# The Relationship between the Physicochemical Properties of Antioxidants and Their Ability to Inhibit Lipid Oxidation in Bulk Oil and Oil-in-Water Emulsions

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Chain-breaking antioxidants differ in their effectiveness at inhibiting lipid oxidation because of their chemical properties and physical location within a food. Our objective was how the physicochemical properties of four structurally related lipid-soluble antioxidants were related to their antioxidant activity. Antioxidants differed in the number of methyl ($\alpha$-tocopherol and $\delta$-tocopherol) or hydroxyl (butylated hydroxytoluene (BHT) and 4-hydroxymethyl-2,6-ditertiarybutylphenol) groups. Surface activity of the antioxidants was in the order of $\delta$-tocopherol > $\alpha$-tocopherol $\approx$ 4-hydroxymethyl-2,6-ditertiary-butylphenol > BHT. Free-radical scavenging activity was similar between $\alpha$-tocopherol and $\delta$-tocopherol as well as BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol. In bulk menhaden oil, BHT was a more effective antioxidant than 4-hydroxymethyl-2,6-ditertiarybutylphenol while $\alpha$-tocopherol was more effective than $\delta$-tocopherol. In menhaden oil-in-water emulsions, BHT was a more effective antioxidant than 4-hydroxymethyl-2,6-ditertiarybutylphenol while $\delta$-tocopherol was more effective than $\alpha$-tocopherol. These results indicate that the surface activity and polarity of lipid-soluble antioxidants were not the only determinants of their antioxidant effectiveness in food lipids.

## INTRODUCTION

Current research suggests that consumption of unsaturated lipids especially $\omega$-3 polyunsaturated fatty acids is beneficial to health (*1*). However, these lipids are susceptible to oxidation reactions that lead to development of undesirable off flavors, nutrient loss, and potential formation of toxic products (*2*). Lipid oxidation can be controlled by a variety of antioxidant technologies such as control of oxidation substrates, control of prooxidants, and addition of antioxidants (*2*−*4*). The effectiveness of antioxidants depends on several factors such as the polarity of the antioxidants, lipid substrate, pH, temperature, concentration of antioxidants, and the physical properties of the food (*5*). Because of the influence of many factors on the ability of antioxidants to inhibit lipid oxidation, great variations in antioxidant activity can be seen in different food systems. In fact, some antioxidants retard lipid oxidation under certain conditions but promote it under other conditions and therefore act as prooxidants (*6*).

Much work has compared the ability of various antioxidants to inhibit lipid oxidation in bulk oil and in oil-in-water emulsions (*7*−*15*). Most results have shown that polar antioxidants are more effective in bulk oil than in oil-in-water emulsions and nonpolar antioxidants are more effective in emulsions than in bulk oil as described by the "antioxidant polar paradox" (*8*−*9*,

*14*−*18*). The antioxidant polar paradox has been postulated to be due to retention of nonpolar antioxidants in the lipid phase of oil-in-water emulsions or the ability of polar antioxidant to concentrate at oil/air or oil/water interfaces in bulk oils since they are surface active molecules.

In this study, phenolic antioxidants with similar chemical structures (**Figure 1**) but different polarities were used to compare their effectiveness in bulk oil and oil-in-water emulsions. Tocopherols are considered to be a major group of natural lipid-soluble chain-breaking antioxidants. Therefore, $\alpha$-tocopherol was chosen to compare with $\delta$-tocopherol. These two tocopherol homologues are structurally similar except for their methyl groups in benzene ring, with $\alpha$-tocopherol containing three methyl groups and $\delta$-tocopherol containing only one methyl group. Thus, $\delta$-tocopherol is more polar than $\alpha$-tocopherol. The antioxidant activity of BHT (butylated hydroxytoluene) was compared with 4-hydroxymethyl-2,6-ditertiary-butylphenol. These two phenolics are structurally similar except that 4-hydroxymethyl-2,6-ditertiarybutylphenol has one more hydroxyl group than BHT making it more polar. Interfacial tension measurements were used to determine the affinities of these antioxidants toward the oil−water interfaces. The antioxidant activity of phenolic antioxidants can also be dependent on their ability to scavenge free radicals (*19*−*20*). In general, free-radical scavenging activity of phenolic antioxidants improves as the number of hydroxyl (OH) and methyl ($CH_3$) groups increases with the number of hydroxyl groups being more important (*21*−*23*). The free-radical scavenging activity of

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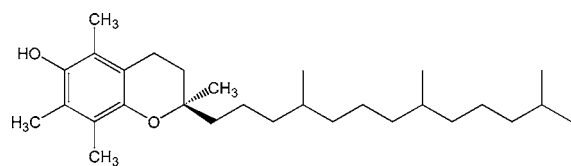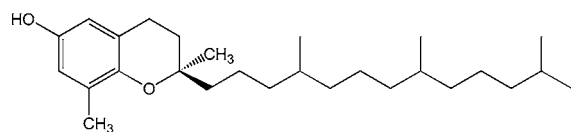
α-tocopherol



δ-tocopherol
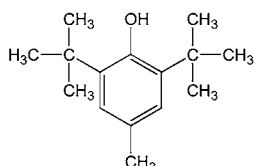


BHT (butylated hydroxytoluene)
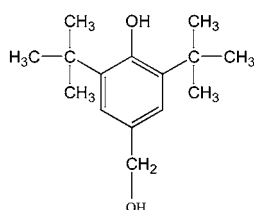


4-hydroxymethyl-2,6-ditertiarybutylphenol or
3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol

**Figure 1.** Chemical structures of phenolic antioxidants used in this study.

antioxidants in this study were measured using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) in methanol.

