Effect of Ascorbic Acid on Iron Release from the Emulsifier Interface and on the Oxidative Flavor Deterioration in Fish Oil Enriched Mayonnaise

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This research examines the effect of ascorbic acid (0−800 ppm) on the sensory perception of mayonnaises containing 16% fish oil and on the levels of iron and copper in the aqueous phase. Ascorbic acid increased the formation of fishy off-flavors in fresh mayonnaise. Simultaneously, the iron concentration increased from below the detection limit (1.8 μM) to 34 μM in the aqueous phase of mayonnaises. Model mayonnaises with various concentrations of egg yolk (1−7% w/w) and ascorbic acid (0−8000 ppm) were prepared. Iron concentrations in the aqueous phase increased with increasing ascorbic acid levels, whereas iron concentrations in the assumed interfacial layer decreased. It is proposed that ascorbic acid is able to complex and reduce Fe^{3+} to Fe^{2+} from phosvitin in the egg yolk, whereby iron is released from the interface. The ascorbic acid−iron complex subsequently reacts with lipid hydroperoxides, resulting in increased lipid oxidation and in the immediate formation of rancid and fishy off-flavors.

Keywords: *Mayonnaise; fish oil; ascorbic acid; iron; oxidation; egg yolk; off-flavors; discriminant partial least-squares regression*

INTRODUCTION

Oxidation of lipid-bearing foods such as vegetable oil, mayonnaise, and spreads causes flavor deterioration. Furthermore, oxidation may reduce the nutritional quality of the product as oxidation depletes the level of potentially beneficial polyunsaturated fatty acids, and dietary lipid oxidation products may even accelerate atherosclerotic processes, coronary heart disease (Kubow, 1990), and carcinogenesis (McBrien and Slater, 1982). Therefore, antioxidants, including aqueous compounds such as ascorbic acid, are widely employed to delay oxidative flavor deterioration of food products containing oxidizable lipids (Löliger, 1991). The antioxidative effect of ascorbic acid is mainly due to its radical scavenging effect, as ascorbic acid reacts directly with hydrophilic free radicals (Niki, 1991), but ascorbic acid may also act as an O_2 scavenger (Kläui and Pongrancz, 1981). Ascorbic acid is frequently employed in combination with tocopherol, as several studies have shown a synergistic effect of these two antioxidants (Lambelet et al., 1985). Thus, ascorbic acid appears to regenerate the tocopheroxyl radical to tocopherol (Lambelet et al., 1985). In low concentrations ascorbic acid may, however, also act as a prooxidant, especially in metal-catalyzed oxidation processes (Kanner and Mendel, 1977).

In our previous studies on oxidative stability of bulk fish oil and on fish oil containing mayonnaise, we employed the antioxidant system ascorbic acid/lecithin/tocopherol (A/L/T) (Jacobsen, 1995). When used in bulk oils, the A/L/T system exerted strong antioxidant effects when the oil was evaluated both by chemical analyses and by sensory analysis (Meyer and Jacobsen, 1996). However, when the A/L/T system was employed in emulsified systems, for example, mayonnaise, strong fishy and rancid off-flavors rapidly developed despite low peroxide and anisidine values (Jacobsen, 1995). When the A/L/T system was added to the oil phase in mayonnaise, ascorbic acid partitioned into the aqueous phase of mayonnaise, and we therefore proposed that this disintegration of the A/L/T system partly explained the loss of antioxidant efficacy, but the data did not explain the concomitant acceleration of oxidative flavor deterioration of mayonnaise (Meyer and Jacobsen, 1996). Further studies in our laboratory indicated that the formation of fishy and rancid off-flavors in mayonnaise enriched with fish oils were accelerated in particular by the ascorbic acid supplementation (unpublished data). To investigate this phenomenon in more detail, we initiated the study reported in this paper.

It is well-known that ascorbic acid is able to reduce Fe^{3+} to Fe^{2+} (Kanner and Mendel, 1977). The reduced form of iron (Fe^{2+}) catalyzes the breakdown of hydroperoxides (LOOH) to reactive free radicals: $LOOH + Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$. In addition, Fe^{3+} may also decompose LOOH, but at a much lower rate ($LOOH + Fe^{3+} \rightarrow LOO^{\bullet} + Fe^{2+}$). These types of reactions ultimately result in the production of secondary oxidation products (aldehydes, ketones, acids) that cause undesirable off-flavor development (Frankel et al., 1989).

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Table 1. Design of Model Mayonnaise Experiment and pH Values Measured in the Model Mayonnaise

code name[a]	egg yolk content, % w/w	ascorbic acid content, ppm (mM)[b]	pH value after mayonnaise production[c]
$egg_{low}Asc_{low}$	1	0 (0)	5.8
$egg_{low}Asc_{med}$	1	80 (0.45)	5.2
$egg_{low}Asc_{high}$	1	800 (4.54)	3.9
$egg_{med}Asc_{low}$	4	0 (0)	6.1
$egg_{med}Asc_{med}$	4	80 (0.45)	5.8
$egg_{med}Asc_{high}$	4	800 (4.54)	4.5
$egg_{med}Asc_{exthigh}$	4	8000 (45.4)	3.3
$egg_{high}Asc_{low}$	7	0 (0)	6.2
$egg_{high}Asc_{med}$	7	80 (0.45)	6.0
$egg_{high}Asc_{high}$	7	800 (4.54)	4.9

[a] Abbreviations: egg, egg yolk; Asc, ascorbic acid; low, low concentration; med, medium concentration; high, high concentration; exthigh, extra high concentration. [b] Values in parentheses show the concentration in mM. [c] There was no pH adjustment.

Recently, Mei et al. (1998) found that iron ions (both Fe^{2+} and Fe^{3+}) can associate with negatively charged sodium dodecyl sulfate (SDS) emulsion droplets, but not with positively charged or neutral droplets, and that oxidation of such SDS-stabilized emulsion droplets increased with increasing iron association.

In mayonnaise several of the ingredients (oil, water, salt, vinegar, and lemon juice) contain Fe and Cu, albeit in low concentrations (<14.3 μM Fe and <0.8 μM Cu) (Jacobsen et al., 1999a). However, egg yolk, which is traditionally used as the emulsifying agent in mayonnaise and in other oil-in-water food emulsions, is the richest source of iron in mayonnaise as it contains >720 μM iron, but only ~17 μM copper. If metal ions (Fe or Cu) are involved in the development of off-flavors in mayonnaise containing ascorbic acid, the major part of the metal ions most likely stems from egg yolk. In native egg yolk, the major proportion of iron is chelate bound to the protein phosvitin (Causeret et al., 1991). The complex between iron and phosvitin is strong and does not dissociate in the presence of 0.01 M citric acid or in the presence of 1 M NaCl. However, EDTA (0.15 M) was demonstrated to be able to dissociate the iron−phosvitin complex, assumably via competitive complexation of the iron (Albright et al., 1984).

