Partitioning of Selected Antioxidants in Mayonnaise

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This study examined partitioning of α-, β-, and γ-tocopherol and six polar antioxidants (Trolox, ferulic acid, caffeic acid, propyl gallate, gallic acid, and catechin) in mayonnaise. Partitioning of antioxidants between different phases was determined after separation of mayonnaise by either (a) centrifugation + ultracentrifugation or (b) centrifugation + dialysis. Antioxidants partitioned in accordance with their chemical structure and polarity: Tocopherols were concentrated in the oil phase (93−96%), while the proportion of polar antioxidants in the oil phase ranged from 0% (gallic acid and catechin) to 83% (Trolox). Accordingly, proportions of 6% (Trolox) to 80% (gallic acid and catechin) were found in the aqueous phase. Similar trends were observed after dialysis. After ultracentrifugation, large proportions of polar antioxidants were found in the "emulsion phase" and the "precipitate" (7−34% and 2−7%, respectively). This indicated entrapment of antioxidants at the oil−water interface in mayonnaise. The results signify that antioxidants partitioning into different phases of real food emulsions may vary widely.

Keywords: *Partitioning; emulsion; mayonnaise; tocopherol; polar antioxidants*

INTRODUCTION

The understanding of oxidation and antioxidation mechanisms in complex systems such as oil−water (o/w) food emulsions is limited. However, the available knowledge indicates that the chemical environment of the antioxidants and the physical state of lipid systems have a major impact on the activity and efficacy of antioxidants (Roginsky, 1990; Frankel et al., 1994; Huang et al., 1996a, 1997). Thus, the hydrogen-donating ability and stability of the antioxidant is affected by the environment where the antioxidant is located (Roginsky, 1990; Frankel et al., 1996; Huang et al., 1997). Furthermore, a polar paradox appears to exist with respect to antioxidant efficacy in bulk oils and emulsions (Porter, 1993). In bulk oils, the hydrophilic antioxidants ascorbic acid and Trolox were thus better antioxidants than their lipophilic analogues, ascorbyl palmitate and α-tocopherol. Contrary to this, in emulsions the order of efficiency was reversed (Frankel et al., 1994). This polar paradox was explained by differences in the affinity of the antioxidants toward the different phases: air, oil, water, and the interface between oil and water in emulsions (Frankel et al., 1994).

In heterophasic food emulsions, such as mayonnaise, antioxidants may partition into at least three different phases, namely, the aqueous phase, the oil phase, and the interface between oil and water (Schwarz et al., 1996). Furthermore, micelles may also be present in emulsion systems if the concentration of emulsifier is above the critical micelle concentration (Friberg and Kayali, 1991). If this is the case, antioxidants may also partition into micelles. The high activity of hydrophobic antioxidants in o/w emulsions as compared with the low activity of hydrophilic antioxidants in o/w emulsions has been suggested to be due to the finding that hydrophobic antioxidants were located in the oil phase and at the oil−water interface. The hydrophilic antioxidants remained in the water phase where they apparently were less efficient (Huang et al., 1996a,b). Subsequently, it was found that more than 90% of the lipophilic antioxidants α-tocopherol, methyl carnosate, and carnosol were present in the oil phase of corn oil−water mixtures (1:1 w/w) (Huang et al. 1997). In analogy, it was shown that more than 90% of the hydrophilic antioxidants propyl gallate, rosmarinic acid, catechin, and gallic acid and 68% of Trolox were localized in the water phase of a 10% (w/w) corn oil−water mixture (Huang et al., 1997).

The partitioning properties of a particular antioxidant apparently depend not only on the chemical structure and relative polarity of the antioxidant but also on the lipid substrates, surfactants, pH, temperature, and composition of the phases (Cornell et al., 1970; Pryor et al., 1993; Barclay and Vinqvist, 1994; Schwarz et al., 1996). In an o/w emulsion containing 20% corn oil emulsified with either the cationic dodecyl trimethylammonium bromide (DTABr), the anionic sodium dodecyl sulfate (SDS), or the nonionic polyoxyethylene-sorbitan monolaurate (Tween 20), the charge of the surfactants affected partitioning of ferulic acid, caffeic acid, propyl gallate, gallic acid, Trolox, and catechin (Schwarz et al., 1996).

In real foods, complex emulsifiers such as egg yolk are frequently employed. Although, the protein and lipid

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composition of egg yolk is well-known (Li-Chan et al., 1994), there are no data available on how egg yolk affects the partitioning properties of antioxidants in real food emulsions. Partitioning studies on Trolox in egg lecithin liposomes showed, however, that the bulk of Trolox (approximately 80%) would reside in the aqueous phase of the liposomes but that Trolox exerted antioxidant activity in liposomes even when oxidation was initiated in the lipid phase (Barclay and Vinqvist, 1994). On the basis of these observations, it was therefore suggested that Trolox at least partially diffused into the lipid bilayer phase of egg lecithin liposomes (Barclay and Vinqvist, 1994). Thus, the available reports support the concept that the localization of antioxidants is important for their activity in emulsion systems.

In our current research program on oxidation mechanisms in real food emulsions, we have chosen mayonnaise enriched with fish oil as a model for o/w food emulsions (Meyer and Jacobsen, 1996; Jacobsen et al., 1998). Mayonnaise is a complex emulsion system with a low pH (<4.2) where egg yolk is used as surfactant/emulsifier. Egg yolk is a mixture of water, proteins, and lipids plus other minor constituents. The emulsifying properties of egg yolk is generally believed to be due to a phospholipid−protein complex formed by egg yolk constituents (Mine and Bergougnoux, 1998). The proteins involved in this complex include the egg yolk granule proteins phosvitin (Chung and Ferrier, 1995), lipovitellin, and lipovitellenin, the plasma protein livetin (Carrillo and Kokini, 1988), and the low-density lipoprotein (LDL) complex from both the egg yolk plasma and the egg yolk granules (Mine, 1998). The LDL complex is considered to make the major contribution to the emulsifying properties of egg yolk (Kiosseoglou and Sherman, 1983). The LDL proteins contain up to 90% lipids of which approximately 26% consists of phospholipids (Martin et al., 1963). Due to the high NaCl concentration in the continuous phase of mayonnaise and the low pH (<4.2), LDL micelles with a diameter of 250 Å are assumingly degraded into subunits with a particle diameter of 42−50 Å (Chang et al., 1972). Together with intact egg yolk LDL particles, these LDL subunits are envisaged to surround the oil droplets in the mayonnaise. Egg yolk granules may also be disrupted due to the high NaCl concentration and the low pH in mayonnaise. The constituents of the egg yolk granules, lipovitellins, phosvitin, and granular LDL also take part in interface formation as mentioned above. In the continuous phase, Chang et al. (1972) found filaments that may stem from livetins.

The main aim of our work was to examine the partitioning properties in mayonnaise of a number of hydrophilic and lipophilic antioxidants that had previously been studied in model systems. We therefore chose α-tocopherol, Trolox, and propyl gallate as well as the hydrophilic, phytochemical antioxidants caffeic acid, ferulic acid, gallic acid, and catechin for our study. Furthermore, two commercial tocopherol preparations, Toco 70 and Grindox 1032, that are widely used in food systems were included. These two tocopherol preparations were made either oil-soluble (Toco 70) or water-dispersible (Grindox 1032) by incorporation of different carrier systems by the manufacturer (see Materials and Methods). Thus, an additional objective was to examine whether the carriers used in the two different tocopherol preparations would affect the partitioning properties of tocopherol. The chemical structures of the selected antioxidants are shown in Figure 1.