The objective of this research was to better understand the "antioxidant polar paradox" in bulk oil and oil-in-water emulsion systems by measuring the chemical and physical properties of α-tocopherol, δ-tocopherol, butylated hydroxytoluene (BHT), and 4-hydroxymethyl-2,6-ditertiarybutylphenol. Gaining a better understanding how the chemical and physical properties of phenolic antioxidants influence their antioxidant activity in bulk oil and oil-in-water emulsion could lead to development of new food additives to increase the use of nutritionally important polyunsaturated lipids in food industry.

## MATERIALS AND METHODS

**Materials.** Commercial menhaden oil that was unstabilized, deodorized, refined, and bleached (eicosapentaenoic acid or EPA 10−17%; docosahexaenoic acid or DHA 7−12%) was donated by Omega Protein (Reedville, VA). Menhaden oil was stored in 50-mL glass containers at −80 °C in the dark until used. Hexadecane, α-tocopherol, δ-tocopherol, butylated hydroxytoluene (BHT), polyoxyethylene 100 stearyl ether (Brij 700), sodium acetate, and imidazole were purchased from Sigma-Aldrich Co. (St. Louis, MO). 4-Hydroxymethyl-2,6-ditertiary-butylphenol or 3,5-di-*tert*-butyl-4-hydroxy-benzyl alcohol was purchased from Aldrich Chemical Co. (Milwaukee., WI). All other chemicals were reagent grade or better and were obtained from Sigma-Aldrich Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

**Methods.** *Measurement of Interfacial Tension.* Interfacial tension was determined using a digital tensiometer K 10 ST (Kruss USA, Charlotte, NC) equipped with a platinum−iridium Du Noüy ring (*24*). Phenolic antioxidants at different concentrations (0−5 mmol/kg) were dissolved in hexadecane (40 g) prior to being layered over double

distilled water (40 g) followed by equilibration at 30 °C for 24 h. Preliminary measurements showed that the interfacial tension decreased during the first 24 h and then it reached a plateau; therefore, measurements were taken after 24 h.

*Measurement of Antioxidant Partitioning Behavior.* Antioxidant partitioning behavior was determined in the hexadecane−water bilayer described above. Phenolic antioxidant concentrations in the water phase after 24 h at 30 °C were determined using the Folin−Ciocalteu method according to Eguchi et al. (*25*) with 120 $\mu$L of the 50% Folin−Ciocalteu reagent being added to 2 mL of the water phase. Absorbance at 725 nm was measured immediately after addition of Folin−Ciocalteu reagent (to correct for sample turbidity if present) and again after 60 min of incubation at room temperature in the dark. Concentrations were determined from standard curves of α-tocopherol, δ-tocopherol, BHT, and 4-hydroxymethyl-2,6-ditertiarybutylphenol.

*Measurement of Free-Radical Scavenging Activity.* The free-radical scavenging activity of the phenolic antioxidants was determined using the 2,2-diphenyl-1-picrylhydrazyl free-radical (DPPH•) method (*26−27*). Antioxidant in methanol was added to methanolic DPPH• solution to make the initial DPPH• concentration $6 \times 10^{-5}$ mol/L. Absorbance was measured at 515 nm using an Ultrospec 3000 *pro* UV/visible spectrophotometer (Biochrom Ltd., Cambridge, England) every 15 min until the reaction reached completion (e.g., no more loss of DPPH•). The exact DPPH• concentration at the completion of the reaction was determined using a DPPH• standard curve. The concentration of DPPH• after the completion of the reactions was plotted versus the molar ratios of antioxidant and DPPH•. The concentration of antioxidant that decreased the DPPH• concentration 50% was calculated from this graph and expressed as the $EC_{50}$ (*26*, *28*).