The overall aim of the present study was to investigate the mechanism of the promoting effect of ascorbic acid on the development of fishy and rancid off-flavors in mayonnaise with fish oil. The first objective was to determine how different concentrations of ascorbic acid ([0, 40, 80, 200, 400, and 800 ppm (0, 0.23, 0.45, 1.14, 2.27, and 4.45 mM)] affected the sensory perception of mayonnaise enriched with fish oil and to evaluate if ascorbic acid influenced the concentrations of iron and copper in the aqueous phase of mayonnaise. The second objective was to investigate possible dose−response effects of ascorbic acid and egg yolk on the levels of iron and copper in the different phases of a model mayonnaise composed of only oil and water (80:20) with egg yolk as emulsifier (Table 1). To quantify metal ions in the different phases, both model emulsions and mayonnaises were separated into different phases by ultracentrifugation (Figure 1) (Jacobsen et al., 1998).

MATERIALS AND METHODS

Materials. Refined, unhydrogenated rapeseed oil was obtained from Aarhus Olie A/S, Aarhus, Denmark [peroxide value = 0.6 mequiv/kg; anisidine value = 1.6; iron content = 0.1 ppm (1.8 μM); and copper content = <0.05 ppm (<0.8 μM) as analyzed by atomic absorption spectrophotometry (Nordic Committee on Food Analysis, No. 139)]. 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 Department of Biotechnology, Technical University of Denmark [peroxide value = 0.9 mequiv/kg; anisidine value = 1.5; 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)]. Tarragon vinegar (7%) was purchased from A/S Dansk Eddikecentral, Copenhagen, Denmark. Lemon juice was from ItalLemon, Milan, Italy [iron content = 1.0 ppm (18 μM) and copper content = 0.2 ppm (3.1 μM)]. Potassium sorbate was purchased from Merck, Darmstadt, Germany. Grindsted FF DC stabilizer was donated by Danisco Ingredients (Brabrand, Denmark). Ascorbic acid [Fe < 0.0002% (<3.6 μM)] was purchased from Merck.

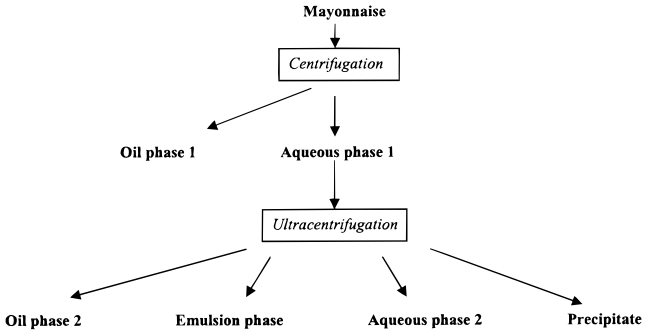


Figure 1. Flow sheet of the separation procedure. In model emulsion experiments a mixed aqueous emulsion phase was also obtained after ultracentrifugation in addition to the four phases depicted in the flow sheet.

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), 40.0 g of vinegar (4.0% w/w), 12.0 g of lemon juice (1.2% w/w), 1.0 g of potassium sorbate (0.1% w/w), and 2.0 g of Grindsted FF DC (0.2% w/w). Depending on the desired concentration, 0.04, 0.08, 0.20, 0.40, or 0.80 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. Mayonnaises were produced as previously described (Meyer and Jacobsen, 1996).

Model Mayonnaise Production. Model mayonnaises were composed of 640.0 g of rapeseed oil (64% w/w), 160 g of fish oil (16% w/w), 10.0, 40.0, or 70.0 g of egg yolk (1, 4, or 7% w/w), and 0.0, 0.08, 0.8, or 8.0 g of ascorbic acid (0, 80, 800, or 8000 ppm). The amount of model mayonnaise was in each case adjusted to 1000.0 g by the addition of distilled water. Model mayonnaises were produced according to the same procedure as mayonnaise.

Separation of Mayonnaise and Model Mayonnaises. Aliquots of 500 g of mayonnaise or model mayonnaise were packed in aluminum bags from Danisco Flexible (Lyngby, Denmark). Mayonnaises were then frozen at −40° C and separated by centrifugation at 25.400*g* for 10 min (Jacobsen et al., 1998). After this centrifugation, an oil phase 1 and an aqueous phase 1 were obtained. The latter was diluted 1:2 with distilled water and was subsequently ultracentrifuged at 85.800*g* for 15 h at 15 °C (Jacobsen et al., 1999b). Thereby, four phases were obtained: a precipitate, aqueous phase 2, an emulsion phase, and oil phase 2 (Figure 1). In the model emulsions, where separation was more difficult, as much as possible was collected of the pure emulsion phase. Subsequently, the remaining emulsion phase together with small amounts of aqueous phase 2 were collected. This phase was termed "aqueous emulsion phase".

Table 2. Sensory Scores for Mayonnaise with Different Concentrations of Ascorbic Acid (±SD, $n = 12$, Sensory Scale from 0 to 9)

amount of ascorbic acid added, ppm (mM)[a]	pH	fishy/train oil aroma[b]	rancid aroma[b]	fishy/train oil flavor[b]	rancid flavor[b]	metallic flavor[b]
0 (0)	3.80	0.4 ± 0.8^{a}	0.5 ± 1.2^{a}	0.2 ± 0.6^{a}	0.3 ± 0.6^{a}	0.3 ± 0.8^{a}
40 (0.23)	3.78	1.1 ± 1.2^{ab}	1.5 ± 1.8^{a}	2.7 ± 2.2^{b}	1.7 ± 2.0^{abcd}	0.4 ± 0.7^{a}
80 (0.45)	3.78	1.9 ± 1.6^{ab}	1.4 ± 1.9^{a}	2.6 ± 1.4^{b}	1.2 ± 1.9^{abc}	0.8 ± 1.3^{a}
200 (1.14)	3.77	1.8 ± 1.4^{ab}	1.4 ± 2.2^{a}	3.2 ± 1.7^{b}	2.5 ± 2.2^{bcd}	0.8 ± 1.3^{a}
400 (2.27)	3.76	2.5 ± 2.2^{b}	1.7 ± 1.7^{a}	4.2 ± 2.4^{b}	3.0 ± 2.1^{d}	1.0 ± 1.5^{a}
800 (4.45)	3.75	1.6 ± 2.2^{ab}	2.0 ± 1.9^{a}	3.5 ± 2.4^{b}	2.3 ± 1.7^{bcd}	1.2 ± 1.5^{a}

[a] Values in parentheses show the concentration of ascorbic acid in mM. [b] Values in the same column followed by the same letter are not significantly different ($p < 0.05$). Samples with more than one letter have overlapping confidence limits.

Table 3. Concentration of Iron and Copper in Mayonnaise Fractions (±SD, $n = 2$)

ascorbic acid added, ppm (mM)[a]	whole mayonnaise		oil phase 1		aqueous phase 2	
	iron, μM	copper, μM	iron, μM	copper, μM	iron, μM[b]	copper, μM
0 (0)	30.4 ± 1.8	<1.6	<1.8	<1.6	$<1.8^{a}$	<1.6
40 (0.23)	32.2 ± 1.8	<1.6	<1.8	<1.6	$<1.8^{a}$	<1.6
80 (0.45)	32.2 ± 1.8	<1.6	<1.8	<1.6	1.8 ± 1.8^{a}	<1.6
200 (1.14)	32.2 ± 1.8	<1.6	<1.8	<1.6	9.0 ± 1.8^{b}	<1.6
400 (2.27)	32.2 ± 1.8	<1.6	<1.8	<1.6	21.5 ± 1.8^{c}	<1.6
800 (4.54)	32.2 ± 1.8	<1.6	<1.8	<1.6	34.0 ± 1.8^{d}	<1.6

[a] Values in parentheses are concentrations of ascorbic acid in mM. [b] Values within the same column followed by the same letter are not significantly different ($p < 0.05$).

