), and the remaining volatiles were separated and quantified by gas chromatography on a Hewlett-Packard 5890 (Avondale, PA) gas chromatograph equipped with a flame ionization detector (FID). The oven temperature program used was as follows: 35 °C, 5 min; increased at 1.5 °C/min to 55 °C; increased at 2.5 °C/min to 90 °C; increased at 12 °C/min to 220 °C; 220 °C, 4 min. A J&W Scientific DB 1701 (Folsom, CA) capillary column was employed (length = 30 m, i.d. = 0.25 mm, film thickness = 1.0 μm).

Nine volatile oxidation compounds were selected for quantification: 2-ethylfuran, 1-penten-3-one, pentanal, 2-*E*-pentenal, hexanal, heptanal, 2-pentylfuran, 2-*E*-heptenal, and 2,4-heptadienal. The criteria for selecting these nine compounds were as follows: (1) the compounds were previously found to be among those that most likely contributed to the fishy off-flavor formed during oxidation in fish oil enriched mayonnaise (*12*−*15*); (2) the compounds were easily identifiable by GC-FID analysis. The identities of the compounds were confirmed by GC-MS and by spiking with external standards. Quantification was performed with the aid of calibration curves obtained for the nine different volatile compounds in seven concentrations (0, 25, 100, 200, 400, 600, and 900 ng/g). The total concentration of selected compounds ("total volatiles") was calculated by summation of concentrations of individual compounds. Analyses were made in triplicate.

Data Analysis. Data obtained from the determination of metal ions in the different phases of mayonnaise as well as data from the storage experiment were analyzed together by multivariate data analysis using the Unscrambler version 7.5 alpha 1 software program (Camo, Norway). The data were analyzed by ANOVA principal least squares regression (APLSR) using design variables as *X* data and the measured variables as *Y* data. The qualitative design variables employed were, for ascorbic acid levels, 0, 400, 800, 1200, 1300, 2000, and 4000 ppm; for pH levels, A:6.0−6.2, B:5.1−5.4, C:4.8−5.1, D:4.4−4.6, E:4.2, and F:3.8; and for replicates, Rep1, Rep2, Rep3, and Av (mean values). Furthermore, quantitative design variables describing the actual concentrations of ascorbic acid (asc acid) and the actual pH level (pH) were included. Finally, interactions (pH**2, ascorbic acid**2, and pH*ascorbic acid) were also included as design variables. The values taken into account for data analysis included PV; concentrations of selected volatiles; iron concentrations in aqueous phase 2, emulsion phase, and precipitate; and recoveries of iron in these three phases. Copper concentrations as well as iron concentrations in the mayonnaise and in oil phase 1 were not included in the analysis as they did not change upon addition of ascorbic acid or with decreasing pH (data not shown). Cross-validation on treatments was used to validate the APLSR model. All variables were weighed by 1/standard deviation. By using the jack-knifing facility in the Unscrambler it was possible to assess whether regression coefficients for the different design variables were significantly positive or negative ($p < 0.1$) for each of the measured variables. The regression coefficients were automatically calculated by the Unscrambler software in connection with the calculations of the APLSR model.

RESULTS

Multivariate Data Analysis. The present study consisted of two parts: (1) evaluation of the influence of pH and ascorbic acid on iron and copper release from the o/w interface into the aqueous phase in freshly prepared rapeseed/fish oil mayonnaises and (2) evaluation of the influence of pH and ascorbic acid levels on the formation of primary and secondary oxidation products in these mayonnaises during storage at 20 °C. Data obtained from both parts of the study were analyzed by APLSR. Four principal components (PCs) were validated. Together, these explained 51% of the variance in the *X* data and 72% of the variance in the *Y* data. Only PC1 and PC2, which together explained 32% of the variance in *X* data and 56% of the variance in *Y* data, will be discussed in the following (Figures 2 and 3) as PC3 and PC4 did not explain the effect of pH and ascorbic acid addition.

Scores Plot. In the scores plot, mayonnaises with high pH values and low ascorbic acid levels were located to the left in the diagram (Figure 2). Mayonnaises moved