*Preparation of Bulk Oil and Oil-in-Water Emulsions for Lipid Oxidation Studies.* Samples were prepared by dissolving α-tocopherol, δ-tocopherol, BHT, and 4-hydroxymethyl-2,6-ditertiarybutylphenol (1.0 mmol/kg oil for bulk oil and 0.5 or 2.8 mmol/kg oil for oil-in-water emulsions) in menhaden oil. Oil-in-water emulsions were prepared by blending menhaden oil (5 wt %/wt) with a polyoxyethylene 100 stearyl ether solution (Brij 700; 1 wt %/wt in 5 mM acetate-imidazole buffer, pH 7.0) and then sonicating the mixture at an amplitude setting of 50%, a 0.5 s on, 0.5 s off repeating duty cycle for 4 min with a standard probe (Sonic Disembrator, Model 500, Fisher Scientific, Pittsburgh, PA), followed by readjustment of pH to 7.0. Particle size distributions (*29*) were measured using laser light scattering (Mastersizer, Malvern Instruments Ltd., Worchester, U.K.). The average droplet diameters (volume-surface mean diameter; $d_{32}$) ranged from 0.26 to 0.28 $\mu$m and did not change during the course of the experiment. Both bulk oil and oil-in-water emulsion samples (1.0 mL) were placed in 10-mL headspace vials, sealed with poly(tetrafluoroethylene) (PTFE)/butyl rubber septa using a crimper and aluminum seals, and then incubated at 37 °C in the dark. Lipid oxidation was followed by measuring lipid hydroperoxides and headspace propanal.

Lipid hydroperoxides were determined using a modified method of Shantha and Decker (*30*). In bulk menhaden oil, the sample was diluted 20−60 times with isooctane depending on the oxidation state of the oil. In menhaden oil-in-water emulsion, the sample (0.3 mL) was added to 1.5 mL of a mixture of isooctane/2-propanol (3:2, v:v), vortexed three times for 10 s each, and centrifuged for 2 min at 2000*g*. The clear upper layer was collected for lipid hydroperoxides analysis. The diluted sample from bulk oil or extracted sample from emulsions was mixed with 2.8 mL of methanol/1-butanol (2:1, v:v) and 30 $\mu$L of thiocyanate/$Fe^{2+}$ solution and then was vortexed. The thiocyanate/$Fe^{2+}$ solution was made by mixing one part 3.94 M thiocyanate solution with one part 0.072 M $Fe^{2+}$ solution (obtained from the supernatant of a mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl). After 20 min of incubation at room temperature, absorbance was measured at 510 nm using an Ultrospec 3000 *pro* UV/visible spectrophotometer (Biochrom Ltd., Cambridge, England). Lipid hydroperoxide concentrations were determined using a cumene hydroperoxide standard curve.

Headspace propanal in bulk oil and oil-in-water emulsion samples was determined using a Shimadzu GC-17A gas chromatograph (Columbia, MD) with a Hewlett-Packard 19395A headspace sampler (Avondale, PA) coupled to a computer. The chromatograms were
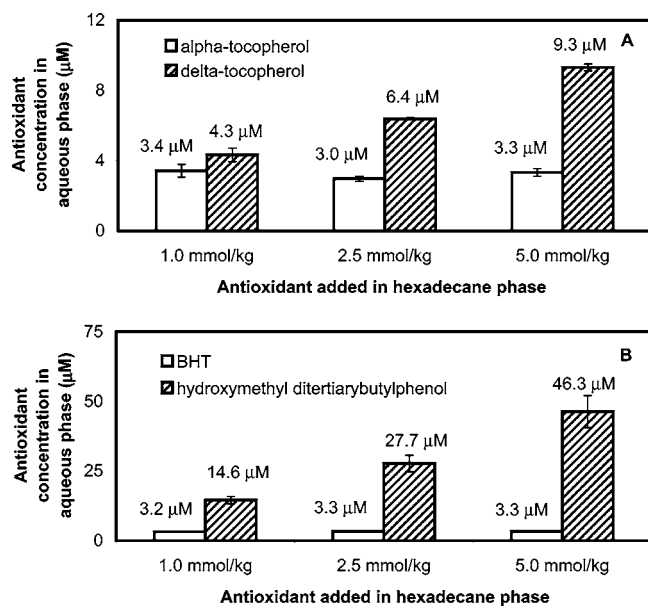
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**Figure 2.** Ability of (**A**) α-tocopherol and δ-tocopherol or (**B**) BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol to diffuse into water phase of hexadecane–water bilayer at 30 °C after 24 h of equilibration. Data points represent means ($n = 2$) ± standard deviations (some error bars may lie within the data points).



**Figure 3.** Effect of (**A**) α-tocopherol and δ-tocopherol or (**B**) BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol on interfacial tension of water–hexadecane interface at 30 °C after 24 h of equilibration. Data points represent means ($n = 2$) ± standard deviations (some error bars may lie within the data points).

integrated using Shimadzu CLASS-VP chromatography data system software. The headspace conditions were as follows: sample temperature, 40 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1 min. The aldehydes were separated isothermally at 70 °C on an HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31-mm i.d., 1.03-μm film thickness). The splitless injector temperature was 180 °C, and the flame ionization detector temperature was 200 °C. Concentrations were determined from peak area using a standard curve made from authentic propanal.

*Statistical Analysis.* Interfacial tension, phase partitioning, and free-radical scavenging experiments were done in duplicate. Lipid hydroperoxide and headspace propanal measurements were run in triplicate. Statistic analysis was performed using one-way analysis of variance (ANOVA). Mean separations were achieved using Duncan's multiple range test (*31*).