Sensory Analysis. Descriptive sensory analysis was used to evaluate freshly produced mayonnaises as previously described (Jacobsen et al., 1999c). The following sensory attributes were evaluated: aroma (vinegar/acidic, fishy/train oil, rancid, oily, dusty, miscellaneous); texture (appearance and mouthfeel); and flavor (vinegar/acidic, fishy/train oil, rancid, oily, dusty/dry, synthetic, metallic, nutty, egg yolk, and miscellaneous). A sensory scale from 0 to 9 was employed; 0 indicated no intensity, and 9 indicated high intensity of the descriptor. For the descriptors describing appearance and mouthfeel, 0 indicated a very thin and 9 a very thick (viscous) mayonnaise.

Determination of Iron and Copper. The samples were incinerated and dissolved in acid, and iron and copper were determined by atomic absorption spectrophotometry (Nordic Committee on Food Analysis, 1991). The analysis did not differentiate between Fe^{2+} ions and Fe^{3+} ions.

Data Analysis. The sensory and iron data from the mayonnaise experiment were evaluated by principal component analysis (PCA). Discriminant partial least-squares regression was performed on the data from the model mayonnaise experiment; ascorbic acid concentrations, egg yolk concentrations, and pH values were defined as design variables. These design variables were used as *Y* variables. The iron concentrations in the unseparated model emulsion, in aqueous phase 1, in aqueous phase 2, in the precipitate, and in the emulsion phase, the iron recoveries in aqueous phase 2, in the precipitate, and in the emulsion phase, and the relative proportion of mass obtained in aqueous phase 2, in the emulsion phase, and in the precipitate were used as *X* variables. In both analyses all variables were standardized by 1/standard deviation and full cross-validation was employed. The software program Unscrambler 7.01 (Camo A/S, Oslo, Norway) was used as an aid in the multivariate data analysis as well as for analysis of variance and Tukey's test.

RESULTS

Mayonnaise. *Sensory Evaluation of Mayonnaise with Ascorbic Acid.* Freshly produced mayonnaises with different concentrations of ascorbic acid (0−800 ppm corresponding to 0−4.54 mM) were evaluated by a sensory panel (12 assessors). The sensory scores for fishy aroma and flavor and for rancid aroma and flavor as well as the score for metallic flavor are summarized in Table 2. The other sensory attributes evaluated will be discussed in conjunction with the results of the multivariate data analysis.

Sensory scores varied significantly ($p < 0.05$) between 0.4 and 2.5 and between 0.2 and 4.2 for fishy/train oil aroma and flavor, respectively. For rancid aroma and flavor sensory scores varied between 0.5 and 2.0 and between 0.3 and 3.0, respectively, and for metallic flavor the scores varied between 0.3 and 1.2 (Table 2).

Mayonnaise without ascorbic acid had very low intensity scores for fishy/train oil aroma and flavor (0.4 and 0.2, respectively) (Table 2). When ascorbic acid was added to mayonnaise, the intensities of fishy aroma and flavor increased significantly. For both fishy aroma and fishy flavor, the intensity scores were highest for mayonnaises supplemented with 400 ppm of ascorbic acid and appeared to decrease slightly, but not significantly, with a doubling of the ascorbic acid addition to 800 ppm. The same was observed for the rancid flavor scores. Furthermore, rancid flavor showed significantly higher intensity scores only at addition levels >200 ppm of ascorbic acid. The sensory intensity of rancid aroma and metallic flavor also tended to increase with ascorbic acid addition, but these trends were not statistically significant (Table 2).

The pH values only varied between 3.75 and 3.80 (Table 2). Thus, pH of the mayonnaises was only slightly affected by the addition of ascorbic acid.

Concentrations of Iron and Copper in Different Fractions in the Mayonnaise Experiment. To investigate if differences in iron and/or copper levels in the various phases of mayonnaise influenced the accelerated development of the fishy, rancid, and metallic off-flavors in mayonnaises with ascorbic acid, concentrations of iron and copper were quantified in the whole mayonnaise, in oil phase 1 obtained after the first centrifugal separation, and in aqueous phase 2 obtained after ultracentrifugation of aqueous phase 1 (Figure 1).

The concentration of copper was below the detection limit (1.6 μM) in all mayonnaise fractions analyzed, irrespective of the ascorbic acid level (Table 3). Thus, copper concentrations in the aqueous phase of mayonnaise were apparently not affected by ascorbic acid addition. In whole mayonnaise, the concentration of iron was also constant for all ascorbic acid levels (30.4−32.2 μM) (Table 3). In oil phase 1, the iron concentration was

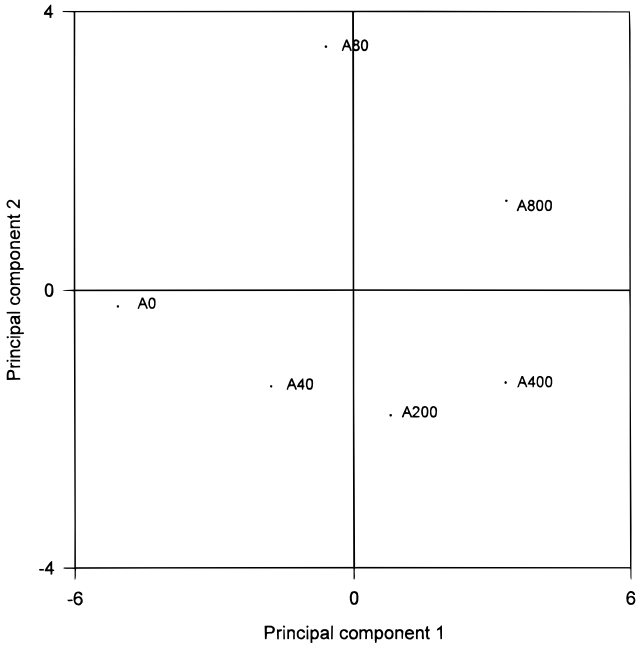


Figure 2. Score plot from the PCA on sensory scores and iron concentration in aqueous phase 2: A0, 0 ppm of ascorbic acid; A40, 40 ppm of ascorbic acid; A80, 80 ppm of ascorbic acid; A200, 200 ppm of ascorbic acid; A400, 400 ppm of ascorbic acid; A800, 800 ppm of ascorbic acid.