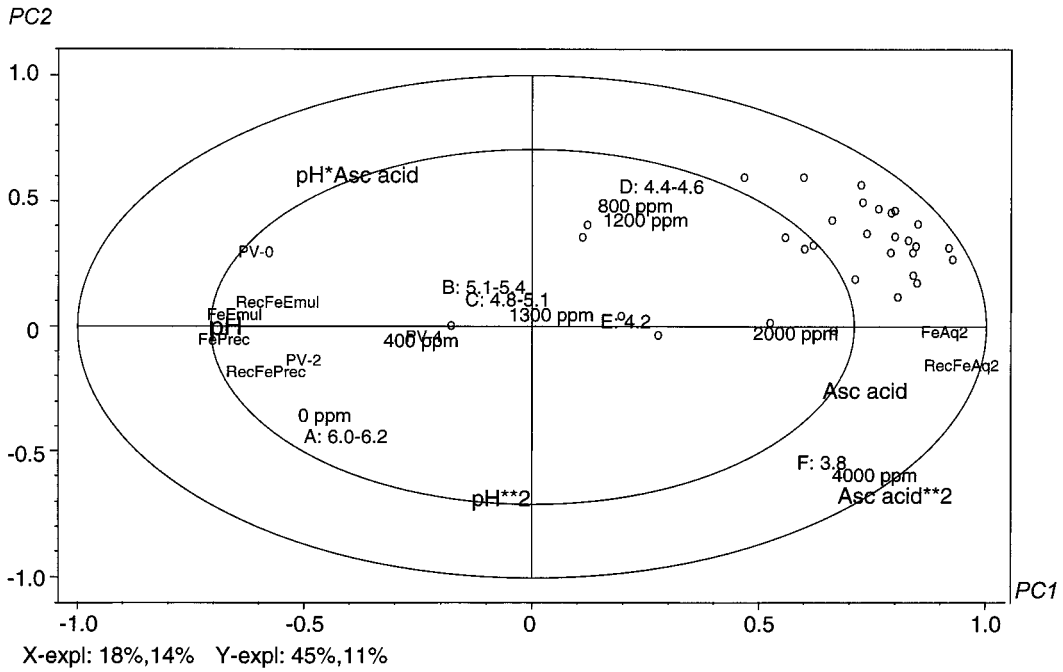


Figure 3. Correlation loadings plot from the APLSR analysis on all variables: Fe, iron concentration; RecFe, recovery of iron in the fraction in question; Emul, emulsion phase; Prec, precipitate; Aq2, aqueous phase 2; o, volatile compounds; the number after the hyphen refers to storage time in weeks; pH, quantitative design variable for actual pH concentration; pH**2, pH squared; Asc acid, quantitative design variable for actual ascorbic acid level; Asc acid**2, ascorbic acid concentration squared; 0, 400, 800, 1200, 1300, 2000 and 4000 ppm, qualitative design variables describing ascorbic acid level; Asc acid*pH, interaction between ascorbic acid and pH. A capital letter followed by pH intervals (e.g., A:6.0−6.2) denotes qualitative design variables that refer to the mayonnaise groups in Table 1. The inner and outer ellipses represent 50 and 100% explained variance, respectively.

to the right with decreasing pH values and increasing ascorbic acid values. Mayonnaises with the same pH value generally moved toward larger PC2 values with increasing ascorbic acid concentration when ascorbic acid concentrations were lower than ∼1200 ppm. When ascorbic acid concentrations were higher, mayonnaises tended to move toward lower PC2 values with increasing ascorbic acid concentrations. This trend was particularly evident for the mayonnaise with pH ∼3.8 and 4000 ppm of ascorbic acid (code F3), which was located in the lower right corner of the plot (Figure 2). In summary, the movement of the mayonnaises from high pH and no ascorbic acid to mayonnaises with a low pH and high ascorbic acid concentration could be described as a half-circle. Thus, PC1 mainly seemed to explain the variation in the data caused by pH and ascorbic acid, whereas PC2 to a certain degree seemed to be related to the interaction between pH and ascorbic acid.

Correlation Loadings Plot: Design Variables. In the loadings plot, the quantitative design variables describing actual pH and ascorbic acid levels were located to the far left and right, respectively (Figure 3). The ascorbic acid design variable thus had a high PC1 value but a slightly negative PC2 value, whereas the pH design variable had a negative PC1 value but was placed on the PC1 axis, resulting in a PC2 value of zero. The qualitative design variables describing pH levels of mayonnaise groups moved from left to right with decreasing pH values: when pH values decreased from pH ∼6.2 to 4.6 (groups A to D), the variables moved almost linearly upward from the third quadrant to the first quadrant (Figure 3), but for values below pH 4.4, the direction shifted to more negative PC2 values, possibly indicating a different type of interference by pH decrease in mayonnaises at these low pH ranges. The qualitative design variables describing ascorbic acid levels moved in a similar half-circular pattern. Thus, they moved from the left to the right and from low PC2 values to high PC2 values and finally back to low PC2 values with increasing ascorbic acid additions. The pattern of pH and ascorbic acid variables together with the location of the pH*Asc acid variable in the upper left corner could indicate an interactive effect between decrease in pH and increase in ascorbic acid in mayonnaises.

Correlation Loadings Plot: Effect of pH and Ascorbic Acid on Iron Concentrations. Iron concentrations and recoveries in the emulsion phase and the precipitate were located to the left near the quantitative pH design variable, whereas iron concentration and recovery in the aqueous phase were located to the right near the quantitative ascorbic acid design variable (Figure 3). These observations indicated that iron concentrations decreased in the precipitate and emulsion phase upon addition of ascorbic acid (i.e., a negative correlation), whereas they increased in the aqueous phase (i.e., a positive correlation). The raw data confirmed this interpretation of the APLSR model (Table 1). Thus, in mayonnaise with pH ∼3.8 iron concentrations decreased from 823 to 90 μM in the precipitate and from 145 to 27 μM in the emulsion phase when ascorbic acid levels were increased from 0 to 4000 ppm, whereas iron concentrations in aqueous phase 2 increased from 0 to 51 μM (Table 1). Also, the data for iron recovery were in agreement with the interpretation that ascorbic acid addition was associated with increased iron levels in aqueous phase 2 and decreased iron levels in the emulsion and precipitate phases obtained after ultracentrifugation of mayonnaises (Table 1). The effect of ascorbic acid addition was particularly strong in mayonnaises with pH <4.4, where the iron recovery in aqueous phase 2 increased to almost 100% upon supplementation