## RESULTS

**Antioxidants Partitioning Behavior.** A range of phenolic antioxidant concentrations in hexadecane (0−5 mmol antioxidant/kg hexadecane) was used to determine the ability of the antioxidants to diffuse into the water phase of a hexadecane−water bilayer at 30 °C after 24 h of equilibration. Hexadecane was used as a nonoxidizable lipid to minimize oxidation of the antioxidants. The partitioning of antioxidants into the aqueous phase of the hexadecane−water bilayer as determined by Folin−Ciocalteu method (*25*) was in order of α-tocopherol (3.0−3.4 μM) ≈ BHT (3.2−3.3 μM) < δ-tocopherol (4.3−9.3 μM) < 4-hydroxymethyl-2,6-ditertiarybutylphenol (14.6−46.3 μM) (**Figure 2**). Increasing α-tocopherol concentrations from 1 to 5.0 mmol/kg hexadecane did not significantly ($p > 0.05$) increase its aqueous phase concentration suggesting that α-tocopherol had reached its solubility limit in the water phase (**Figure 2a**). Increasing δ-tocopherol concentration in the hexadecane (1.0−5.0 mmol/kg hexadecane) significantly ($p \leq 0.05$) increased δ-tocopherol's partitioning into the water phase (4.3−9.3 μM; **Figure 2a**). As with α-tocopherol, increasing BHT concentrations from 1 to 5.0 mmol/kg hexadecane did not significantly ($p > 0.05$) increase aqueous phase BHT concentrations suggesting that the concentrations of BHT in water phase had
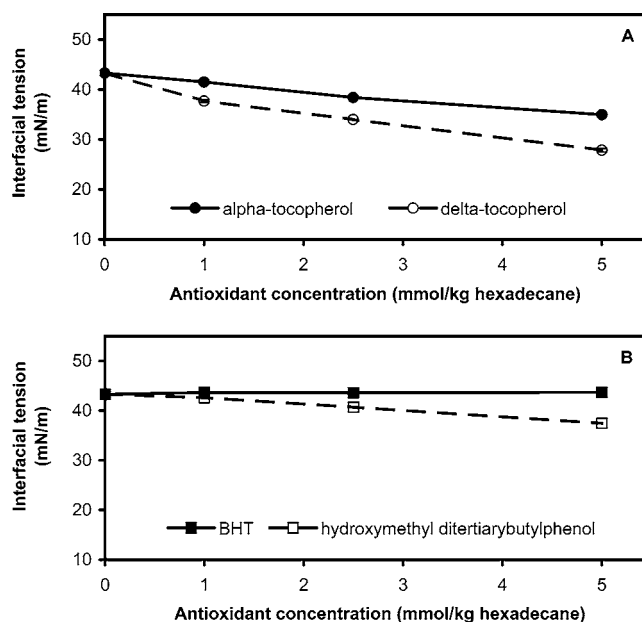
reached its solubility limit (**Figure 2b**). Increasing 4-hydroxymethyl-2,6-ditertiarybutylphenol from 0 to 5.0 mmol/kg hexadecane significantly ($p \leq 0.05$) increased its partitioning into the water phase from 14.6 to 46.3 μM (**Figure 2b**). In all samples, the amount of antioxidants partitioning into the water was very small with aqueous phase concentrations being less than 1.5% of the total antioxidant concentrations.

**Ability of Antioxidants to Alter Interfacial Tension.** The influence of phenolic antioxidant structure on surface activity was determined by measuring interfacial tension at the water−hexadecane interface using the Du Noüy ring method. Hexadecane was again used as a nonoxidizable lipid to minimize any complications that could be caused by lipid oxidation. In addition, hexadecane does not contain high amounts of potentially interfering surface active material that could be found in edible oils.

δ-Tocopherol was able to decrease the interfacial tension of the water−hexadecane bilayer more effectively than α-tocopherol at all concentrations tested ($p \leq 0.05$; **Figure 3A**). Increasing the concentration of both tocopherol homologues in the hexadecane phase (1.0−5.0 mmol tocopherol/kg hexadecane) resulted in a further decrease in interfacial tension ($p \leq 0.05$) indicating that more tocopherol molecules were concentrating at the hexane−water interface. 4-Hydroxymethyl-2,6-ditertiarybutylphenol decreased interfacial tension more than BHT at concentrations greater than 1.0 mmol/kg hexadecane ($p \leq 0.05$; **Figure 3B**). As with the tocopherol homologues, increasing 4-hydroxymethyl-2,6-ditertiarybutylphenol concentrations ($\geq 2.5$ mmol/kg hexadecane) further decreased interfacial tension. However, increasing the concentrations of BHT in hexadecane did not change interfacial tension of water−hexadecane bilayer indicating that it has very low surface activity.