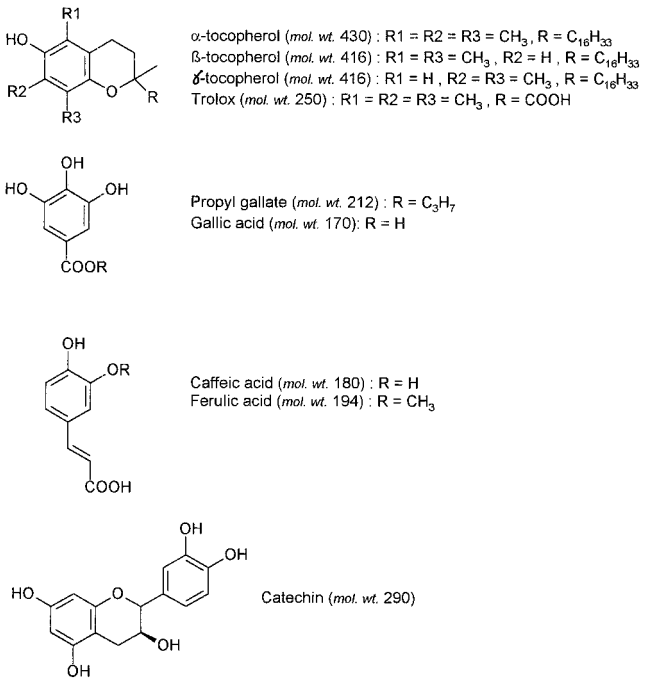

Figure 1. Chemical structure of antioxidants.

Separation of food emulsions to examine distribution of compounds between different phases is notoriously difficult. Previously, an ultrafiltration−centrifugation technique has been employed to study antioxidant partitioning in various heterophasic model systems (Huang et al., 1996b; Schwarz et al., 1996). However, due to the high fat content and the high viscosity and complexity of the mayonnaise system, this technique was not immediately applicable. We therefore developed a centrifugation method followed by ultracentrifugation to separate the mayonnaise in five different phases (Jacobsen et al., 1998). We evaluated antioxidant distribution both after separation of mayonnaises by centrifugation−ultracentrifugation and after separation by centrifugation followed by dialysis.

MATERIALS AND METHODS

Materials. Refined, unhydrogenated rapeseed oil was obtained from Aarhus Olie A/S, Aarhus, Denmark (unsaturated fatty acid composition: 18:1, 61.0%, 18:2, 20.2%, 18:3, 9.2%, 20:1, 1.3%; tocopherol content: α, 201 mg kg^{-1}, β, 67 mg kg^{-1}, γ, 234 mg kg^{-1}). Raw fish oil 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 (unsaturated fatty acid composition: 18:1, 10.5%, 18:2, 2.0%, 18:3, 1.7%, 18:4, 4.6%, 20:1, 6.9%, 20:5, 10.4%, 22:1, 10.5%, 22:6, 10.8%, 24:1, 1.1%; peroxide value, <0.3 mequiv/kg; anisidine value, 3.77; tocopherol content: α, 34 mg kg^{-1}, β, 0 mg kg^{-1}, γ, 0 mg kg^{-1}). Liquid, frozen egg yolk with 3% salt (NaCl) was from Danæg, Copenhagen, Denmark. Tarragon vinegar (7%) was purchased from A/S Dansk Eddikecentral, Copenhagen, Denmark. Lemon juice was from ItalLemon, Milano, Italy. Potassium sorbate was purchased from Merck, Darmstadt, Germany. Grindsted FF DC, Grindox 1032 tocopherol mixture (water-dispersible mixture comprising 20% natural tocopherol concentrate and 80% acetic acid esters of mono- and diglycerides of fatty acids and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids), Toco 70 tocopherol mixture (oil-soluble mixture comprising 70% natural tocopherol concentrate and 30% vegetable oil) and propyl gallate were donated by Danisco

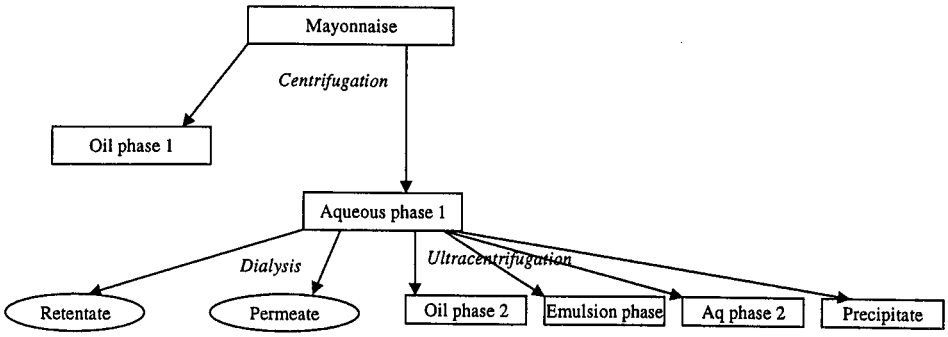

Figure 2. Flow sheet of the experiments.

Ingredients, Brabrand, Denmark. The tocopherol mixtures used in Grindox 1032 and Toco 70 were similar and contained α-tocopherol (16.2% w/w), β- and γ-tocopherol (58.2% w/w), and δ-tocopherol (25.6% w/w). Trolox and ferulic acid were from Aldrich Chemical Co. (Milwaukee, WI); α-tocopherol was purchased from Merck (Darmstadt, Germany); caffeic acid, gallic acid, and catechin were from Sigma (Steinheim, Germany).

Mayonnaise Production and Separation. 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), 2.0 g of Grindsted FF DC (= 0.2% w/w), and 92.0 g of distilled water (= 9.2% w/w). Mayonnaises were produced as previously described (Meyer and Jacobsen, 1996). Values of pH varied between 3.85 and 3.90 for the mayonnaises. Antioxidants (200 mg kg^{-1}) were added before mayonnaise production either to the water phase (Trolox, Grindox 1032, ferulic acid, caffeic acid, gallic acid) or to the oil (α-tocopherol, Toco 70, propyl gallate, and catechin). Subsequently, aliquots of 500 g of mayonnaise were packed in aluminum bags from Danisco Flexible (Lyngby, Denmark). To facilitate separation, mayonnaises were frozen at −40 °C and then separated by centrifugation as previously described (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 separated into two portions. One portion was then ultracentrifuged according to Jacobsen et al. (1999a). Thereby, four phases were obtained: precipitate, aqueous phase 2, emulsion phase, and oil phase 2 (Figure 2). Five milliliters of the other portion of aqueous phase 1 was dialyzed against distilled water (Figure 2) in a custom-built small dialysis cell (2 × 5 mL) made of plastic equipped with a Spectra/Por dialysis membrane (Spectrum Medical Industries Inc., Houston, TX) with a molecular mass cutoff of 6000−8000 Da. The dialysis was carried out in darkness at room temperature for 48 h, whereby a permeate and a retentate were obtained as described by Stöckmann and Schwarz (1999).