Table 2. Peroxide Values in Mayonnaise during Storage at 20 °C ($n = 2$)

group	pH in mayonnaise	ascorbic acid added (ppm)	PV (mequiv/kg) week 0	week 2	week 4
A1	6.0	0	1.6 ± 0.1	2.2 ± 0.6	1.5 ± 0.1
A2	6.2	400 (2.27)[a]	1.4 ± 0.2	1.8 ± 0.1	0.4 ± 0.1
B1	5.1	0	1.1 ± 0.3	1.6 ± 0.3	2.1 ± 0.3
B2	5.4	400 (2.27)	1.4 ± 0.0	0.8 ± 0.3	0.9 ± 0.1
B3	5.4	1200 (6.81)	1.0 ± 0.0	0.5 ± 0.3	0.6 ± 0.3
B4	5.3	1300 (7.38)	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2
C1	4.8	0	1.1 ± 0.1	1.6 ± 0.2	1.8 ± 0.0
C2	5.1	800 (4.54)	1.1 ± 0.3	0.7 ± 0.2	0.8 ± 0.1
D1	4.4	0	1.0 ± 0.0	1.3 ± 0.1	4.2 ± 0.3
D2	4.4	800 (4.54)	1.0 ± 0.1	0.7 ± 0.0	0.5 ± 0.2
D3	4.6	1200 (6.81)	0.9 ± 0.0	0.5 ± 0.2	0.4 ± 0.1
E1	4.2	0	1.2 ± 0.1	5.2 ± 0.5	9.9 ± 2.9
E2	4.2	2000 (11.35)	1.3 ± 0.2	0.9 ± 0.1	0.3 ± 0.1
F1	3.8	0	0.0 ± 0.0	2.7 ± 0.2	2.6 ± 0.1
F2	3.8	2000 (11.35)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
F3	3.8	4000 (22.70)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Values in parentheses are concentrations in mM.

with ascorbic acid. Moreover, the regression coefficient for ascorbic acid was significantly positive ($p < 0.1$) for iron concentration and recoveries in aqueous phase 2 and significantly negative for iron concentrations and recoveries in the emulsion phase and precipitate (data not shown). A positive regression coefficient indicates a positive correlation between the variables, and a negative regression coefficient indicates a negative correlation between the variables.

The location of variables describing iron concentrations and recoveries indicated that iron concentrations and recoveries in aqueous phase 2 increased with decreasing pH values (i.e., a negative correlation), whereas they decreased in the emulsion phase and precipitate (positive correlation). However, the raw data did not show a clear pattern with respect to the effect of pH on iron concentrations in the respective fractions. For example, iron concentrations in the emulsion phase from mayonnaise without ascorbic acid increased from 237 to 371 μM when the pH was decreased from 6.0 to 5.1 but decreased to 281 μM when the pH was further decreased to 4.8. Comparison of iron concentrations in the emulsion phase from mayonnaises with the same ascorbic acid concentrations showed a similar trend. Moreover, the regression coefficient for pH was not significant for any of the variables describing iron concentrations and recoveries. Hence, the effect of pH on iron concentrations in the various fractions was ambiguous.

Correlation Loadings Plot: Effect of pH and Ascorbic Acid on Oxidation Parameters. The variables describing PV were located to the left in the diagram and moved from low PC1 to higher PC1 values with increasing storage time (Figure 3). PV after 4 weeks was located relatively close to the origin. This indicated that PV-4 was not well explained by the model. However, the APLSR model indicated that PVs after 0 and 2 weeks were higher in mayonnaises with high pH and lower in mayonnaises with high ascorbic acid concentrations. Comparison of PV-4 throughout all pH groups in mayonnaises without ascorbic acid showed that PV-4 increased from 1.5 to 9.9 mequiv/kg when the pH was decreased from 6.2 to 4.2, but when the pH was further decreased to 3.8, PV-4 did not increase but rather decreased (from 9.9 to 2.6 mequiv/kg, Table 2). This surprising pattern in PV-4 may reveal why PV-4 was badly explained by the model. Comparison of PVs in mayonnaises with the same pH level showed that the PV generally was lower in mayonnaises supplemented with ascorbic acid during the entire storage period (Table 2). For example, in mayonnaises with pH ~4.5, the PV was 4.2 mequiv/kg after 4 weeks when no ascorbic acid was present but was only 0.5 and 0.4 mequiv/kg when mayonnaises were supplemented with 800 and 1200 ppm of ascorbic acid, respectively (Table 2). As expected, the effect of ascorbic acid on PV was more pronounced after 2 and 4 weeks of storage than in the fresh samples. The regression coefficients for ascorbic acid were significantly negative for PV-2 and PV-4, whereas the regression coefficients for pH were insignificant ($p > 0.1$). The decreasing effect of ascorbic acid on PV could indicate an antioxidative effect but is more likely due to the ability of ascorbic acid to promote the breakdown of lipid hydroperoxides to secondary oxidation products, as will be discussed later.

Most of the variables describing concentrations of volatiles were located to the right in the diagram, and most had positive PC2 values. The APLSR model thus suggested that volatiles generally were formed in higher concentrations in mayonnaises with low pH. Comparison of the formation of volatiles in mayonnaises without ascorbic acid confirmed this interpretation of the model. Selected data are shown in Figure 4. For example, concentrations of 2,4-heptadienal increased from 36 to 180 ng/g during storage in mayonnaise with pH ~3.8 but only from 13 to 19 ng/g in mayonnaise with pH ~6.0. Likewise, the concentration of 2,4-heptadienal after 4 weeks increased with decreasing pH values (Figure 4e). The same pattern was observed for the other volatiles except for 2-pentylfuran and 2-*E*-heptenal, which had slightly higher concentrations in mayonnaises with pH ~4.2 than with pH~3.8 (data not shown). The regression coefficients for pH for the volatiles were significantly negative ($p < 0.1$) for 2-ethylfuran after 2 weeks, for pentanal after 4 weeks, and for hexanal, heptanal, 2-pentylfuran, and total volatiles after 2 and 4 weeks.