**Free-Radical Scavenging Activity.** The 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) in methanol was used to determine the ability of the different antioxidants used in this study to scavenge free radicals. No differences were observed

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**Table 1.** Free-Radical Scavenging Activity of Phenolic Antioxidants as Determined by the 2,2-Diphenyl-1-picrylhydrazyl Free-Radical (DPPH•) Method

| antioxidant | $EC_{50}$ |
|---|---|
| $\alpha$-tocopherol | $0.20 \pm 0.01^a$ |
| $\delta$-tocopherol | $0.20 \pm 0.00^a$ |
| BHT | $0.31 \pm 0.04^b$ |
| 4-hydroxymethyl-2,6-di tertiarybutylphenol | $0.30 \pm 0.00^b$ |

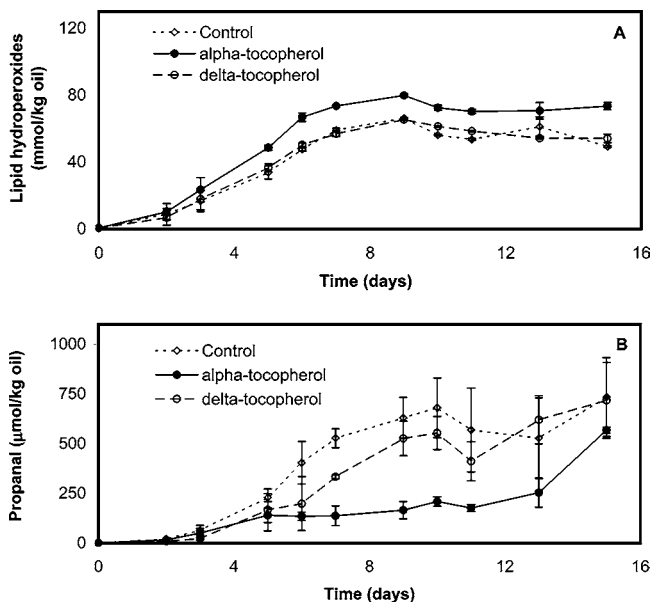$^{a,b}$Represent significantly different at ($p \leq 0.05$).



**Figure 4.** Influence of $\alpha$-tocopherol and $\delta$-tocopherol (1.0 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in bulk menhaden oil at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).
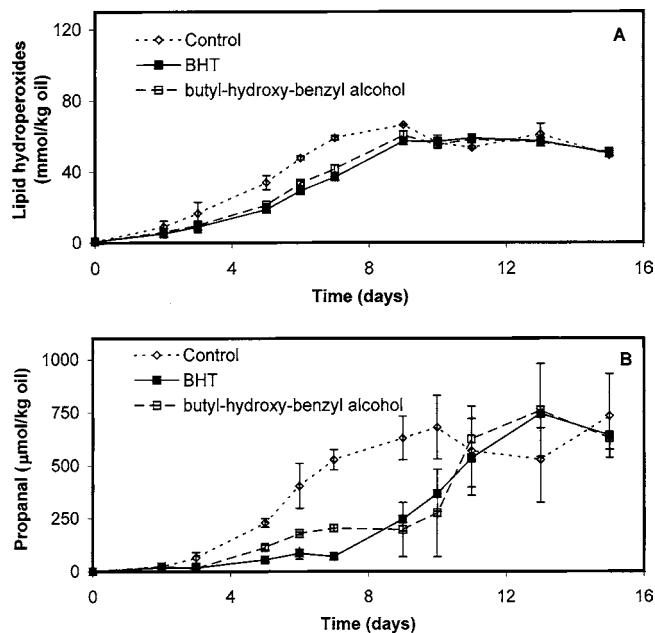


**Figure 5.** Influence of BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol (1.0 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in bulk menhaden oil at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).
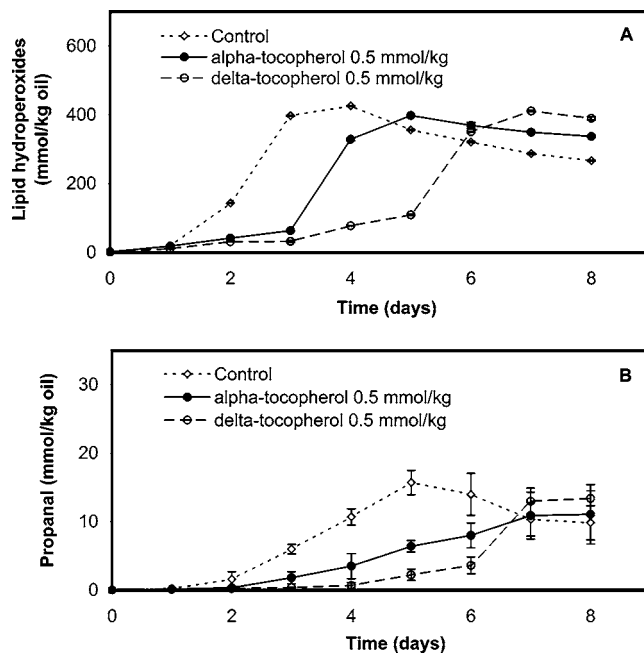


**Figure 6.** Influence of $\alpha$-tocopherol and $\delta$-tocopherol (0.5 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in menhaden oil-in-water emulsions at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

in free-radical scavenging activity between $\alpha$-tocopherol and $\delta$-tocopherol or BHT and 4-hydroxymethyl-2,6-ditertiary-butylphenol ($p > 0.05$; **Table 1**).