Sample Preparation for Determination of Antioxidants. All antioxidants were determined by HPLC in mayonnaise and in all phases obtained after centrifugation, ultracentrifugation, and dialysis (Figure 2). Samples with alpha-tocopherol, Toco 70, and Grindox 1032 were saponified according to Pfalzgraf et al. (1995). The volume of the extract obtained was adjusted to 10.0 mL with isooctane/*tert*-methyl butyl ether (96:4). Propyl gallate, gallic acid, ferulic acid, caffeic acid, and Trolox were diluted (1:4, 1:9, or 1:19 depending on the concentration) in 2-propanol/hexane (70:30) and subjected to HPLC analysis without further preparation. Prior to mixing with 2-propanol/hexane, the precipitate obtained after ultracentrifugation was suspended in distilled water (1:2). Mayonnaise, aqueous phase 1, aqueous phase 2, emulsion phase, and precipitate were centrifuged for 2 min at 2000*g* after mixing with the solvent. The supernatant was used for analysis.

HPLC Analysis of Antioxidants. Polar antioxidants (propyl gallate, gallic acid, ferulic acid, caffeic acid, and catechin) were analyzed by HPLC according to Stöckmann and Schwarz (1999). A chemically modified stationary phase (LiChrosorb 100, CN, 5 μm; 250 × 4 mm i.d.; Knauer, Berlin, Germany) was used, and the eluent was composed of hexane, 2-propanol, ethyl acetate, and acetic acid (25%) in the ratio 55:35:5:5. The flow was 0.7 mL/min, and the injection volume was 20 μL. Antioxidants were detected at the maximum absorbance (propyl gallate and gallic acid, 273 nm; ferulic acid, 320 nm; caffeic acid, 287 nm; catechin, 279 nm) using a UV detector (UVIS-206; Latek, Eppelheim, Germany). Trolox was detected by fluorescence detection (ex 295 nm, em 340 nm; Kratos fluorescence detector; Karlsruhe, Germany). Analyses were made in triplicate. α-tocopherol, Grindox 1032, and Toco 70 were analyzed on a HPLC system with fluorescence (ex 295 nm, em 340 nm) and UV detection (295 nm). Lichrosorb-60Si, 5μ (5 μm, 250 × 4 mm i.d.; Knauer, Germany, Berlin) was used as the stationary phase, and isooctane/*tert*-methyl butyl ether (96:4) was used as the mobile phase. The flow was 1.2 mL/min, and the injection volume was 20 μL. Analyses were made in duplicate. Antioxidants were quantified from calibration curves using authentic standards.

Determination of Droplet Size Distribution. Droplet size was determined in mayonnaise after 4 weeks of storage at 5 °C on a Malvern Mastersizer S (Malvern Instruments, Malvern, U.K.) as previously described by Jacobsen et al. (1999b). The following mean diameters were calculated: volume mean diameter, $D[4,3] = \sum d^4/\sum d^3$; surface mean diameter, $D[3,2] = \sum d^3/\sum d^2$.

Statistical Analysis. Analysis of variance and Duncan tests were carried out using Statgraphics version 7 software (Manugistics Inc., Cambridge, MA) ($p < 0.05$ unless otherwise stated). All results reported are means of duplicate runs of the same experiment.

RESULTS

Distribution of Antioxidants after Ultracentrifugation. The concentrations determined from the different antioxidants after separation of mayonnaise are summarized in Table 1. Concentrations of the different antioxidants varied markedly in the different phases: the concentration of the tocopherols was at least three times higher in oil phase 1 than in aqueous phase 1, whereas the reversed behavior was found for the polar antioxidants ferulic acid, caffeic acid, propyl gallate, gallic acid, and catechin (Table 1). For Trolox, the concentrations in aqueous phase 1 and oil phase 1 were of the same magnitude (109 vs 126 μg/mL). As expected, tocopherols were not present in aqueous phase 2 (Table 1). The rankings of concentration levels in aqueous phases 1 and 2 for the other antioxidants are summarized in Table 3. The concentrations of antioxidants in the precipitate and in the emulsion phase were higher than in aqueous phase 2, except for gallic acid, which had a lower concentration in the emulsion phase than in aqueous phase 2. Thus, for α- and γ-tocopherol the concentrations in the precipitate varied from 27 to 97

Table 1. Analyzed Concentrations and Recoveries of Antioxidants after Centrifugation and Ultracentrifugation of Mayonnaise[a] (± SD, $n = 2, 3$)

antioxidant	oil phase 1 (μg/mL)	aq phase 1 (μg/mL)	aq phase 2[b] (μg/mL)	precipitate[b] (μg/g)	emulsion phase[b] (μg/g)	oil phase 2[b] (μg/mL)	recovery in aq phase 1 + oil phase 1[c] (%)	recovery after ultracentrifugation[d] (%)
				Tocopherols				
α-tocopherol	187.9 ± 20.5	50.4 ± 1.5	−[e]	55.1 ± 19.3	162.2 ± 12.4	nd	51.3 ± 5.3	56.2 ± 6.2
Toco 70 α	167.7 ± 6.0	29.4 ± 1.3	−	27.0 ± 2.6	100.9 ± 4.6	nd	80.5 ± 4.9	55.8 ± 1.3
Toco 70 β	51.5 ± 7.7	7.1 ± 0.8	−	−	20.2 ± 2.2	nd	90.7 ± 16.5	34.1 ± 11.4
Toco 70 γ	182.9 ± 12.0	44.6 ± 2.3	−	51.2 ± 2.0	93.1 ± 23.9	nd	56.0 ± 5.6	43.9 ± 10.4
G1032 α	151.1 ± 10.1	29.2 ± 0.5	−	45.1 ± 0.7	94.5 ± 16.4	nd	74.5 ± 4.9	64.6 ± 6.8
G1032 β	44.9 ± 3.5	−	−	−	14.1 ± 15.7	nd	79.1 ± 6.0	112.7 ± 129.9
G1032 γ	151.2 ± 11.8	44.9 ± 7.9	−	97.0 ± 6.8	80.0 ± 29.2	nd	48.4 ± 4.0	63.4 ± 6.7
				Polar Antioxidants				
Trolox	126.3[f]	108.8 ± 6.6[g]	26.1 ± 3.4	531.5 ± 62.9	355.2 ± 27.1	46.8 ± 3.4	65.6 ± 0.4[g]	186.5 ± 24.8[g]
ferulic acid	151.6 ± 0.9	219.1 ± 6.3	62.5 ± 0.1	387.8 ± 6.9	169.9 ± 21.2	59.3[f]	88.3 ± 0.0	114.4 ± 4.2
caffeic acid	36.3 ± 0.2	659.1 ± 13.8	176.7 ± 8.2	1479.1 ± 127.7	390.5 ± 6.5	14.8 ± 0.1	83.0 ± 0.9	113.4 ± 5.9
propyl gallate	100.9 ± 2.6	540.0 ± 23.1	99.6 ± 1.6	1652.0 ± 0.8	641.8 ± 24.5	49.7[f]	98.5 ± 0.9	96.9 ± 6.3
gallic acid	−	824.0 ± 68.4	286.9 ± 6.4	993.3 ± 126.7	243.1 ± 27.4	−	82.6 ± 7.6	115.9 ± 4.9
catechin	−	1125.1 ± 43.6	213.9 ± 23.7	2107.6 ± 232.9	663.0 ± 10.5	−	110.2 ± 2.5	88.8 ± 8.9