It is more difficult to interpret the effect of ascorbic acid on the formation of volatiles from Figure 3, because most of the volatiles were located at the other side of the PC1 axis than the ascorbic acid design variable. However, the model could indicate that volatile formation increased upon the addition of ascorbic acid. To investigate this further, regression coefficients for ascorbic acid versus each of the volatiles were examined. It was observed that the regression coefficient was significantly positive for 2-pentylfuran and 2,4-heptadienal after 2 weeks and for pentanal, hexanal, heptanal, 2-*E*-heptenal, and total volatiles after both 2 and 4 weeks. Therefore, ascorbic acid addition seemed to increase the formation of volatiles.

Scatter Plots of Measured Data. Because the APLSR model above indicated interactions between the decrease in pH and the addition of ascorbic acid, scatter plots were constructed to illustrate the combined effect of pH and ascorbic acid on iron concentrations in different phases and on the formation of the selected nine volatile oxidation products (Figures 5 and 6).

Scatter Plots: Effect of pH and Ascorbic Acid on Iron Concentrations. The scatter plots for iron concentrations and iron recoveries in the respective fractions after ultracentrifugation were similar. Moreover, plots of the iron concentrations in the precipitate and emulsion phase also showed the same trends. Therefore, only the plots of the iron concentrations in aqueous phase 2 and the precipitate are shown (Figure 5a,b). The scatter plot

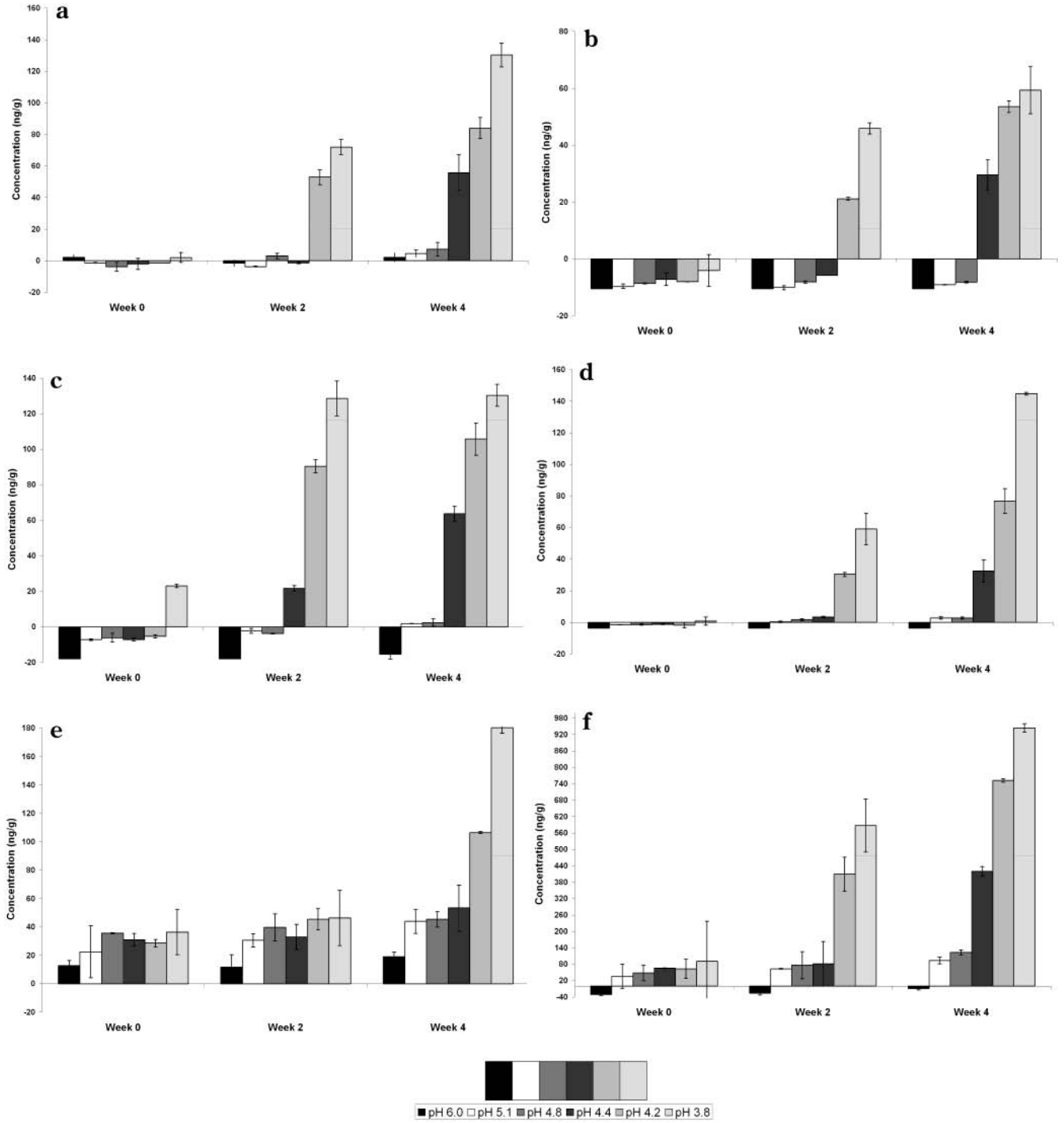


Figure 4. Concentrations of selected volatiles during storage in mayonnaise without ascorbic acid with different pH levels: (a) 2-ethylfuran; (b) 1-penten-3-one; (c) pentanal; (d) 2-*E*-pentenal; (e) 2,4-heptadienal; (f) total volatiles.

of iron concentrations measured in aqueous phase 2 (Figure 5a) clearly showed that the level of iron was more affected by ascorbic acid concentration than by pH. Moreover, the effect of ascorbic acid on iron levels tended to be higher at lower pH values (Figure 5a). This can also be deduced from the observation that the regression coefficient for the pH*ascorbic acid interaction was negative, although it was not significant ($p > 0.1$).