**Antioxidant Activity in Bulk Menhaden Oil.** To determine the impact of the polarity of lipid-soluble phenolic antioxidants on the oxidation of bulk menhaden oil, the tocopherol homologues, BHT, and 4-hydroxymethyl-2,6-ditertiarybutylphenol were added to the oil at 1.0 mmol/kg oil, and lipid hydroperoxides and propanal were measured during storage in the dark at 37 °C. Lipid hydroperoxides were observed to increase in the no antioxidant control after 2 days of storage (**Figure 4A**). No difference was observed in lipid hydroperoxide concentrations between the control and $\delta$-tocopherol ($p > 0.05$) during the entire incubation period. Lipid hydroperoxides in the $\alpha$-tocopherol-containing samples were greater than the control after 5 days of storage. In contrast to lipid hydroperoxide formation, headspace propanal formation was significantly ($p \leq 0.05$) lower than the control in the $\alpha$-tocopherol (from 5 to 10 days of incubation) and $\delta$-tocopherol (from 7 days of incubation) treatments (**Figure 4B**). Headspace propanal formation was lower in $\alpha$-tocopherol than $\delta$-tocopherol treated oil from 7 to 13 days of storage.

Both BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol were able to significantly lower ($p \leq 0.05$) the formation of lipid hydroperoxides and headspace propanal (days 5−9) compared to the control (**Figure 5A** and **B**). Little difference in lipid hydroperoxide concentrations was observed between the

BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol. BHT was more effective than 4-hydroxymethyl-2,6-ditertiarybutylphenol at decreasing headspace propanal at 5−7 days of incubation ($p \leq 0.05$).

**Antioxidant Activity in Menhaden Oil-in-Water Emulsions.** Menhaden oil-in-water emulsions were used to determine the impact of the polarity on the effectiveness of the different lipid-soluble antioxidants at concentrations of 0.5 (**Figures 6** and **8**) and 2.8 mmol/kg oil (**Figures 7** and **9**) during storage in
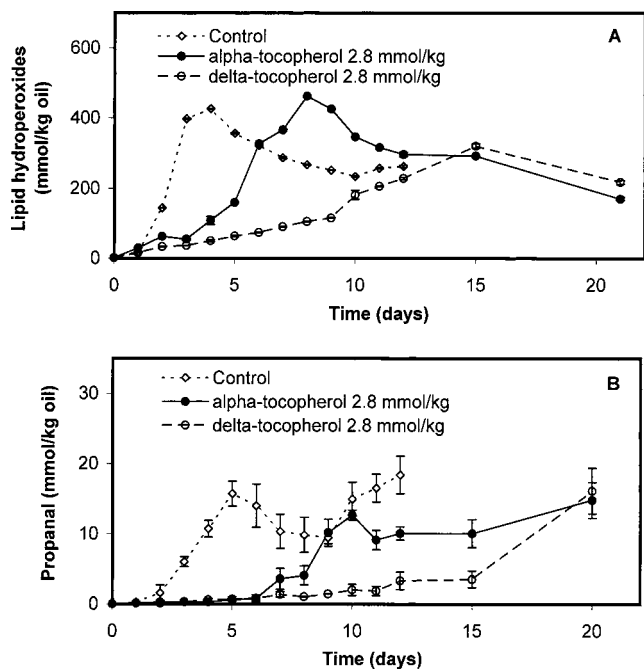
**Figure 7.** Influence of $\alpha$-tocopherol and $\delta$-tocopherol (2.8 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in menhaden oil-in-water emulsions at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).