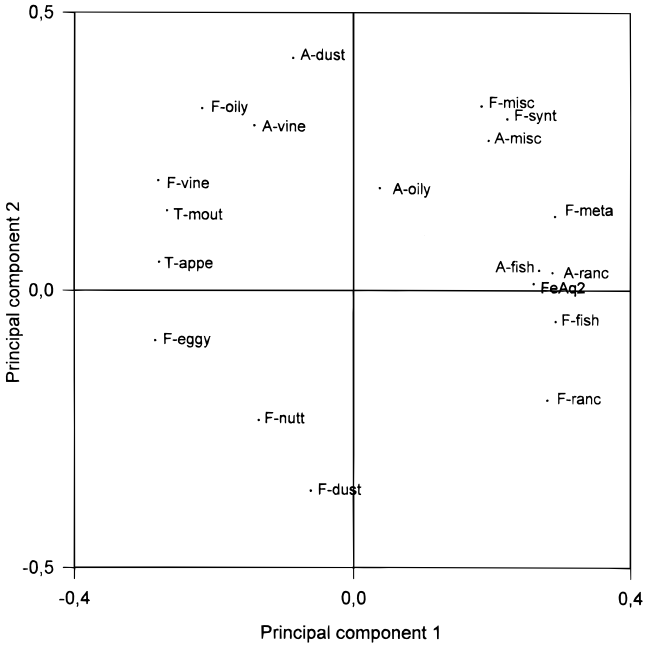


Figure 3. Loading plot from the PCA on sensory scores and iron concentrations in aqueous phase 2: F-, refers to flavor; A-, refers to aroma; T-, refers to texture; Vine, vinegar/acidic; fish, fishy/train oil; ranc, rancid; oily, oily; dust, dusty/dry; nutt, nutty; eggy, egg yolk; meta, metallic; synt, synthetic; misc, miscellaneous; appe, appearance; mout, mouthfeel; FeAq2, iron concentration in aqeuous phase 2.

below the detection limit (1.8 μM) for all levels of ascorbic acid. In contrast, the concentration of iron in aqueous phase 2 varied significantly: Concentrations ranged from <1.8 to 34.0 μM and increased significantly with increasing additions of ascorbic acid (Table 3).

PCA of Mayonnaise Experiment. To study the correlation between sensory scores and iron concentrations in aqueous phase 2 of mayonnaises with different ascorbic acid additions, PCA was carried out on these variables. Two principal components (PC1 and PC2) were validated. These two principal components explained 61% of the variance. PC1 explained 43% and PC2 18% (Figures 2 and 3). Mayonnaise without ascorbic acid was located to the far left in the score plot, whereas the two mayonnaises containing high levels of ascorbic acid were located to the far right (Figure 2). Thus, PC1 mainly described differences between mayonnaises caused by the ascorbic acid addition. There was no clear pattern in the location of the mayonnaises with respect to PC2. The sensory scores for fishy, rancid aroma and flavor (A-fish, A-ranc, F-fish, F-ranc) were located to the right in the loading plot together with metallic flavor (F-meta) and iron concentration in aqueous phase 2 (FeAq2) (Figure 3). These observations corroborated the interpretation of the sensory scores data (Table 2) and associated them with an increased iron concentration in the aqueous phase of mayonnaises. Taken together, these results indicate a strong correlation between iron concentration in aqueous phase 2 and occurrence of fishy, rancid, and metallic off-flavors and off-odors. As mayonnaises with high ascorbic acid additions were also located to the right in the score plot (Figure 2), there appeared to be a strong correlation between ascorbic acid concentration and these variables. The miscellaneous flavor and aroma attributes as well as synthetic flavor (F-misc, A-misc, and F-synt) were also located to the right in the loading plot. This indicates that mayonnaises containing high ascorbic acid levels also had higher intensities of these attributes. Surprisingly, vinegar/acidic aroma and flavor (A-vine and F-vine) were located to the left in the loading plot. This finding indicates that mayonnaise without ascorbic acid had more vinegar/acidic flavor and aroma than mayonnaises with ascorbic acid even though ascorbic acid itself has a sour taste. Our present experiment does not offer a mechanistic explanation to this phenomenon.

The egg yolk flavor and the attributes describing the physical appearance and mouthfeel of the mayonnaise were located to the left in the loading plot. Thus, mayonnaises without ascorbic acid were perceived as having more egg yolk flavor and as being thicker and more viscous than mayonnaises with ascorbic acid. The attributes for dusty aroma and flavor as well as for nutty flavor and oily aroma (A-dust, F-dust, F-nutt, A-oily) were located close to the PC2 axis. Thus, these attributes were not described by PC1 and were therefore not related to the addition of ascorbic acid.

Model Mayonnaise Emulsions. *Effects of Egg Yolk and Ascorbic Acid Levels on pH Values in Model Mayonnaises.* The values for pH varied between 3.3 and 6.2 in the different model emulsions (Table 1). As expected pH values increased with increasing egg yolk levels and decreased with increasing ascorbic acid levels. The effect of ascorbic acid on pH decrease was greatest in emulsions with low egg yolk levels.

Concentrations of Iron and Copper in Different Fractions from Model Mayonnaises. In unseparated model emulsions, iron concentrations varied significantly between 5.4 and 57.3 μM. As expected, iron concentrations in model emulsions increased significantly with increasing levels of egg yolk added ($p < 0.05$) but did not vary with ascorbic acid concentrations (Table 4). The same was observed for copper concentrations, although the copper concentrations were below the detection limit for low egg yolk levels (1 and 4%).

In oil phase 1, iron and copper concentrations were below the detection limits, 1.8 and 0.8 μM, respectively,

Table 4. Concentrations of Iron and Copper in Model Emulsion Fractions (±SD, $n = 1, 2$)[a]

	whole model mayonnaise		oil phase 1		undiluted aqueous phase 1[b]		precipitate[b]		aqueous phase 2		emulsion phase[b]	
code name[c]	iron, μM	copper, μM	iron, μM	copper, μM	iron, μM	copper, μM	iron, μM	copper, μM	iron, μM	copper, μM	iron, μM	copper, μM
$egg_{low}Asc_{low}$	5.4 ± 0.0[a]	<0.8	<1.8	<0.8	23	<0.8			<1.8[a]	<0.8		
$egg_{low}Asc_{med}$	7.2 ± 0.0[a]	<0.8	<1.8	<0.8	32	1.6			<1.8[a]	<0.8		
$egg_{low}Asc_{high}$	9.0 ± 0.0[a]	<0.8	<1.8	<0.8	41	1.6			9.0 ± 0.0[c]	<0.8		
$egg_{med}Asc_{low}$	32.2 ± 1.8[b]	<0.8	<1.8	<0.8	111	3.1	663	13	<1.8[a]	<0.8	269	4.7
$egg_{med}Asc_{med}$	34.0 ± 3.6[b]	<0.8	<1.8	<0.8	113	3.1	967	5	<1.8[a]	<0.8	412	4.7
$egg_{med}Asc_{high}$	32.2 ± 1.8[b]	<0.8	<1.8	<0.8	124	3.1	645	6	12.5 ± 1.8[d]	<0.8		
$egg_{med}Asc_{exthigh}$	34.0 ± 1.8[b]	<0.8	<1.8	<0.8	134	3.1	39	19	46.6 ± 1.8[e]	<0.8		
$egg_{high}Asc_{low}$	55.5 ± 0.0[c]	0.8 ± 0.0	<1.8	<0.8	215	6.3	1039	13	<1.8[a]	<0.8	304	1.4
$egg_{high}Asc_{med}$	57.3 ± 0.0[c]	1.1 ± 0.0	<1.8	<0.8	197	4.7	716	8	<1.8[a]	<0.8	287	0.8
$egg_{high}Asc_{high}$	57.3 ± 0.0[c]	1.1 ± 0.0	<1.8	<0.8	215	6.3	501	661	3.6 ± 0.0[b]	<0.8	1110	12.6