The scatter plots of iron concentrations in the precipitate and emulsion phase were similar as mentioned above. In both fractions, iron concentrations decreased upon the addition of ascorbic acid, but the effect was stronger in the precipitate (Figure 5b) than in the emulsion phase; moreover, in the precipitate, the pH almost had no effect at low ascorbic acid concentrations, whereas iron concentrations in the emulsion phase increased with increasing pH values irrespective of the ascorbic acid concentration (Figure 5b). This difference between the interaction effects of pH and ascorbic acid was confirmed by calculation of the regression coefficients: for the pH*ascorbic acid interaction, the regression coefficient was positive for the iron concentration in the precipitate, but zero for the iron concentrations in the emulsion phase.

Scatter Plots: Effect of pH and Ascorbic Acid on Oxidation. The scatter plots after 2 and 4 weeks for both PV and the volatiles were similar. Furthermore, com-

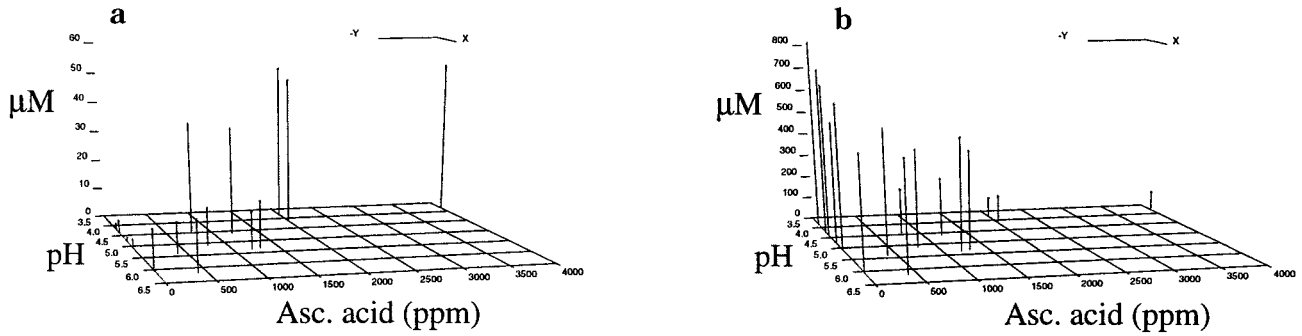


Figure 5. Scatter plots of the effect of pH and ascorbic acid on iron concentrations in mayonnaise fractions: (a) aqueous phase 2; (b) precipitate.

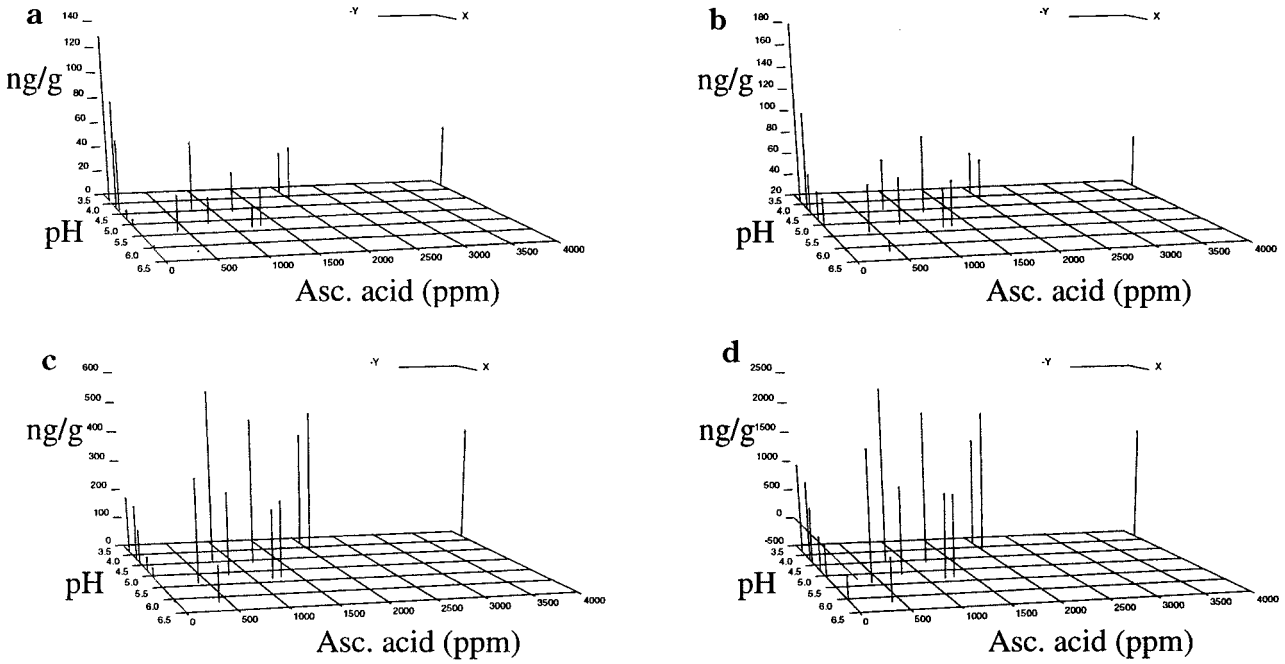


Figure 6. Scatter plots of the effect of pH and ascorbic acid on selected volatiles after 4 weeks of storage: (group 1) (a) 2-ethylfuran and (b) 2,4-heptadienal; (group 2) (c) heptanal and (d) total volatiles.

parison of the scatter plots of PV and the nine selected volatile oxidation compounds plus the "total volatiles" showed that the plots could be categorized in two groups. Therefore, only two selected plots after 4 weeks from each group are shown here (Figure 6). Group 1: the concentrations of compounds in this group decreased with increasing ascorbic acid levels when the pH was $<\sim 5.0$, whereas the concentrations seemed to increase or remain constant with increasing concentrations of ascorbic acid when the pH was >5.0 (Figure 6a,b). This group of compounds included 2-ethylfuran, 1-penten-3-one, 2,4-heptadienal, 2-*E*-pentenal, and 2-pentylfuran. Group 2: the concentrations of compounds increased with increasing ascorbic acid levels, irrespective of the pH level (Figure 6c,d). This group of compounds included pentanal, hexanal, heptanal, 2-*E*-heptenal, and total volatiles.