**Figure 8.** Influence of BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol (0.5 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in menhaden oil-in-water emulsions at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).
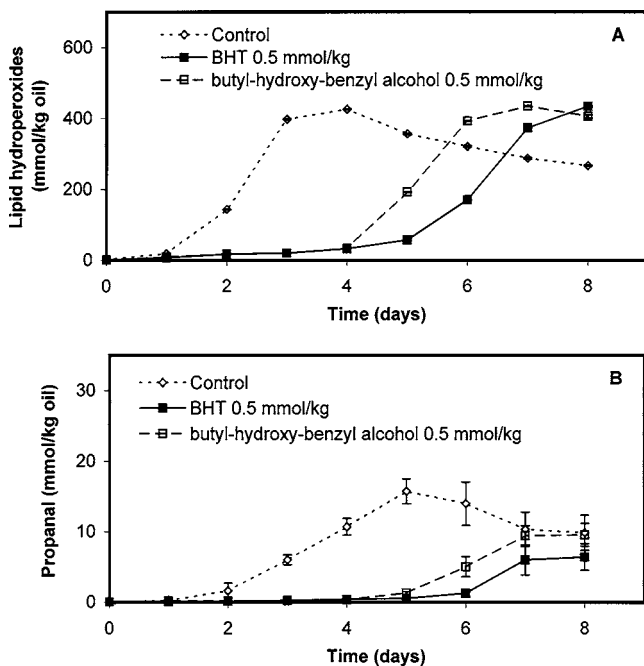
the dark at 37 °C. Lipid oxidation was again monitored by measuring lipid hydroperoxides and headspace propanal. All antioxidants were able to significantly ($p \le 0.05$) decrease the formation of both lipid hydroperoxides and headspace propanal compared to the control. At both concentrations, $\delta$-tocopherol was significantly ($p \le 0.05$) better than $\alpha$-tocopherol at decreasing the formation of both lipid oxidation markers. At
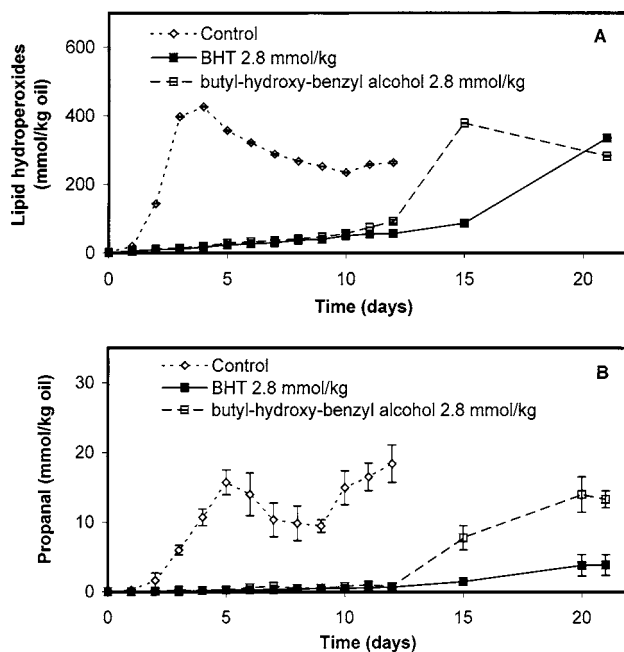


**Figure 9.** Influence of BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol (2.8 mmol/kg oil) on formation of (**A**) lipid hydroperoxides and (**B**) headspace propanal in menhaden oil-in-water emulsions at 37 °C in the dark. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

0.5 mmol/kg oil, $\delta$-tocopherol was more effective than $\alpha$-tocopherol at decreasing lipid hydroperoxides from 1 to 5 days of incubation and headspace propanal from 5 to 6 days of incubation (**Figure 6**). Increasing tocopherol concentration from 0.5 to 2.8 mmol/kg increased oxidative stability of emulsions as observed by longer induction period (**Figure 7**). $\delta$-Tocopherol was significantly ($p \le 0.05$) better than $\alpha$-tocopherol at decreasing lipid hydroperoxides from 1 to 12 days of incubation and headspace propanal from 9 to 15 days of incubation (**Figure 7**).

BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol were also tested at 0.5 and 2.8 mmol/kg oil in the menhaden oil-in-water emulsions. Both antioxidants were able to significantly ($p \le 0.05$) decrease lipid oxidation compared to the control as determined by lipid hydroperoxides and headspace propanal (**Figure 8** and **9**). Increasing the antioxidant concentrations from 0.5 to 2.8 mmol/kg increased induction period of oxidation and thus increased the oxidative stability of emulsions. At both concentrations, BHT was more effective than 4-hydroxymethyl-2,6-ditertiarybutylphenol. BHT decreased lipid hydroperoxide formation more than 4-hydroxymethyl-2,6-ditertiarybutylphenol from 5 to 7 days (0.5 mmol/kg oil) and 5 to 15 days (2.8 mmol/kg oil). Headspace propanal formation was significanty lower in emulsions containing BHT compared to 4-hydroxymethyl-2,6-ditertiarybutylphenol emulsions at 5 days (0.5 mmol/kg oil) and 15−21 days (2.8 mmol/kg).

## DISCUSSION

$\alpha$-Tocopherol and $\delta$-tocopherol have similar chemical structures with both of them having one hydroxyl group in their benzene ring. Their difference in structure is due to $\delta$-tocopherol having only one methyl group on the benzene ring making it more polar than $\alpha$-tocopherol which has three methyl groups (**Figure 1**). The higher polarity of $\delta$-tocopherol was confirmed by its higher water solubility (**Figure 2A**) and greater surface

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activity (**Figure 3A**). Even though α-tocopherol and δ-tocopherol had structural differences, this did not seem to alter their ability to scavenge free radicals with both having similar ability to inactivate the DPPH radical (**Table 1**).

BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol are also structurally similar with 4-hydroxymethyl-2,6-ditertiarybutylphenol having one more hydroxyl group than BHT (**Figure 1**), thus making 4-hydroxymethyl-2,6-ditertiarybutylphenol more polar than BHT. The higher polarity of 4-hydroxymethyl-2,6-ditertiarybutylphenol made it more water soluble (**Figure 2B**) and surface active (**Figure 3B**) than BHT. Again, the structural differences between BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol did not alter their ability to scavenge the DPPH radical. Even though 4-hydroxymethyl-2,6-ditertiarybutylphenol contains an additional hydroxyl group, it may not effectively inactivate free radicals since it is not directly connected to the benzene ring (**Table 1**).