[a] Antioxidants were added to mayonnaise at a concentration of 200 ppm (mg/kg mayonnaise). [b] Absolute concentrations obtained after ultracentrifugation of diluted aqueous phase 1. [c] Recoveries in oil phase 1 + aqueous phase 1 = $(M_{o1}C_{o1} + M_{aq1}C_{aq1}) \times 100\%/M_mC_m$ where M refers to mass, C refers to concentration, o1 refers to oil phase 1, aq1 refers to aqueous phase 1, and m refers to mayonnaise. [d] Recoveries in precipitate (P) + aqueous phase 2 (Aq2) + emulsion phase (E) + oil phase 2 (O2) = $\Sigma(M_iC_i) \times 100\%/M_{aq1u}C_{aq1u}$, where i refers to the four phases (precipitate, aqueous phase 2, emulsion phase, and oil phase 2) and M_{aq1u} and C_{aq1u} refer to mass of diluted aqueous phase 1, which was used for ultracentrifugation, and concentration in diluted aqueous phase 1, respectively. [e] −, not detected; nd, not determined. [f] Only one determination was made. [g] Assuming that recovery after ultracentrifugation was 100%, the concentration of Trolox in aqueous phase 1 was estimated to be 198 μg/mL, and recovery after centrifugation was estimated to be 74% (see Results).

Table 2. Analyzed Concentrations and Recoveries of Antioxidants after Dialysis of Aqueous Phase 1[a] (± SD, $n = 2, 3$)

antioxidant	permeate[b] (μg/mL)	retentate[b] (μg/mL)	recovery after dialysis[c] (%)
	Polar Antioxidants		
Trolox	14.0 ± 0.6 (28.0)[d]	34.1 ± 0.7	132.8 ± 4.4
ferulic acid	32.8 ± 0.0 (65.6)	57.2 ± 1.7	123.3 ± 5.9
caffeic acid	94.9 ± 1.9 (189.8)	76.1 ± 3.6	77.8 ± 4.1
propyl gallate	146.2 ± 2.1 (292.4)	51.3 ± 1.1	109.8 ± 6.5
gallic acid	157.8 ± 3.1 (314.6)	201.7 ± 1.9	131.3 ± 11.3
catechin	103.2 ± 5.4 (206.4)	223.5 ± 4.2	87.1 ± 0.9

[a] Antioxidants were added to mayonnaise at a concentration of 200 mg/kg mayonnaise. [b] Absolute concentrations obtained after dialysis of diluted aqueous phase 1. [c] Recovery in permeate plus retentate = $(M_pC_p + M_rC_r)/(M_{aq1d}C_{aq1d}) \times 100\%$, where M refers to mass, C refers to concentration, p refers to permeate, r refers to retentate, and aq1d refers to diluted aqueous phase 1 used for the dialysis. [d] Values in parentheses are concentrations compensated for dilution of the permeate (see also Results).

μg/g, whereas it varied from 80 to 162 μg/g in the emulsion phase independent of the type of preparation of tocopherol (G1032 or Toco 70). β-Tocopherol was not detected in the precipitate and occurred only at low levels (14−20 μg/g) in the emulsion phase.

For the more hydrophilic antioxidants (Trolox, ferulic acid, caffeic acid, propyl gallate, gallic acid, and catechin), concentrations in the precipitate varied from 388 to 2108 μg/g and in the emulsion phase they varied from 170 to 663 μg/g. Among these more polar antioxidants, the rankings of the concentration levels in the precipitate and in the emulsion phase were the same except for the ranking of Trolox and gallic acid (Table 3). The concentrations of antioxidants determined in oil phase 2 varied from 15 to 59 μg/mL with ferulic acid and propyl gallate as the highest levels and caffeic acid at the lowest level. It was not possible to determine antioxidant concentrations in oil phase 2 for the tocopherols as the amount of sample available was too small.

Distribution of Antioxidants after Dialysis. The dialysis experiments reflect the equilibrium of antioxidants between the aqueous phase (permeate) and other constituents of aqueous phase 1 (retentate). Hence the antioxidant concentrations in the retentate reflect the retainment or binding of antioxidants by the egg yolk emulsifier and the lipids present in aqueous phase 1. The antioxidant concentration in the retentate is represented by the concentrations in the emulsion phase, the precipitate, and oil phase 2 when ultracentrifugation was employed. The concentrations determined in the permeate for the polar antioxidants varied between 14 and 158 μg/mL (Table 2). These values were the actual concentrations analyzed in the permeate. However, distilled water was added in the permeate compartment of the dialysis cell prior to dialysis. The retentate and permeate compartments of the dialysis cell were of equal size. Therefore, the antioxidant concentrations obtained for the permeate were multiplied by 2 to compare the levels with the concentrations obtained in aqueous phase 2 after ultracentrifugation. After this compensation for dilution, the antioxidant levels in the permeate after dialysis were very similar to those obtained in aqueous phase 2 after ultracentrifugation—the main exception being propyl gallate, where the level was much lower in aqueous phase 2 than in the permeate (Tables 1 and 2). Thus, the ranking of antioxidant levels in the permeate was similar to the ranking of antioxidant concentrations in aqueous phase 2: Trolox lowest and gallic acid highest (Table 3). Concentrations in the retentate varied from 34 to 224 μg/mL, with Trolox lowest and gallic acid and catechin highest (both above 200 μg/mL) (Table 2). Dialysis was not carried out on samples with tocopherol, as the tocopherols were not detectable in aqueous phase 2 after ultracentrifugation.

Recovery after Initial Centrifugation. On the basis of the analyzed concentrations, the calculated recoveries of tocopherols in oil phase 1 plus aqueous phase 1 varied from 48 to 91% (Table 1). The highest recoveries were found for α- and β-tocopherols in mayonnaise with Toco 70 and Grindox 1032, namely, 75−91% (Table 1). Recoveries in oil phase 1 plus aqueous phase 1 for the more hydrophilic antioxidants

Table 3. Oil−Water Partition Coefficients and Ranking of Concentrations of Polar Antioxidants in Aqueous Phase 2, Permeate, Precipitate, and Emulsion Phase[a]

antioxidant	$P_{o/w}$[b]	ranking of concn in aq phase 1	ranking of concn in aq phase 2	ranking of concn in precipitate	ranking of concn in emulsion phase	ranking of concn in permeate
Trolox	3.55	1	1	2	3	1
propyl gallate	0.89	3	3	5	5	5
ferulic acid	0.74	2	2	1	1	2
gallic acid	0.12	5	6	3	2	6
caffeic acid	0.11	4	4	4	4	3
catechin	0.08	6	5	6	6	4

[a] The lowest concentration has been assigned the lowest ranking value. [b] Detetermined in oil−water (20:80 w/w) mixtures in unbuffered water by Schwarz et al. (1996). The horizontal lines in columns 2−4 indicate that the $P_{o/w}$ values and antioxidant concentrations in aqueous phases 1 and 2 could be categorized in three groups.