The regression coefficients calculated from the APLSR analysis showed that compounds in group 1 always had positive regression coefficients for the pH*ascorbic acid interaction. This meant that the combined effect of pH and ascorbic acid was greater than one would expect from the effects of each of these two parameters; for example, the combined effect caused higher values than expected when the values of both parameters were increased. In group 2, only three compounds (2-*E*-heptenal, heptanal, and total volatiles) had positive regression coefficients for the pH*ascorbic acid interaction, whereas the remaining two compounds (pentanal and hexanal) had negative regression coefficients.

DISCUSSION AND CONCLUSION

The results obtained demonstrated that the addition of ascorbic acid increased iron concentrations in the aqueous phase of mayonnaises. Furthermore, increased iron concentrations in the aqueous phase were accompanied by decreased iron concentrations in both the precipitate and the emulsion phase. These results were in perfect agreement with the results from our previous study (*5*). Obviously, for the data to reflect events occurring in intact mayonnaise it is important that the phases obtained by ultracentrifugation relate to different phases in mayonnaise: For our studies mayonnaises were separated into different fractions by centrifugation and ultracentrifugation (Figure 1). On the basis of previous detailed analysis of protein, fat, and phosphorus in the different fractions (*7*), we believe that aqueous phase 2 represents the "real" aqueous phase in mayonnaise, whereas the emulsion phase and precipitate together represent the o/w interface in mayonnaise (*16*). Therefore, the result of the present study confirmed and

expanded our previous proposition that ascorbic acid supplemented during mayonnaise production can induce flavor deterioration via release of iron from the o/w interface into the aqueous phase of mayonnaise at all pH levels between 3.8 and 6.2. In our earlier study (*5*) the effect of ascorbic acid addition on this iron release was confounded with the decreasing effect of ascorbic acid on the pH level. The present study was designed to minimize this confounding effect and showed that ascorbic acid affected iron concentrations in the various fractions at all tested pH levels but that ascorbic acid supplementation had the largest effect at low pH values.

The present study showed that the concentration of all nine selected volatile oxidation products increased with increasing ascorbic acid levels when the pH was $>\sim$5.0. The concentrations of four of the volatiles (pentanal, hexanal, heptanal, and 2-*E*-heptenal) as well as total volatiles (group 2) also increased with ascorbic acid addition when the pH was <5.0, but the other selected volatiles [2-ethylfuran, 1-penten-3-one, 2,4-heptadienal, 2-*E*-pentenal, and 2-pentylfuran (group 1)] decreased rather than increased with increasing ascorbic acid concentrations at pH values $<\sim$5. This unexpected phenomenon may be related to the differences in the molecular structure of the compounds in the two groups. Thus, whereas the compounds in group 2 were simple saturated aldehydes, except for 2-*E*-heptenal, the compounds in group 1 were either furans, unsaturated ketones, or aldehydes. The latter compounds may be more prone to further reaction than saturated aldehydes, and the decrease in their concentration may be a result of such reactions; for example, they may react directly with egg yolk proteins or with other lipid oxidation products or oxidation compounds formed from the oxidation of these proteins by ascorbic acid at low pH values. Clearly, further experimental data are required to elucidate this phenomenon in more detail. The PV decreased upon addition of ascorbic acid, and the effect of ascorbic acid was greatest at low pH values. Because the decrease in PV, also during storage, was accompanied by an increase in total volatiles, the data indicate that ascorbic acid may cause increased breakdown of lipid hydroperoxides at low pH values in the presence of iron.

In mayonnaises without ascorbic acid, both the PV and the concentration of the selected volatiles increased during storage when the pH was decreased from 6.0 to 4.2, which indicated a strong pro-oxidant effect of decreasing pH. When the pH was further decreased to 3.8, the PV decreased, maybe due to an increased breakdown of peroxides. However, further studies are necessary to clarify this. The present experimental setup does not explain why the concentrations of 2-pentylfuran and 2-*E*-heptenal decreased when the pH was lowered from 4.2 to 3.8, when this was not the case for the other volatiles. Taken together, the data nonetheless demonstrated that in fish oil enriched mayonnaise without ascorbic acid low pH promoted oxidation. This observation was in accordance with previously reported data, which showed that free radicals could be detected only in mayonnaise with a low pH ($\sim$3.8) and not in mayonnaise with a high pH ($\sim$6.0) (*6*).

Egg yolk, which is very rich in iron (734 μM), was used as an emulsifier in mayonnaise. Egg yolk components, including the iron-carrying protein, phosvitin, are therefore suggested to be located at the o/w interface in mayonnaise (*17*). The high iron-binding capacity of phosvitin is due to the existence of many phosphoserine residues in the protein (*18*), where iron ions are assumed to be bound by strong ionic bonding (*19*). In native egg yolk, iron ions also participate in iron ion bridges between phosvitin and the egg yolk constituents, low-density lipoprotein (LDL) and lipovitellin (*19*). These ion bridges may break when the pH decreases from the natural pH in egg yolk of 6.0 to 4.0 (*19*). Ferric ions are generally insoluble at neutral pH values, whereas they become soluble at low pH values. Therefore, lowering the pH could also lead to increased solubilization of iron ions from other mayonnaise ingredients.