[a] Values in the same column followed by the same letter are not significantly different ($p < 0.05$). [b] Values without standard deviation were obtained with only one determination, or the concentration was below the detection limit. [c] Abbreviations: egg, egg yolk; Asc, ascorbic acid; low, low concentration; med, medium concentration; high, high concentration; exthigh, extra high concentration. Please refer to Table 1 for exact concentration levels.

in all samples analyzed (Table 4). In aqueous phase 1, concentrations varied between 23 and 215 μM for iron and from <0.8 to 6.3 μM for copper (Table 4). Both iron and copper concentrations tended to increase with increasing egg yolk levels. In aqueous phase 1's with the same level of egg yolk, iron concentrations appeared to increase with increasing additions of ascorbic acid. However, in model emulsions with high levels of egg yolk (7%), the iron concentrations were found to be similar, containing 197−215 μM irrespective of the level of ascorbic acid addition. In aqueous phase 1, copper concentrations appeared to be unrelated to the level of ascorbic acid added but tended to increase with increased amounts of egg yolk (Table 4).

In the precipitate, iron and copper concentrations varied between 39 and 1039 μM and between 6 and 661 μM, respectively (Table 4). For emulsions containing medium amounts of egg yolk (egg_{med}, 4% w/w), iron concentrations in the precipitate decreased from 967 to 39 μM when ascorbic addition increased from 80 to 8000 ppm (Table 4). For emulsions with high levels of egg yolk (egg_{high}, 7% w/w) the iron concentrations in the precipitate fractions decreased from 1039 to 501 μM when ascorbic acid addition increased from 0 to 800 ppm (Table 4). Because we assume that the precipitate represents some of the oil−water interfacial fraction of the model mayonnaise emulsions, these observations indicate that ascorbic acid induced iron release from the oil−water interface in the emulsions.

The analyzed concentration of copper in the precipitate from the emulsion with high egg yolk and high ascorbic acid ($egg_{high}Asc_{high}$) was much higher, 661 μM, than what was found in the other precipitate fractions (5−19 μM) (Table 4). However, the data obtained did not show a clear pattern with respect to the effect of ascorbic acid on the copper concentrations in the precipitate.

In aqueous phase 2, iron concentrations varied significantly in response to both egg yolk and ascorbic acid levels added. Copper concentrations were all below the detection limit (Table 4). Iron concentrations increased significantly with increasing ascorbic acid concentrations in aqueous phase 2 from emulsions with the same egg yolk concentration. Thus, for emulsions with low amounts of egg yolk, egg_{low}, iron increased from <1.8 to 9.0 μM ($p < 0.05$) with increased ascorbic acid addition from 0 to 800 ppm (Table 4); for model mayonnaise emulsions with medium, normal amounts of 4% w/w egg yolk, egg_{med}, iron concentrations in aqueous phase 2 increased from <1.8 to 46.6 μM with increased ascorbic acid addition (0−8000 ppm) ($p < 0.05$), and in the emulsions with 7% egg yolk by weight, egg_{high}, iron in the aqueous phase increased from <1.8 to 3.6 μM ($p < 0.05$) with increased ascorbic acid addition (Table 4).

In the emulsion phase, iron and copper concentrations varied between 269 and 1110 μM and between 0.8 and 12.6 μM, respectively. Iron concentrations in the emulsion phase seemed to increase with increasing ascorbic acid levels. However, it was difficult to make any firm conclusions as only 5 of 10 emulsion phase samples were analyzed, due to low amounts of sample available. This was also the case for the copper concentrations in the emulsion phase.

Relative Mass Distributions of Model Emulsion Fractions. Ultracentrifugation of model emulsions caused a separation of the emulsion into five fractions. Proportions of masses found in the different fractions were calculated by dividing the mass of a particular fraction with the total mass recovered in all fractions as previously described (Jacobsen et al., 1999d). The mass fraction of oil phase 1 varied between 75.7 and 77.6% (Table 5). There was no general trend with respect to effect of egg yolk or ascorbic acid levels.

The mass fraction of precipitate varied between 0.1 and 1.2% and increased both with increasing egg yolk and with increasing ascorbic acid levels (Table 5). The increased amounts of precipitate recovered with increased ascorbic acid addition hint at an influence (or interaction) of ascorbic acid on the interfacial egg yolk constituents.

In the emulsion phase, mass fractions varied between 0 and 1.7%. As expected, it was observed that egg yolk addition caused increasing recoveries of emulsion phase. However, as opposed to the precipitate, mass fractions of emulsion phase did not increase with increasing ascorbic acid concentration. Rather, there was a tendency that ascorbic acid addition decreased the mass fraction of emulsion phase (Table 5). Iron and copper concentrations reported in Table 4 for the emulsion phase were based on analysis of the emulsion phase, not including the aqueous emulsion phase.

There was no clear trend with respect to the effect of egg yolk or ascorbic acid additions on the mass of

Table 5. Relative Distributions of Masses after Ultracentrifugation (%, n =1)[a]

code name[b]	oil phase 1, %	precipitate, %	aqueous phase 2, %	aqueous emulsion phase,[c] %	emulsion phase, %	oil phase 2, %
$egg_{low}Asc_{low}$	76.9	0.1	14.9	7.3	0.0	0.7
$egg_{low}Asc_{med}$	76.1	0.1	11.7	10.5	0.5	1.0
$egg_{low}Asc_{high}$	77.6	0.3	11.4	10.1	0.1	0.5
$egg_{med}Asc_{low}$	76.8	0.6	13.3	7.8	0.8	0.7
$egg_{med}Asc_{med}$	77.1	0.7	10.3	10.5	0.6	0.9
$egg_{med}Asc_{high}$	76.5	1.1	12.1	10.1	0.0	0.4
$egg_{med}Asc_{exthigh}$	77.5	1.2	10.8	9.4	0.4	0.7
$egg_{high}Asc_{low}$	76.4	0.8	13.0	7.2	1.7	0.8
$egg_{high}Asc_{med}$	75.9	1.0	13.7	6.9	1.5	1.0
$egg_{high}Asc_{high}$	75.7	1.2	11.0	9.8	1.1	1.1

[a] The sum of masses obtained in precipitate + aqueous phase 2+ aqueous emulsion phase + emulsion phase + oil phase 2 equals the total mass obtained in aqueous phase 1. [b] Abbreviations: egg, egg yolk; Asc, ascorbic acid; low, low concentration; med, medium concentration; high, high concentration; exthigh, extra high concentration. Please refer to Table 1 for exact concentration levels. [c] Aqueous emulsion phase refers to the emulsion phase that was "polluted" with constituents from aqueous phase 2 (see Materials and Methods).