Table 4. Relative Masses of Mayonnaise Fractions ($n = 2$)[a,b]

antioxidant	oil phase 1 (%)	aq phase 2 (%)	precipitate (%)	emulsion phase (%)	oil phase 2 (%)	emulsion phase plus precipitate (%)
			Tocopherols			
α-tocopherol	79.4^{bcd}	18.7^{bc}	1.2^{cd}	0.8^{ab}	0.0^{a}	2.0^{bc}
Toco 70	79.1^{ab}	18.5^{bc}	1.2^{d}	0.8^{bc}	0.0^{a}	2.0^{cd}
G1032	79.9^{de}	18.2^{abc}	1.1^{a}	0.9^{bcd}	0.0^{a}	2.0^{bc}
			Other Antioxidants			
Trolox	78.6^{a}	18.9^{c}	1.1^{abcd}	1.1^{e}	0.2^{b}	2.2^{e}
ferulic acid	80.2^{e}	17.5^{a}	1.1^{abcd}	1.0^{de}	0.0^{a}	2.1^{de}
caffeic acid	79.3^{bc}	18.5^{bc}	1.2^{bcd}	1.0^{cde}	0.1^{a}	2.1^{de}
propyl gallate	79.7^{cde}	18.3^{abc}	1.1^{abc}	0.8^{b}	0.0^{a}	1.9^{bc}
gallic acid	79.7^{bcde}	18.2^{abc}	1.2^{cd}	0.7^{a}	0.3^{c}	1.8^{ab}
catechin	80.1^{e}	18.0^{ab}	1.1^{ab}	0.7^{a}	0.1^{a}	1.8^{a}
standard errors (pooled values)	0.16	0.23	0.02	0.04	0.03	0.04

[a] Distributions were calculated as recovered mass in each phase divided with the total amount of recovered mass in all phases multiplied by 100 (%). [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$). Statistical analysis was made on values corrected to two decimals.

varied between 66 and 110%, but only mayonnaise with Trolox had a recovery lower than 83% (Table 1).

Recovery after Ultracentrifugation. Recoveries of antioxidants after ultracentrifugation (precipitate + aqueous phase 2 + emulsion phase + oil phase 2) varied between 34 and 113% for the tocopherols, whereas the variation for the polar antioxidants was from 89 to 187% (Table 1). The exceptionally high recovery of 187% was observed for Trolox. However, as already mentioned, the recovery after the first centrifugation was only 66% for this antioxidant. Taken together, these results could indicate that the concentration analyzed in aqueous phase 1 was lower than the "real" concentration in aqueous phase 1 for this antioxidant. If the amount of Trolox analyzed in aqueous phase 2 plus precipitate plus emulsion phase plus oil phase 2 is used to estimate indirectly the "real" concentration in aqueous phase 1, the concentration of Trolox was 198 μg/mL. By using this value to calculate the recovery after centrifugation, a more realistic value of 74% is obtained.

Recovery after Dialysis. The recovery of antioxidants in the permeate plus retentate after dialysis varied between 78% and 133% (Table 2). Only caffeic acid and catechin had recoveries lower than 100%.

Mass Distribution of Mayonnaise Phases. To evaluate if different antioxidants affected the phase distribution, the recovered masses of phases (in %) obtained after centrifugation and ultracentrifugation were calculated for the different mayonnaise phases (Table 4). The calculations were based on the absolute amounts of mass of each phase obtained after separation (centrifugation or ultracentrifugation). For example, the total mass recovered after centrifugation of 450 g of mayonnaise with Trolox was 445.7 g, of which 350.5 g was recovered in oil phase 1 (data not shown). Hence, the mass recovered in oil phase 1 was 350.5/445.7 × 100% = 78.6% (Table 4). In all emulsions, approximately 80% of the mass of mayonnaise constituted oil phase 1 (total average 79.6 ± 0.5%), 18% constituted aqueous phase 2 (total average 18.3 ± 0.4%), 1% was found as precipitate (total average 1.1 ± 0.0%), and 1% was found as emulsion phase (total average 0.9 ± 0.1%) (Table 4). Only 0.1% of the mayonnaise was found as oil phase 2 (total average 0.1 ± 0.1%) (Table 4). Even though the differences among distributions of phase masses were small, the differences varied significantly with different antioxidant additions, notably for the more polar antioxidants. Thus, ferulic acid addition increased mass recovery of oil phase 1 (80.2 ± 0.2%), whereas Trolox decreased oil phase 1 recovery (78.6 ± 0.2%) (Table 4). Moreover, these antioxidants affected the size of aqueous phase 2 in the opposite direction. Thus, the highest mass recovery for aqueous phase 2 was obtained for mayonnaise with Trolox added (18.9 ± 0.1%) while the smallest aqueous phase 2 was found for mayonnaise containing ferulic acid (17.5 ± 0.1%) (Table 4). For the precipitate and emulsion phase, the highest mass recoveries were found for mayonnaises containing Toco 70 (1.2 ± 0.0%) and Trolox (1.1 ± 0.0%), respectively (Table 4).

Mass Distributions of Antioxidants in Mayonnaise. Distributions of antioxidant masses were calculated based on the masses of recovered antioxidants in the different phases (Table 5). For example, the total amount of ferulic acid recovered after centrifugation of 438 g of mayonnaise was 77.37 mg, of which 58.37 mg was found in oil phase 1 (data not shown). Hence, 75.7% (= 58.37/77.37 × 100%) of the ferulic acid was localized in oil phase 1 (Table 5). As expected, the distribution of antioxidants between phases varied significantly de-

Table 5. Mass Distribution of Antioxidants between Phases of Mayonnaise (% Mean, $n = 2$)[a,b]

antioxidant	oil phase 1 (%)	aq phase 2 (%)	precipitate (%)	emulsion phase (%)	oil phase 2 (%)
		Tocopherols[c]			
α-tocopherol	94.3^{fgh}	0.0^{a}	1.9^{a}	3.8^{cd}	nd[d]
Toco 70 α	95.6^{h}	0.0^{a}	1.2^{a}	3.3^{bc}	nd
Toco 70 γ	93.9^{fg}	0.0^{a}	2.5^{ab}	3.1^{abc}	nd
G1032 a	95.3^{gh}	0.0^{a}	1.7^{a}	2.9^{ab}	nd
G1032 γ	93.1^{f}	0.0^{a}	4.2^{ac}	2.7^{ab}	nd
		Other Antioxidants			
Trolox	82.9^{e}	5.7^{b}	7.0^{d}	4.3^{d}	0.1^{b}
ferulic acid	75.7^{d}	15.6^{c}	6.2^{cd}	2.4^{a}	0.0^{a}
caffeic acid	19.0^{b}	49.5^{e}	25.8^{f}	5.7^{e}	0.0^{a}
propyl gallate	44.9^{c}	23.9^{d}	24.2^{f}	7.0^{f}	0.0^{a}
gallic acid	0.0^{a}	79.9^{g}	17.7^{e}	2.4^{a}	0.0^{a}
catechin	0.0^{a}	58.6^{f}	34.5^{g}	6.8^{f}	0.0^{a}
standard errors (pooled values)	0.46	0.66	0.68	0.23	0.01