The above results show that the antioxidants used in this study had different polarities and surface activity but similar ability to scavenge free radicals. Even though differences were observed in the water solubility of these antioxidants, they were all largely lipid soluble with only 0.1−1.5% partitioning into the aqueous phase. The "antioxidant polar paradox" is based on the hypothesis that polar antioxidants are more effective in bulk oil and nonpolar antioxidants are more effective in oil-in-water emulsions. Several theories have been presented to explain the antioxidant polar paradox in bulk oils. In bulk oils, more polar or more surface active antioxidants could have a higher affinity toward the air−oil interface or reverse micelles and thus could be able to concentrate at locations where oxidative reactions would be greatest.

In this study, we found that both δ-tocopherol and 4-hydroxymethyl-2,6-ditertiarybutylphenol were more surface active than α-tocopherol and BHT, respectively, suggesting that they should be more effective at concentrating at the oil−air interface or in reverse micelles within the oil. Even though δ-tocopherol and 4-hydroxymethyl-2,6-ditertiarybutylphenol were more surface active, they were not a more effective antioxidant than the more nonpolar α-tocopherol and BHT (**Figures 4** and **5**). Huang et al. (*6*) also found that the more nonpolar α-tocopherol was more effective than γ-tocopherol at inhibiting hexanal formation in tocopherol-stripped corn oil. Research that has supported the antioxidant polar paradox in bulk oils has generally used antioxidants with large differences in polarity (e.g., α-tocopherol versus Trolox (*15*)). It is possible that the small differences in surface activity between the antioxidants used in this study were not enough to alter their physical location and thus antioxidant activity. Surface activity would be a major factor in antioxidant activity if it allowed antioxidants to concentrate at interfaces where oxidative reactions were occurring. However, bulk oils would be expected to contain other surface active material such as free fatty acids, mono- and diacylglycerols, and lipid hydroperoxides. If the surface activity of these components is much greater than the antioxidants, the antioxidants would not be able to concentrate at the interfaces where oxidation was occurring. However, this does not explain why the nonpolar antioxidants are more effective than the polar antioxidants. Thus, other factors such as the ability of the antioxidants to interact with other endogenous antioxidants or differences in the stability and reactivity of the antioxidant radicals could be responsible for the observed differences in antioxidant activity. In addition, it is possible that oxidative reactions in bulk oils does not primarily occur at interfaces such as the oil−air interface or reverse micelles.

When the same antioxidants were evaluated in menhaden oil-in-water emulsions, the more polar δ-tocopherol was more effective at inhibiting both lipid hydroperoxide and headspace propanal formation than α-tocopherol (**Figures 6** and **7**). Wanger et al. (*32, 33*) also found that the antioxidant activity of the more polar δ-tocopherol was better than α-tocopherol in rapeseed oil triacylglycerols-in-water emulsions. The more polar γ-tocopherol was also reported to be a more effective antioxidant than α-tocopherol in corn oil-in-water emulsions as followed by lipid hydroperoxides and hexanal (*6*). Lipid oxidation in oil-in-water emulsions is thought to be accelerated by the iron-promoted decomposition of lipid hydroperoxides (*34*). Since lipid hydroperoxides are surface active, this reaction will occur at the water−lipid interface (*24*). Therefore, it is possible that the increased effectiveness of the more polar tocopherols (δ and γ) was due to their higher surface activity which would allow them to concentrate at the emulsion droplet interface where they could inactivate free radicals produced by hydroperoxide decomposition. However, we observed that the more nonpolar BHT was a more effective antioxidant than the more surface active 4-hydroxymethyl-2,6-ditertiarybutylphenol at inhibiting the formation of both lipid hydroperoxides and headspace propanal in menhaden oil-in-water emulsions (**Figures 8** and **9**). Unlike the tocopherols which are structurally different at the benzene end of the molecule with a large phytol chain at the opposite end of the molecule, BHT and 4-hydroxymethyl-2,6-ditertiarybutylphenol are structurally different at opposite ends of the molecule (**Figure 1**). Thus, the orientation of 4-hydroxymethyl-2,6-ditertiarybutylphenol at the emulsion droplet interface could be different than BHT which could influence its ability to inactivate free radicals produced by hydroperoxide decomposition.

In conclusion, although the antioxidant polar paradox has been used to help predict the type of antioxidants that are effective in bulk oils and oil-in-water emulsions, not all antioxidants behave in the manner proposed by this hypothesis. In this study, the physical and chemical properties of four structurally related lipid-soluble antioxidants were evaluated, and then the antioxidant activity of the compounds was measured in bulk oil and in oil-in-water emulsions. In the bulk oil, the more nonpolar antioxidants were more effective which disagrees with the antioxidant polar paradox. In the oil-in-water emulsions, results were mixed with the polar tocopherol being most effective and the nonpolar BHT derivative being more effective. These results indicate that antioxidant polarity is not the sole criteria that can predict antioxidant activity even when the free-radical scavenging activity of the antioxidants is similar.

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