Table 6. Recoveries of Iron in Aqueous Phase 1, Precipitate, and Aqueous Phase 2 of Model Mayonnaises (%, n = 1)[a]

code name[b]	aqueous phase 1, %	aqueous phase 2, %	precipitate, %	emulsion phase, %
$egg_{low}Asc_{low}$	98.4	0.0[c]		
$egg_{low}Asc_{med}$	106.2	0.0[c]		
$egg_{low}Asc_{high}$	102.1	33.5		
$egg_{med}Asc_{low}$	79.2	0.0[c]	37.2	19.3
$egg_{med}Asc_{med}$	75.0	0.0[c]	60.1	20.1
$egg_{med}Asc_{high}$	89.2	13.7	61.5	
$egg_{med}Asc_{exthigh}$	87.6	43.4	3.9	
$egg_{high}Asc_{low}$	90.3	0.0[c]	45.3	26.1
$egg_{high}Asc_{med}$	81.9	0.0[c]	37.3	21.4
$egg_{high}Asc_{high}$	90.4	2.0	30.9	63.6

[a] Recoveries were calculated relative to the total amount of iron in the model mayonnaise. Recoveries of iron in the aqueous emulsion phase were not calculated as iron concentrations were not analyzed in this phase (see Materials and Methods). Therefore, the sum of recoveries in aqueous phase 2 plus in the emulsion phase plus in the precipitate does not equal the recovery of iron in aqueous phase 1. [b] Abbreviations: egg, egg yolk; Asc, ascorbic acid; low, low concentration; med, medium concentration; high, high concentration; exthigh, extra high concentration. Please refer to Table 1 for exact concentration levels. [c] The iron concentration was below the detection limit.

aqueous phase 2, oil phase 2, and the aqueous emulsion phase (Table 5).

Distribution of Iron between Different Fractions of Model Emulsions. Recoveries of iron in aqueous phase 1 varied between 75 and 106.2% (Table 6). This observation signifies that almost all iron available for analysis was recovered in aqueous phase 1 after centrifugation.

Recoveries of iron in the precipitate varied between 3.9 and 61.5% (Table 6) and except for the emulsion with medium amounts and no ascorbic acid, they generally decreased with increasing ascorbic acid levels. In contrast, there was no clear trend with respect to the effect of egg yolk levels (Table 6).

In aqueous phase 2, the recoveries of iron varied between 0 and 43.4% (Table 6). Recoveries increased with increasing levels of ascorbic acid; for example, for the emulsions containing medium amounts of egg yolk (egg_{med}), the relative proportions of iron recovered in aqueous phase 2 increased from 0 to 43% when ascorbic acid addition was increased from 0 to 8000 ppm in the model mayonnaises (Table 6). It was not possible to point out a significant effect of the egg yolk level on the recoveries of iron in aqueous phase 2.

No clear effect of egg yolk or ascorbic acid levels on the iron recovery in the emulsion phase was determined, perhaps due to the limited number of samples analyzed.

Discriminant Partial Least-Squares Regression (DPLSR) of Model Emulsion Data. DPLSR was performed on iron concentrations in whole model mayonnaise, aqueous phase 1, aqueous phase 2, emulsion phase, and precipitate and on iron recoveries in the three latter as well as on proportions of mass found in aqueous phase 2, the emulsion phase, and the precipitate. Ascorbic acid and egg yolk concentrations as well as pH values of the different emulsions were used as design variables (Y variables). In this analysis, two principal components were validated. PC1 explained 50% and PC2 33% of the variance in the X variables and 23 and 19% of the variance in the Y variables, respectively. Hence, these two components explained 83% of the variance in the X variables and 42% of the variance in the Y variables.

In the score plot (Figure 4) emulsions with lowest egg yolk levels (egg_{low}) had negative PC2 values. Emulsions with the highest egg yolk levels (egg_{high}) had positive PC2 values and were located diagonally to the egg_{low} emulsions. Emulsions with intermediate egg yolk levels (egg_{med}) were located between egg_{low} and egg_{high} mayonnaises. Mayonnaises with higher ascorbic acid levels generally had lower PC2 values within each group of egg yolk levels. The model mayonnaise with 8000 ppm of ascorbic acid ($egg_{med}Asc_{exthigh}$) was located to the far right in the plot (Figure 4). This means that mayonnaise $egg_{med}Asc_{exthigh}$ had a very high influence on the model, and this should be taken into account when the model is interpreted.

In the loading plot (Figure 5), iron concentrations and recoveries in aqueous phase 2 (RecAq2 and FeAq2) were located in the fourth quadrant as opposed to iron concentrations and recoveries in the precipitate (FePrec and RecPrec), which were located in the second and third quadrants, respectively. This localization of the variables signifies that iron concentrations and recoveries in the precipitate were negatively correlated to the iron concentrations and recoveries in aqueous phase 2 and that the negative correlation was stronger between iron concentrations than between iron recoveries. Hence, the DPLSR analysis verified the indications of a correlation that could also be observed from the chemical analyses (Table 4).

The variables describing egg yolk levels (egg 1%, egg 4%, and egg 7%) moved from the third quadrant to the

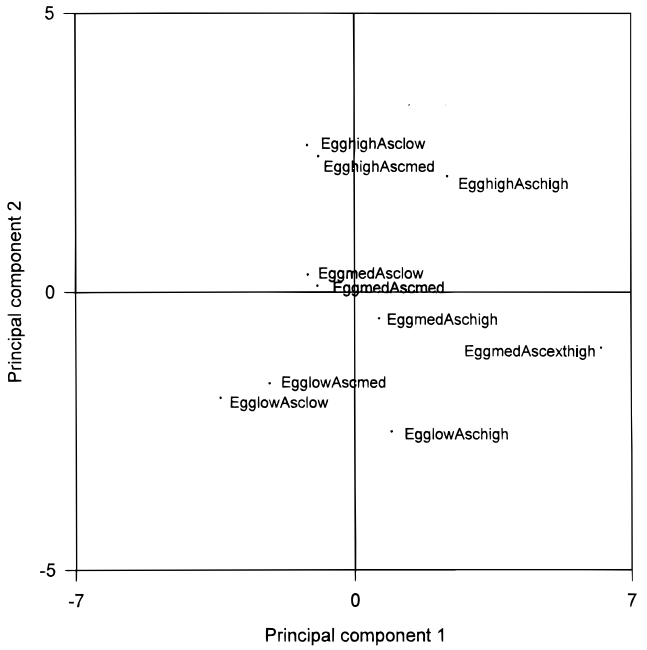


Figure 4. Score plot from the DPLSR on iron concentrations and recoveries in mayonnaise, precipitate, and aqueous phases: egg, egg yolk; Asc, ascorbic acid; low, low concentration; med, medium concentration; high, high concentration; exthigh, extra high concentration. For exact concentration levels, please refer to Table 1.