[a] Distributions were calculated as milligram of recovered antioxidant in each phase divided by milligram of antioxidant recovered in all phases multiplied by 100. [b] Values within each column followed by the same letter are not statistically different ($p < 0.05$). The statistical analysis was made on values corrected to two decimals. [c] The distribution for β-tocopherol was not calculated due to low recoveries of this antioxidant. [d] nd, not determined.

pending on the type of antioxidant. In mayonnaises with α-tocopherol, Grindox 1032, and Toco 70, respectively, almost all α- or γ-tocopherols were found in oil phase 1 (>93%) and 0% was found in aqueous phase 2 as expected (Table 5). The distribution among the remaining two phases only differed slightly for the tocopherols. Thus, for all three antioxidant preparations 1.2−1.9% of the recovered α-tocopherol was found in the precipitate, whereas the variation for γ-tocopherol was higher, namely, between 2.5 and 4.2% depending on whether Toco 70 or Grindox 1032 had been used (Table 5). In the emulsion phase of the mayonnaise, the proportion of α- or γ-tocopherol varied between 2.7 and 3.8%. The majority of added Trolox was present in oil phase 1 (83%), but as compared to the tocopherols, larger fractions of the recovered Trolox were found in aqueous phase 2, in the precipitate, and in the emulsion phase, namely, 5.7, 7.0, and 4.3%, respectively (Table 5). In mayonnaise with ferulic acid, a higher proportion of antioxidant (15.6%) was found in aqueous phase 2, and a lower proportion of 76% was found in oil phase 1. Compared to the other polar antioxidants, the relative proportions of ferulic acid found in the precipitate and emulsion phases were low at 6.2% and 2.4%, respectively (Table 5). By contrast, the proportions of catechin, caffeic acid, gallic acid, and propyl gallate in the precipitate ranged from 18 to 34%. In oil phase 1, proportions for these antioxidants varied from 0 to 45%, and in aqueous phase 2 they varied from 24 to 80% (Table 5). A total of 6−7% of the recovered catechin, caffeic acid, and propyl gallate was localized in the emulsion phase, whereas only 2.4% gallic acid was found in the emulsion phase (Table 5).

Droplet Size Distributions. The measured droplet size diameters $D[4,3]$, which is the volume mean diameter, and $D[3,2]$, which is the surface mean diameter, were relatively similar for the tocopherols (Table 6). $D[4,3]$ varied from 4.2 to 4.9 μm, and $D[3,2]$ varied from 2.4 to 2.7 μm. However large variations were recorded for droplet sizes in mayonnaises containing different polar antioxidants (Table 6). Thus, Trolox addition produced significantly bigger mean diameters than the other antioxidants irrespective of whether the volume mean ($D[4,3]$) or the surface mean diameters ($D[3,2]$ were considered (Table 6). By contrast, ferulic acid addition resulted in a small volume mean diameter (5.3 μm) and surface diameter (2.6 μm). The ranking of the $D[4,3]$ levels was α-tocopherol (4.2 μm) < G1032 < Toco 70 < ferulic acid < catechin < gallic acid < propyl gallate < caffeic acid < Trolox (9.01 μm). The ranking of the $D[3,2]$ levels was only slightly different, namely, α-tocopherol (2.4 μm) < G1032 = ferulic acid < catechin < caffeic acid = gallic acid < Toco 70 < propyl gallate < Trolox (2.9 μm). Differences between mayonnaises were smaller for $D[3,2]$ than for $D[4,3]$.

Table 6. Volume and Surface Mean Diameters of Mayonnaises ($n = 2$)[a]

antioxidant	vol mean diameter, $D[4,3]$ (μm)	surface mean diameter, $D[3,2]$ (μm)
	Tocopherols	
α-tocopherol	4.2^{a}	2.4^{a}
Toco 70	4.9^{ab}	2.7^{bc}
G1032	4.5^{a}	2.6^{ab}
	Polar Antioxidants	
Trolox	9.0^{e}	2.9^{d}
ferulic acid	5.3^{abc}	2.6^{ab}
caffeic acid	6.8^{d}	2.6^{bc}
propyl gallate	6.5^{cd} [b]	2.8^{cd} [b]
gallic acid	5.9^{bcd}	2.6^{bc}
catechin	5.5^{abcd}	2.6^{b}
standard errors (pooled values)	0.38	0.06

[a] Values within each column followed by the same letter are not significantly different ($p < 0.05$). The statistical analysis was made on values corrected to two decimals. [b] Only one measurement was made.

Partition Coefficients. Partition coefficients were calculated for partitioning between oil phase 1 and aqueous phase 1 ($P_{o1/aq1}$) and between oil phase 1 and aqueous phase 2 ($P_{o1/aq2}$) (Table 7). In principle, the two different calculations ought to produce similar partition coefficients. The $P_{o1/aq1}$ values varied between 0 and 7.2 with the polar antioxidants all having $P_{o1/aq1}$ values of 0−1.2. Catechin and gallic acid had the lowest coefficients, and β-tocopherol in Toco 70 had the highest (7.2) (Table 7). As no tocopherols were detected in aqueous phase 2 (Table 1), $P_{o1/aq2}$ values were not calculated for the tocopherols. The $P_{o1/aq2}$ values of the polar antioxidants were similar to the $P_{o1/aq1}$ values, and most were in the range of 0−1. Trolox had a $P_{o1/aq2}$ of 1.6, which was significantly higher than the others.

DISCUSSION

Complex heterophasic food systems such as mayonnaise comprise at least three phases: an oil phase, an aqueous phase, and an interface between the oil and

Table 7. Oil Phase 1−Aqueous Phase 1 and Oil Phase 1−Aqueous Phase 2 Partition Coefficients ($n = 2$)[a,b]

antioxidant	$P_{o1/aq1}$	$P_{o1/aq2}$
Tocopherols		
α-tocopherol	3.7[de]	−[c]
Toco 70 α-tocopherol	5.7[f]	−
Toco 70 β-tocopherol	7.2[g]	−
Toco 70 γ-tocopherol	4.1[e]	−
G1032 α-tocopherol	5.2[f]	−
G1032 β-tocopherol	−	−
G1032 γ-tocopherol	3.4[d]	−
Polar Antioxidants		
Trolox	1.2[c]	1.6[d]
ferulic acid	0.7[bc]	0.8[c]
caffeic acid	0.1[a]	0.1[a]
propyl gallate	0.2[ab]	0.3[b]
gallic acid	0[a]	0[a]
catechin	0[a]	0[a]
standard errors (pooled values)	0.18	0.06