Iron was recently demonstrated to associate with negatively charged sodium dodecyl sulfate (SDS)-stabilized emulsion droplets in 5% o/w model emulsions, and it was observed that increased association of iron to the droplets resulted in increased oxidation of the emulsion (*20*). It was therefore proposed that only small amounts of iron are needed at the o/w surface to promote peroxide breakdown, which subsequently promotes oxidation. Recent results have shown that the addition of 0.5 mM ascorbic acid or ascorbyl palmitate to egg yolk dispersions (at pH 7.4) caused increased levels of free Fe^{2+} in the aqueous fractions of these dispersions (*21*). These observations led the authors to propose that ascorbic acid and ascorbyl palmitate are able to react with the the phosvitin$-Fe^{3+}$ complex in egg yolk to release Fe^{2+} that subsequently can catalyze lipid peroxidation via decomposition of lipid hydroperoxides to reactive radicals ($LOOH + Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$). Other studies have shown that the rate of Fe^{3+} reduction by ascorbic acid in solution increases drastically when the pH is decreased from 6.0 to 2.6 (*22*). In addition, the reduction of Fe^{3+} by ascorbic acid appears to be of zero order with respect to ascorbic acid, at least at pH $\sim$5$-$6 (*23*).

On the basis of the above literature reports and the data obtained in the present study and in our previous work (*5*), we suggest the following hypotheses to explain the observed pro-oxidative effects of ascorbic acid and a low pH in mayonnaise:

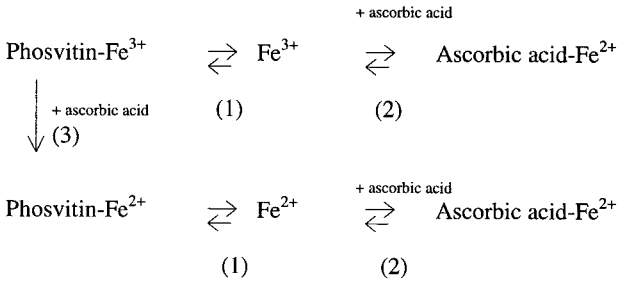
(1) When the pH is $\sim$6.0, iron is located at the o/w interface of mayonnaise, where it participates in the iron bridges between phosvitin, LDL, and lipovitellin in egg yolk. Therefore, it is inaccessible as an initiator of oxidation.

(2) When the pH is decreased from 6.0, the iron bridges between phosvitin, lipovitellin, and LDL gradually break up, and iron ions become more accessible as oxidation initiators than at higher pH levels. Release of iron also makes the iron redox active and therefore pro-oxidative.

(2a) When ascorbic acid is added to the aqueous phase in the mayonnaise system, it reduces Fe^{3+} to Fe^{2+}, which is more active as an oxidation catalyst than Fe^{3+} (*4*). This may already happen when Fe^{3+} is bound to phosvitin as indicated in Scheme 1 (equilibrium 3).

(2b) Ascorbic acid forms a complex with iron, whereby iron is released from phosvitin as shown in Scheme 1 (equilibria 1 and 2). This reaction is suggested to occur more readily at a low pH than at high pH values, because iron ions are more accessible at low pH values than at high pH values as described above (*19*). Thereby, significant amounts of iron are removed from the o/w interface into the aqueous phase of mayonnaise.

Scheme 1. Proposed Mechanism for the Release of Iron by Ascorbic Acid (*5*)



$$K_{(1)} = [\text{Phosvitin-Fe}^{3+}]/([\text{Fe}^{3+}][\text{Phosvitin}])$$

(3) Increased accessibility of iron at the o/w interface (as with low pH) or increased concentrations of iron in the aqueous phase (as when ascorbic acid is present) increase the possibility of interactions between iron and already existing lipid hydroperoxides (LOOH) located at the emulsion droplet surface, and this induces lipid oxidation via LOOH breakdown at the droplet surface (*20*). Due to the many double bonds in polyunsaturated $n-3$ fatty acids, LOOH from such fatty acids may be relatively polar and preferentially localized at or near the oil/emulsifier/water interface or perhaps partition into the aqueous phase of the mayonnaise system. Therefore, it cannot be ruled out that LOOH breakdown partly occurs also in the aqueous phase of mayonnaise.

(4) The decomposition of LOOH from polyunsaturated marine fatty acids rapidly produces volatile oxidation compounds and undesirable rancid and fishy off-flavors.

Moreover, when the ascorbic acid$-Fe^{2+}$ complex catalyzes the decomposition of LOOH, it is oxidized to an Fe^{3+}−ascorbic acid complex, but free ascorbic acid may reduce the Fe^{3+}−complex back to an Fe^{2+}-complex as described by Fukuzawa et al. (*24*).

The proposed mechanism behind the observed pro-oxidative effect of low pH values, which suggest that oxidation is mainly metal catalyzed, is supported by the finding that the metal chelator EDTA efficiently inhibits the formation of free radicals, LOOH, volatile oxidation compounds, and fishy and rancid off-flavors in fish oil enriched mayonnaise (pH ∼3.8) (*15*).