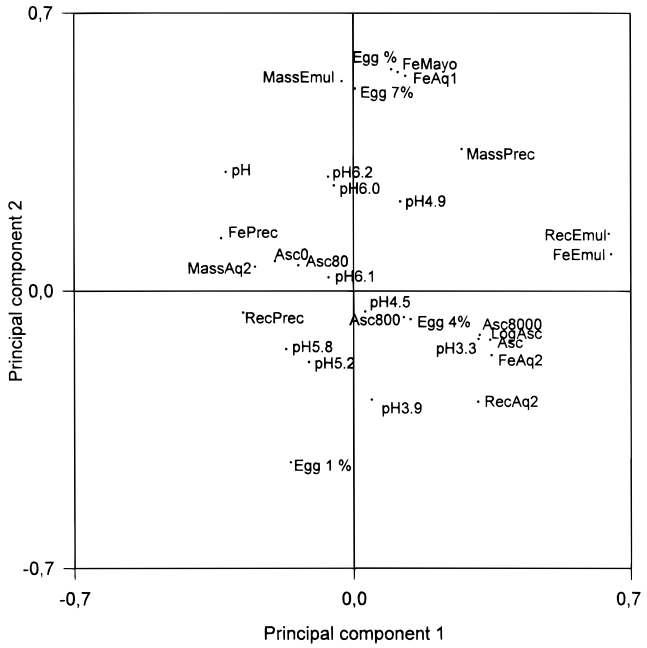


Figure 5. Loading plot from the DPLSR on iron concentrations and recoveries in mayonnaise, precipitate, and aqueous phases: Egg followed by a number refers to egg yolk concentration; Asc followed by a number refers to ascorbic acid concentration; Egg% refers to the qualitative design variable describing egg yolk concentration; Asc and logAsc refer to the qualitative design variables describing ascorbic acid and log ascorbic acid concentrations, respectively; pH refers to the qualitative design variable describing pH values; pH followed by a number refers to the actual pH value; Fe refers to iron concentration; Rec refers to recovery of iron; Mass refers to mass obtained; Mayo refers to whole model mayonnaise; Aq1 refers to aqueous phase 1; Aq2 refers to aqueous phase 2; Emul refers to emulsion phase; Prec refers to precipitate.

fourth quadrant and finally to the first quadrant with increasing egg yolk levels. Thus, the iron concentrations in the unseparated model mayonnaises and aqueous phase 1 correlated positively to the egg yolk concentration as expected. Furthermore, egg yolk concentrations influenced iron concentrations in mayonnaise and aqueous phase 1 more than they influenced concentrations and recoveries of iron in aqueous phase 2 and in the precipitate.

The diagonal movement of the ascorbic acid levels with increasing ascorbic acid concentrations and the location of the quantitative variables describing ascorbic acid levels (Asc and logAsc) in the fourth quadrant confirmed that increasing ascorbic acid concentrations decreased the concentrations and recoveries of iron in the precipitate and increased the concentrations and recoveries of iron in aqueous phase 2.

Iron concentrations and recoveries in the emulsion phase (FeEmul and RecEmul) were located to the far right in the plot (Figure 5). Thus, iron concentrations and recoveries in the emulsion phase correlated to a certain extent with the ascorbic acid concentration, but data from $egg_{high}Asc_{high}$ may have influenced the model to a great extent.

The variables describing the pH values generally moved in the reverse direction of the ascorbic acid variables (Figure 5). Furthermore, the quantitative pH variable (pH) was located in the second quadrant diagonally to the ascorbic acid and logAsc variables and to the variables describing iron recoveries and concentrations in aqueous phase 2. Hence, concentrations and recoveries of iron in aqueous phase 2 increased with decreasing pH, and pH values correlated negatively to the ascorbic acid levels, as expected.

The location of the mass fraction in aqueous phase 2 (MassAq2) to the left in the second quadrant indicated that the mass fraction of aqueous phase 2 apparently decreased with increasing ascorbic acid concentrations. In contrast, the mass fraction in the emulsion phase MassEmul was located very close to Egg%. Hence, as expected, the mass fraction found of this phase increased with increasing egg yolk concentrations. The location of MassPrec in the center of the first quadrant indicated that the mass fraction in the precipitate seemed to be influenced both by the egg yolk and by the ascorbic acid concentrations.

DISCUSSION AND CONCLUSIONS

The results obtained demonstrated that ascorbic acid addition affected negatively the sensory perception of mayonnaise with fish oil and indicated that ascorbic acid promoted lipid oxidation in mayonnaise with fish oil in a dose-dependent matter. Furthermore, ascorbic acid addition gave rise to mayonnaises that were perceived as being thinner and having less vinegar/acidic flavor than mayonnaise without ascorbic acid. Moreover, the iron concentration in the aqueous fraction of mayonnaise increased with increasing addition levels of ascorbic acid. The egg yolk employed as an emulsifier was the primary source of iron among mayonnaise constituents. These findings implied that ascorbic acid promoted the release of iron from the egg yolk constituents at the oil−water interface into the water phase in mayonnaise. As iron is a potent accelerator for lipid oxidation, we suggest that the flavor deterioration caused by ascorbic acid addition to mayonnaise is linked to this iron release. Ascorbic acid was apparently not able to release copper from the egg yolk proteins. Hence, free copper

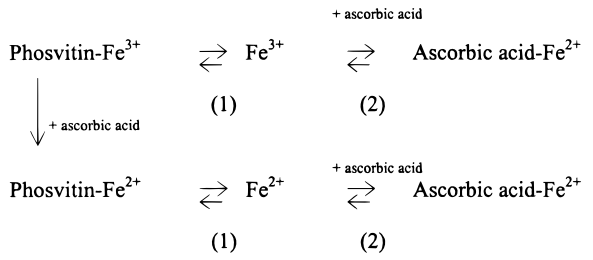
does not seem to play a significant role in the formation of fishy and rancid off-flavors upon addition of ascorbic acid.

The model mayonnaise experiment supported and expanded the findings from the real mayonnaise experiment, notably that ascorbic acid addition gave rise to increased iron concentrations in the aqueous fraction and that the iron concentrations simultaneously decreased in the assumed interfacial layers. Obviously, it is crucial for the interpretation of the data that the different phases obtained by ultracentrifugation (Figure 1) relate to the different phases in mayonnaise. On the basis of previous detailed analyses of fat, protein, and phosphorus in the different fractions obtained by ultracentrifugation, we believe that aqueous phase 2 represents the "real" aqueous phase in mayonnaise and that the precipitate together with the emulsion phase constitutes a representative fraction of the oil−water interface of mayonnaise (Jacobsen et al., 1999d). This interpretation of the various fractions obtained after ultracentrifugation is supported by the result that the relative masses of precipitate and emulsion phase increased with increasing concentrations of egg yolk employed in the model mayonnaises (Table 5).

Taken together, the results obtained in both mayonnaises and mayonnaise models indicate that ascorbic acid is capable of releasing iron from the oil−water interfacial layer into the water phase of emulsions and that this iron release accelerates oxidative flavor deterioration. In mayonnaise, the oil−water interface mainly consists of egg yolk constituents. The egg yolk employed contained 734 μM iron. As previously mentioned, phosvitin, a protein located in the egg yolk granules, is the iron carrier in egg yolk (Li-Chan et al., 1994). The high iron-binding capacity of phosvitin is due to the existence of many phosphoserine residues in the protein (Webb et al., 1973), where iron ions (Fe^{3+}) are assumed to be bound by strong ionic bonding (Causeret et al., 1991). Previous studies have shown that a decrease in pH from the initial value of 6.3. to a value <4.2 or addition of 0.58 M NaCl caused a breakdown of the structure in egg yolk granules (Causeret et al., 1991). Thereby, the granule proteins, lipovitellins, phosvitin, and low-density lipoproteins (LDL) were completely solubilized (Causeret et al., 1991). The effect of ascorbic acid observed in our study was confounded with the pH level that was lowered by the ascorbic acid addition. When pH decreases from 7.0 to 4.0, the phosphate monoester groups become increasingly protonized, resulting in a reduction in the net charge of phosvitin (Vogel, 1983). Furthermore, at low pH values the carboxylic groups are neutralized and the repulsive forces increase between the positive charges (NH_3^+). These factors together induce the breakup of ionic bridges at low pH, which results in a breakdown of the granule structure (Causeret et al., 1991). Furthermore, Lu and Baker (1987) showed that phosvitin was able to inhibit Fe^{2+}-catalyzed oxidation at pH 6.1 and 7.8. At pH 3.8 the antioxidative efficacy of phosvitin was poor (Lu and Baker, 1987), but no explanation for this observation was proposed. Our results agree with the data indicating (Causeret et al., 1991; Lu and Baker, 1987) that the iron binding capacity of egg yolk decreases significantly with decreasing pH values.