[a] Values within each column followed by the same letter are not significantly different ($p < 0.05$). [b] Partition coefficients for partition of antioxidants were calculated by $P_{x/y} = C_x/C_y$, where x refers to phase x, y refers to phase y, and C refers to the concentration. [c] −, not detected,

aqueous phase (Schwarz et al., 1996; Coupland and McClements, 1996). At present, it is not possible to measure antioxidant concentrations directly in the different phases of mayonnaise. Hence, to determine partitioning properties of antioxidants, it is necessary to separate the mayonnaise in fractions and subsequently measure the concentrations of antioxidants in each fraction. We have developed a separation technique that by centrifugation and ultracentrifugation separated mayonnaise into five phases; two oil phases, an aqueous phase (aqueous phase 2), an emulsion phase, and a precipitate (Jacobsen et al., 1998) (Figure 2). We proposed that the two oil phases represent the oil droplets in the mayonnaise and that aqueous phase 2 represents the "real" aqueous phase in mayonnaise. We also suggested that the emulsion phase and the precipitate represent the interface between the oil and aqueous phase in mayonnaise (Jacobsen et al., 1998). However, the freeze−thaw cycle of mayonnaise prior to separation may damage the yolk structure. Furthermore, the separation by ultracentrifugation is based on differences in density between phases. Therefore, the precipitate and the emulsion phase may contain egg yolk constituents that were not originally located at the oil−water interface. Thus, the proposal that the emulsion phase and the precipitate represent the oil−water interface can be modulated to propose that they constitute a representative fraction of the oil−water interface. To mirror the chemical equilibrium between emulsion phases without physical separation, a dialysis technique applicable to the preseparated aqueous phase (aqueous phase 1) was recently developed (Stöckmann and Schwarz, 1999). Therefore, a comparison of antioxidant partitioning data based on measurements of phases obtained after different separation principles was relevant. In the present study, we thus employed this centrifugation−ultracentrifugation method to investigate antioxidant partitioning in mayonnaise and compared the results to data obtained after centrifugation−dialysis (Figure 2).

The polarity of the antioxidants has previously been assessed by Schwarz et al. (1996) by determining the partition coefficient ($P_{o/w}$) for oil−water mixtures (20:80 w/w) using distilled water (Table 3). $P_{o/w}$ was much higher for Trolox (3.55) than for the other antioxidants. $P_{o/w}$ values for propyl gallate and ferulic acid were both approximately 0.8, whereas gallic acid, caffeic acid, and catechin had almost identical, low values of approximately 0.1. Thus, $P_{o/w}$ values for the polar antioxidants could apparently be categorized in three groups, as indicated by the horizontal lines in Table 3. The order of antioxidant concentrations determined in both aqueous phase 1 and aqueous phase 2 could be categorized in the same three groups as the $P_{o/w}$ values. Hence, the ranking of antioxidant concentrations in these two phases was similar to the ranking of antioxidant polarity (Tables 1 and 3).

Previously, we found that aqueous phase 2 contained small amounts of lipids (0.14%) and small amounts of phosphorus (Jacobsen et al., 1998). We originally proposed that the lipid and phosphorus found in aqueous phase 2 might represent phospholipid micelles formed from egg yolk constituents, not bound at the interface (Jacobsen et al., 1998). We now believe that the phospholipids mainly stem from LDL proteins present in the aqueous phase (Li-Chan et al., 1994). Ultracentrifugation and dialysis of aqueous phase 1 gave relatively similar results with respect to both the antioxidant concentrations and the order of the antioxidant concentrations in aqueous phase 2 and permeate (Tables 1−3). This observation supports our hypothesis that aqueous phase 2 represents the aqueous phase of mayonnaise. Furthermore, the permeate assumingly did not contain any egg yolk constituents. Therefore, this finding also indicates that if egg yolk constituents were present in aqueous phase 2 they apparently did not associate with the antioxidants.

The reason for the relatively low recoveries of γ-tocopherol after centrifugation and ultracentrifugation may be that tocopherols were bound to micelles/yolk protein structures making them less accessible for analysis. It should also be mentioned that no δ-tocopherol could be detected even though the antioxidant mixtures Grindox 1032 and Toco 70 did contain δ-tocopherol.

The recoveries after dialysis were higher than 100% for all antioxidants except for ferulic acid and catechin (Table 1). This observation may indicate that the concentrations analyzed in aqueous phase 1 are slightly lower than the "real" concentration. The finding that also the recoveries after ultracentrifugation for the polar antioxidants generally were higher than 100% whereas the recoveries after centrifugation were lower than 100% is consistent with this hypothesis.

Surprisingly, mass distribution of phases after centrifugation and ultracentrifugation as well as droplet size distributions were significantly affected by antioxidant addition (Tables 4 and 6). It was particularly observed that Trolox gave rise to high mean diameters ($D[4,3]$ and $D[3,2]$) and that Trolox also gave rise to the highest proportions of mass recovered in aqueous phase 2 and in the emulsion phase. On the other hand, the mass recovered in the precipitate for Trolox mayonnaise was not significantly different from the mass recovered for G1032 mayonnaise, which gave rise to the smallest droplets.

As previously mentioned, we proposed that the emulsion phase and the precipitate obtained after ultracentrifugation together represent the interface in the mayonnaise (Jacobsen et al., 1998). Therefore, to compare mass distributions for the assumed interface, we summed up the mass recoveries for the precipitate and

the emulsion phase (Table 4). The mass recovered in the precipitate plus in the emulsion phase for Trolox was significantly higher than for α-tocopherol, Toco 70, G1032, propyl gallate, gallic acid, and catechin, indicating that Trolox addition affected the composition of the emulsion phase and the precipitate. The minimum amount of emulsifier necessary to cover the surface of the oil droplets is inversely proportional to the diameter of the droplets (Kuhn, 1974). Hence, it is expected that the total mass of interface in mayonnaise with big droplets (Trolox mayonnaise) may be lower than the interface mass for mayonnaise with small droplets (e.g., α-tocopherol mayonnaise), assuming that the interfacial layers of small and big droplets have the same thickness. However, Trolox addition produced increased masses of emulsion phase and precipitate simultaneously with bigger droplets in mayonnaise. On the basis of these data, it is therefore tempting to conclude that Trolox mayonnaise had a thicker interfacial layer around bigger droplets than the other mayonnaises with smaller droplets. The available data do not permit us to favor whether Trolox caused increased thickness of the interfacial layer or if Trolox addition just happened to increase the amounts of components with densities equal to the emulsion phase or precipitate after ultracentrifugation. Despite these reservations, the results obtained strongly indicate that Trolox interacted differently than the other antioxidants with the mayonnaise constituents.