On the basis of the results obtained it is concluded that when ascorbic acid is present, iron ions, presumably Fe^{2+}, are liberated from egg yolk, which significantly promotes and accelerates oxidation in fish oil enriched mayonnaise. Furthermore, this catalytic activity of iron ions is greatly enhanced when the pH is lowered from 6.0 to 3.8, and thus at low pH iron-induced oxidation becomes the most important oxidation mechanism in egg yolk stabilized mayonnaises, even when ascorbic acid is not present. These events and oxidative mechanisms may also dominate in other egg yolk emulsified food systems such as dressings and mayonnaise-based salads. If this is the case, the oxidative stability of such foods may be greatly enhanced only by the addition of suitable metal chelators rather than antioxidants working as radical chain breakers.

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LITERATURE CITED

(1) Löliger, J. The use of antioxidants in foods. In *Free Radicals and Food Additives*; Arouma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, U.K., 1991; pp 121−150.

(2) Niki, E. Vitamin C as an antioxidant. In *Selected Vitamins, Minerals and Functional Consequences of Maternal Malnutrition*; Simopoulos, A. P., Ed.; World Rev. Nutr. Diet: Basel, Switzerland, 1991; Vol. 4, pp 1−30.

(3) Kläui, H.; Pongracz, G. Ascorbic acid and its derivatives as antioxidants in oils and fats. In *Vitamin C, Ascorbic Acid*; Consell, J. N., Horning, D. H., Eds.; Applied Science Publishers: London, U.K., 1981; pp 139−166.

(4) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, Scotland, 1998.

(5) Jacobsen, C.; Adler-Nissen, J.; Meyer, A. S. Effect of ascorbic acid on iron release from the emulsifier interface and on the oxidative flavor deterioration in fish oil enriched mayonnaise. *J. Agric. Food Chem.* **1999**, *47*, 4917−4926.

(6) Thomsen, M. K.; Jacobsen, C.; Skibsted, L. H. Initiation mechanisms of oxidation in fish oil enriched mayonnaise. *Eur. Food Res. Technol.* **2000**, *211*, 381−386.

(7) Jacobsen, C.; Meyer, A. S.; Adler-Nissen, J. Oxidation mechanisms in real food emulsions: Method for separation of mayonnaise by ultracentrifugation. *J. Food Lipids* **1998**, *5*, 87−101.

(8) Nordic Committee on Food Analysis. No. 139. *Metals. Determination by Atomic Absorption Spectrophotometry in Food Stuffs*; Statens Tekniska Forskningscentral: Esbo, Finland, 1991.

(9) Meyer, A. S.; Jacobsen, C. Fate of the synergistic antioxidants system ascorbic acid, lecithin and tocopherol in mayonnaise: Partition of ascorbic acid. *J. Food Lipids* **1996**, *3*,139−148.

(10) Jacobsen, C.; Meyer, A. S.; Adler-Nissen, J. Oxidation mechanisms in real food emulsions: Oil-water partition coefficients of selected volatile off-flavor compounds in mayonnaise. *Z. Lebensm. Unters. Forsch.* **1999**, *208*, 317−327.

(11) Asakawa, T.; Matsushita, S. A colorimetric microdetermination of peroxide values utilizing aluminium chloride as the catalyst. *Lipids* **1980**, *15*, 965−967.

(12) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Hølmer, G. Characterization of volatiles from fish oil enriched mayonnaise during storage by dynamic headspace GC/MS. *J. Agric. Food Chem.* **2000**, *48*, 4858−4867.

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

(14) Jacobsen, C.; Hartvigsen, K.; Lund, P.; Thomsen, M. K.; Skibsted, L. H.; Hølmer, 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*, 308−318.

(15) Jacobsen, C.; Hartvigsen, K.; Thomsen, M. K.; Hansen, L. F.; Lund, P.; Skibsted, L. H.; Hølmer, 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.

(16) Jacobsen, C.; Schwarz, K.; Stöckmann, H.; Meyer, A. S.; Adler-Nissen, J. Partitioning of selected antioxidants in mayonnaise. *J. Agric. Food Chem.* **1999**, *47*, 3601−3610.

(17) Kiosseoglou, V. D.; Sherman, P. Influence of egg yolk lipoproteins on the rheology and stability of o/w emulsions and mayonnaise, 1. Viscoelasticity of groundnut oil-in-water emulsions and mayonnaise. *J. Texture Stud.* **1983**, *14*, 397−417.

(18) Webb, N. B.; Multani, J. S.; Saltam, P.; Beach, N. A.; Gray, H. B. Spectroscopic and magnetic studies of iron-(III) phosvitins. *Biochemistry* **1973**, *12*, 1797.
(19) Causeret, D.; Matringe, E.; Lorient, D. Ionic strength and pH effects on composition and microstructure of yolk granules. *J. Food Sci.* **1991**, *56*, 1532−1536.
(20) Mei, L.; Decker, E. A.; McClements, D. J. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. *J. Agric. Food Chem.* **1998**, *46*, 5072−5077.
(21) Nielsen, J. H.; Kristiansen, G. H.; Andersen, H. J. Ascorbic acid and ascorbic acid 6-palmitate induced oxidation in egg yolk dispersions. *J. Agric. Food Chem.* **2000**, *48*, 1564−1568.
(22) Hsieh, Y.-H. P.; Hsieh, Y. P. Valence state of iron in the presence of ascorbic acid and ethylenediaminetetraacetic acid. *J. Agric. Food Chem.* **1997**, *45*, 1126−1129.
(23) Hsieh, Y.-H. P.; Hsieh, Y. P. Kinetics of Fe(III) reduction by ascorbic acid in aqueous solutions. *J. Agric. Food Chem.* **2000**, *48*, 1569−1573.
(24) Fukuzawa, K.; Seko, T.; Minami, K.; Terao, J. Dynamics of iron-ascorbate-induced lipid peroxidation in charged and uncharged phospholipid vesicles. *Lipids* **1993**, *28*, 497−503.

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