Was the effect of ascorbic acid on iron release, then, a pure pH effect, or did ascorbic acid itself also have a major effect? Addition of 800 ppm of ascorbic acid in the real mayonnaise decreased pH to 3.75 compared with 3.80 in the mayonnaise without ascorbic acid (Table 2), but the iron concentration in aqueous phase 2 increased from <1.8 to 34 μM. This strongly suggests that ascorbic acid had an effect itself that is independent of its decreasing effect on pH. This interpretation is supported by the following: (1) Ascorbic acid apparently enhanced the iron bioavailability from egg yolk by solubilizing iron as ascorbic acid complexes due to the similar apparent association values of the egg yolk−iron and ascorbic acid−iron complexes at pH values 2, 4, and 6 (Galdi and Valencia, 1988). (2) The relative biological value (RBV) of iron in egg yolk is 30% in rats. Upon addition of ascorbic acid to the diet, RBV was increased to 100%. Gel permeation chromatography experiments indicated that ascorbic acid induced release of iron from egg yolk phosvitin (Morris and Greene, 1972).

Scheme 1. Proposed Mechanism for the Release of Iron by Ascorbic Acid



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

These findings coupled with the results reported in the present paper lead us to suggest that when pH decreases below 6.0 in mayonnaises, a gradual breakup of iron and other cation bridges between phosphoserine residues in phosvitin and LDL plus lipovitellin occurs (Scheme 1). This results in a decrease in the equilibrium association constant ($K_{(1)}$) between iron and phosvitin with decreasing pH, but the equilibrium 1 between iron bound to phosvitin and free iron has not yet shifted completely to the right (Scheme 1). Thus, the concentration of free iron is still low and below the detection limit in our iron analysis method. Upon ascorbic acid addition, ascorbic acid reduces Fe^{3+} to Fe^{2+} and a complex between ascorbic acid and Fe^{2+} may be formed (equilibrium 2, Scheme 1). The formation of this complex shifts equilibrium 1 between phosvitin-bound iron and free iron to the right, and thereby iron is released into the aqueous phase of the mayonnaise. The water solubility of Fe^{2+} is higher than that of Fe^{3+}, which may further increase the release of Fe^{2+} into the aqueous phase of mayonnaise. The iron concentration we measured in aqueous phase 2 is the sum of free iron and iron bound to ascorbic acid. The reduction of Fe^{3+} to Fe^{2+} by ascorbic acid may take place either when iron is still bound to phosvitin or after iron has been freed as indicated in Scheme 1. Thus, it is inherent that in both reactions ascorbic acid is involved in a redox cycle with Fe^{3+} and Fe^{2+}.

It is well-known that the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid may promote oxidation by decomposing lipid hydroperoxides to alkoxyl radicals that upon further breakdown form off-flavor products (Frankel, 1991). The vegetable oil and especially the fish oil employed in the mayonnaises in the present study unavoidably contained small amounts of lipid hydro-

peroxides (peroxide values for the two oils were 0.6 and 0.9 mequiv/kg, respectively). Therefore, it is likely that the accelerated development of fishy, rancid, and metallic off-flavors was formed via the iron-catalyzed decomposition of already existing lipid hydroperoxides.

Mei et al. (1998) recently found that iron associated with negatively charged SDS-stabilized emulsion droplets in model emulsions containing 5% lipid and that oxidation of the emulsion increased with increasing association of iron to the droplets. On the basis of these observations they therefore proposed that only small amounts of iron are needed at the droplet surface to promote peroxide breakdown whereby oxidation is promoted.

On the basis of the available theoretical knowledge coupled with the findings reported in this paper, we propose the following mechanism for the prooxidant effect of ascorbic acid in mayonnaise:

(1) Iron is bound to phosvitin in native egg yolk, and iron ions are therefore inaccessible as lipid oxidation initiators in mayonnaise as long as the pH is close to 6.0, which is the natural pH of egg yolk (Li-Chan et al., 1994). Apart from being bound to phosvitin, iron ions also participate in iron ion bridges between phosvitin and the egg yolk constituents LDL and lipovitellin at the oil−water interface.

(2) When the pH is decreased by vinegar addition in real mayonnaise (or by ascorbic acid addition in model emulsions), the cation bridges (including iron bridges) between phosvitin and LDL or lipovitellin are broken. Thereby, iron becomes dissociated from LDL and lipovitellin, but most of the iron is still associated with phosvitin and is thus located at the water−oil interface (Scheme 1).

(3) Ascorbic acid reduces Fe^{3+} to Fe^{2+}. This may already happen when Fe^{3+} is bound to phosvitin.

(4) When ascorbic acid is present in the aqueous phase in mayonnaise with a low pH value, it forms a complex with iron, whereby iron is released from phosvitin by the mechanism described in Scheme 1. Thereby, significant amounts of iron are removed from the interface into the aqueous phase of mayonnaise.

(5) Higher levels of iron in the aqueous phase increase the possibility of interactions between iron and the LOOH located at the emulsion droplet surface, and this induces lipid oxidation via LOOH breakdown at the droplet surface (Mei et al., 1998). We assume that it is the ascorbic acid−Fe^{2+} complex which reacts with LOOH and not free Fe^{2+} ions.

(6) The decomposition of LOOH from polyunsaturated marine fatty acids rapidly produces undesirable rancid and fishy off-flavors. When the Fe^{2+}−ascorbic acid 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+}−ascorbic acid complex back to an Fe^{2+}−ascorbic acid complex as described by Fukuzawa et al. (1993).

It was previously shown that preparation of an 85% w/w water-in-oil emulsion containing slightly oxidized lipids (0.5−2.0 mequiv/kg) led to unavoidable hydrolysis of triglycerides and immediate extraction/partitioning of long-chain fatty acid hydroperoxides into the aqueous phase (Refsgaard et al., 1992). These results suggest that lipid hydroperoxides may also be present in the aqueous phase in our mayonnaise system. Therefore, an alternative hypothesis for step 5 is that the reaction between the iron−ascorbic acid complex and LOOH takes place in the aqueous phase of mayonnaise. It is also possible that the reaction may take place both at the interface and in the aqueous phase. This matter obviously deserves further investigation.

The significantly increased off-flavors occurring upon addition of ascorbic acid may also occur in other low-pH food emulsion systems, notably egg yolk emulsified oil−water emulsions. The realization that metal catalysis is the most important factor in initiation and further oxidation in oil−water emulsions gives a new rational basis for targeted protection of such vulnerable systems.

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