Distributions of antioxidant masses among the different phases obtained after ultracentrifugation of mayonnaises (calculated as % of total recovered antioxidant mass) were significantly different for the different antioxidants (Table 5). The differences were much larger than the differences between mass distributions of the phases. Each proportion of recovered antioxidant found in aqueous phase 2 varied according to the same pattern as the concentrations in aqueous phase 2. There was a clear relation between the chemical structure and polarity of the antioxidant and the distribution. In a study by Schwarz et al. (1996), the proportions of ferulic acid, caffeic acid, propyl gallate, gallic acid, Trolox, and catechin in the aqueous phase were determined in 20% oil/80% water emulsions with either SDS, Tween, or DTABr as emulsifiers. When SDS was used, the lowest proportion of antioxidant in the aqueous phase was found for Trolox followed by ferulic acid, propyl gallate, caffeic acid, gallic acid, and catechin. The order of antioxidants proportion obtained in aqueous phase 2 obtained in the present study is thus in accordance with these results. This similarity in the antioxidant partitioning pattern between emulsions with the anionic SDS emulsifier and mayonnaise with egg yolk as an emulsifier may be due to the anionic character of some of the proteins in egg yolk. Thus, phosvitin is for example an anionic protein due to its high content of phosphoserine (Causeret et al., 1991). In the previous study by Schwarz et al. (1996), it was found that, when Tween 20 was used as an emulsifier in the 20% oil/80% water emulsion, the proportion of antioxidant in the aqueous phase varied between 27% and 68% depending on the type of antioxidant. In a similar study by Huang et al. (1997), it was found that the proportions of Trolox, propyl gallate, catechin, and gallic acid in the aqueous phase of a 10% oil/90% water emulsion with 1% Tween 20 were 36%, 17%, 22%, and 70%, respectively. Thus, the relative proportions of these antioxidants recovered in aqueous phase 2 in the present study (Table 5) were in the same range as reported previously for different Tween-emulsified model systems. However, the differences in antioxidant polarity were reflected more significantly after ultracentrifugal separation of the mayonnaise as compared with the results obtained with Tween 20; hence, propyl gallate, catechin, and gallic acid were found in relatively higher proportions in aqueous phase 2 (24−80% of recovered amounts), but Trolox was found in much lower proportion (6%) (Table 5) as compared to what has been reported for Tween-emulsified emulsion systems that contained less oil than mayonnaise. It should be emphasized that (theoretically) the absolute amount of the polar antioxidants in the aqueous phase of mayonnaise containing 20% water is much lower than the absolute amount of the polar antioxidants in model emulsions with 80% or 90% water. This means that higher proportions in the aqueous phase could be expected for the model emulsions. Disparities are most likely related to the different solubilization capacities of Tween emulsifier and egg yolk.

The proportion of antioxidant found in the precipitate and emulsion phase in our experiment also varied substantially among different antioxidants (1.2−34.5% in the precipitate and 2.4−7.0% in the emulsion phase (Table 5)). The lower proportion of γ-tocopherol found in the precipitate for Toco 70 as compared with Grindox 1032 may be due to lower recovery obtained after ultracentrifugation of the Toco 70 aqueous phase 1. We previously observed that the two carrier systems employed for the tocopherol systems affected the distribution of volatile oxidation compounds (Jacobsen et al., 1999a). However, the present experiment on antioxidant partitioning did not point out a significant influence of the tocopherol carriers on antioxidant partitioning.

Comparison of the partitioning behavior of tocopherols versus their hydrophilic analogue, Trolox, clearly showed that a higher proportion of Trolox partitioned into the precipitate and the emulsions phase. This finding is in accordance with the results obtained by Huang et al. (1996a,c) for Tween-containing emulsions. It was also a general trend that the other hydrophilic antioxidants partitioned into the precipitate and emulsion phase to a much higher extent than did the tocopherols. Gallic acid had, however, a relatively low proportion of antioxidant in the emulsion phase and the precipitate and a very high proportion in aqueous phase 2 (Table 5). Due to the alkyl chain, propyl gallate is less polar than gallic acid and was present to a higher extent in the precipitate and in the emulsion phase than gallic acid (Tables 1 and 5).

Partition coefficients, $P_{o1/aq1}$ and $P_{o1/aq2}$, for the different antioxidants also varied according to their chemical structure (Figure 1). Within the tocopherols, $P_{o1/aq1}$ values generally were lower for γ-tocopherol than for α-tocopherol. However, as recoveries after centrifugation were also lower for γ-tocopherol than for α-tocopherol, it is difficult to make any firm conclusions on the exact mechanisms behind this finding. $P_{o1/aq1}$ and $P_{o1/aq2}$ values for the hydrophilic antioxidants were generally in agreement with the values obtained previously with oil and water mixtures without surfactants (Schwarz et al., 1996; Table 3). Only the partition coefficients for Trolox and propyl gallate were lower than the values obtained with oil−water mixtures.

It may be speculated how the different distribution patterns for the different antioxidants will influence

their antioxidative efficacy in emulsions. As already touched upon, several studies have shown that hydrophilic antioxidants had low antioxidant or prooxidant activities in 10% oil−water emulsions with Tween 20 (1%) as emulsifier (Frankel, 1994; Frankel et al., 1996; Huang and Frankel, 1997). Low antioxidant activities were explained by the higher partitioning of hydrophilic compounds into the Tween 20 micelles and the oil−water interface (Huang et al., 1997). Tocopherols and other hydrophobic antioxidants have been shown to be more efficient than the hydrophilic compounds in the same oil−water emulsion systems (Huang et al., 1996a,b). On this basis, it was suggested that hydrophobic antioxidants were located in the oil phase or at the oil−water interface while, in contrast, hydrophilic antioxidants remained in the aqueous phase. In our mayonnaise system, we may expect a different behavior of the antioxidants as compared with in the model emulsions due to the different emulsifiers employed (egg yolk vs Tween). Egg yolk may thus cause different interactions with antioxidants as compared with Tween. Furthermore, the low pH in mayonnaise may also affect the partitioning and antioxidative properties of antioxidants due to protonization of reactive groups at low pH. Huang et al. (1996c) thus reported that pH significantly affected the efficacy of Trolox and tocopherol in inhibiting oxidation of 10% corn oil emulsions with 1% (w/w) Tween 20. Hence, when pH was varied between 3 and 7, Trolox was most efficient at pH 4 whereas tocopherol was most efficient at pH 6.

In the present study, we have also demonstrated that the proportion of antioxidant that partitioned into the emulsion phase and precipitate, i.e., assumingly the interface, varied significantly depending on the chemical structure of the antioxidant. In a previous investigation we found prooxidant effects of commercial propyl gallate preparations in the same fish oil-enriched mayonnaise system as employed in the present study (Jacobsen et al., 1999b), and this could be due to the high concentrations of propyl gallate at the interface, where it may interact with iron ions present in the egg yolk to generate free radicals in a Fenton-like reaction (Halliwell et al., 1995). However, the effect on antioxidant activity of antioxidant entrapment/binding and antioxidant interaction with interfacial layers in food emulsion systems deserves further exploration.

CONCLUSION

The present study confirmed and expanded the concept that antioxidants partition into different phases of oil−water emulsions in accordance with their chemical structure and polarity and demonstrated that antioxidants partitioned into different phases also in a real food emulsion system such as mayonnaise. Additionally, high proportions of up to 40% of the more polar antioxidants (caffeic acid, catechin, gallic acid, and propyl gallate) were apparently localized at the interfacial layers of mayonnaise. Furthermore, even more lipophilic antioxidant compounds, such as tocopherols, also distributed into the oil−water interface in mayonnaise, albeit only to a limited extent (3−7%). On the basis of the data obtained, we therefore propose that antioxidants apparently interact significantly with the interfacial layers in emulsified systems and that egg yolk constituents participate in such interactions in real mayonnaise. The degree of interaction depends on the nature of the antioxidant. In the present study, the apparent partitioning of antioxidants into the interface also unexpectedly affected the oil droplet size and mass distribution of mayonnaise fractions. These observations signify a possible influence of antioxidant partitioning on the physicochemical properties of mayonnaise. Although significant differences in partitioning behavior among different antioxidant structures have now been demonstrated in various emulsion systems, more research is obviously required to study the correlation between partitioning of antioxidants between the different phases in real food emulsions and the activity and stability of the antioxidants in these systems.

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