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Relationships between Free Radical Scavenging and Antioxidant Activity in Foods

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Numerous attempts have been made to relate the free radical scavenging capacity of compounds to their antioxidant activity in foods even though antioxidant activity is dependent on both physical and chemical properties. The objective of this study was to compare the free radical scavenging activity of various compounds to their ability to inhibit lipid oxidation in foods. The order of free radical scavenging activity of polar compounds was ferulic acid > coumaric acid > propyl gallate > gallic acid > ascorbic acid as determined by a modified oxygen radical absorbance capacity, while the order of nonpolar compounds was rosmarinic acid > butylated hydroxytoluene ≥ *tert*-butylhydroquinone (TBHQ) > α-tocopherol as determined by the 2,2-diphenyl-1-picrylhydrazyl assay. Of these compounds, only propyl gallate and TBHQ were found to inhibit lipid oxidation in cooked ground beef as determiend by thiobarbituric acid reactive substances, while only propyl gallate, TBHQ, gallic acid, and rosmarinic acid inhibited lipid oxidation in an oil-in-water emulsion as determined by lipid hydroperoxides and headspace hexanal. These data indicate that the free radical scavenging assays tested have limited value in predicting the antioxidant activity in complex foods.

KEYWORDS: Lipid oxidation; antioxidants; emulsion; ground beef; ORAC; DPPH and free radical scavenging

INTRODUCTION

Lipid oxidation is a serious problem in foods because it produces rancid odors and flavors, decreases shelf life, alters texture and color, and decreases nutritional value. For example, lipid oxidation has been found to be one of the major causes of quality deterioration in processed muscle foods (*1*). Processes such as grinding disrupt the cellular integrity of muscle tissues, exposing lipids to oxidative catalysts and oxygen (*1, 2*). Thermal processing causes even more rapid acceleration of lipid oxidation of muscle foods by dislodging iron from heme proteins, disrupting cellular integrity, inactivating endogenous antioxidants, and breaking down preexisting hydroperoxides (*1*−*3*). Food emulsions are another example of a food that can rapidly degrade by lipid oxidation reactions. Lipid oxidation chemistry in oil-in-water emulsions is highly dependent on the interfacial membrane of the emulsion droplet since this is where prooxidants such as iron can interact with surface-active lipid hydroperoxides (*4*−*6*).

There have been numerous methods developed to control the rate and extent of lipid oxidation in foods, with one of the most effective being the addition of antioxidants. In brief, an antioxidant is a synthetic or natural compound that has the ability to slow lipid oxidation. Most commercial food antioxidants work by scavenging free radicals or chelating metals (*7*). Free radical scavengers, such as tocopherols, butylated hydroxytoluene (BHT), and plant phenolics, inhibit lipid oxidation by reducing peroxyl and alkoxyl radicals into stable compounds. Through these pathways, free radical scavengers can inhibit chain propagation and fatty acid scission, thus decreasing the formation of volatile fatty acid decomposition products (e.g., aldehydes and ketones) that cause rancidity (*7, 8*). In foods, the effectiveness of an antioxidant is dependent on both its chemical reactivity and its physical properties, which can determine the environment in which the antioxidant partitions (*8, 9*).

Many simplistic one-dimensional assays that use a wide range of conditions, oxidants, and methods to measure end points of oxidation have been developed to investigate the free radical scavenging or "antiradical" ability of natural and synthetic compounds. Free radical scavenging capacity assays can generally be classified into two types: hydrogen atom transfer (HAT) reactions or electron transfer (ET) assays. HAT assays, such as oxygen radical absorbance capacity (ORAC) and total radical trapping antioxidant parameter (TRAP), utilize a competitive reaction scheme where a thermal radical generator is used to produce a steady production of peroxyl radicals that in turn oxidize a probe, which is used to monitor the peroxyl radicals in the assay. When the test compound is added to these assays, it competes with the probe for the peroxyl radicals, thus inhibiting probe oxidation, thereby allowing determination of the free radical scavenging activity of the test compound. In ET assays,

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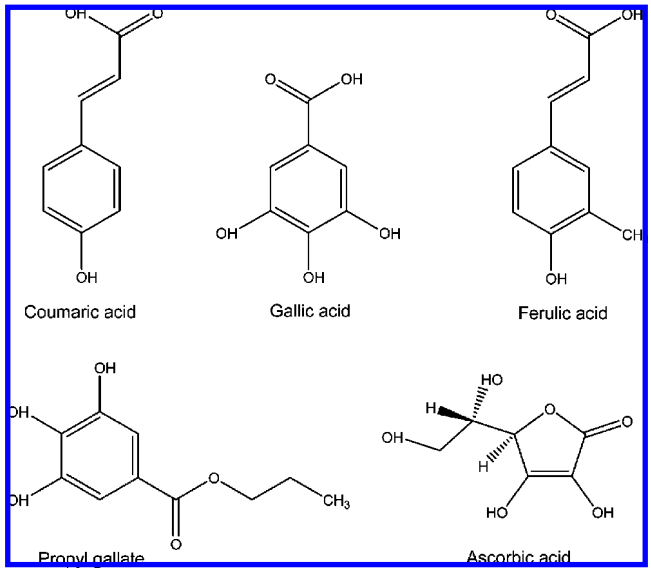

Figure 1. Structures of polar test compounds.

including the Trolox equivalence antioxidant capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assays, the colormetric probe is also a free radical. The test compound reduces the probe radicals, causing a color change that is used to determined free radical scavenging activity (*10*−*12*).

While there are many publications on the ability of HAT and ET assays to measure the free radical scavenging activity of natural and synthetic compounds, very little research has been conducted to determine if these assays can be used to predict the ability of a compound to inhibit lipid oxidation in a complex food system. Such comparisons are important because the ability of a compound to inhibit lipid oxidation in foods is thought to not only be related to its free radical scavenging activity but also its physical location (e.g., does the compound concentrate where oxidative reactions are most prevalent) and ability to participate in other oxidative pathways (e.g., metal inactivation and regeneration of endogenous food antioxidants). Therefore, the objective of this research was to utilize the ORAC and the DPPH• free radical assays to determine the free radical scavenging activity of polar and nonpolar compounds, respectively. The ability of each compound to inhibit lipid oxidation in cooked ground beef and oil-in-water emulsions was also evaluated to determine if the free radical scavenging activity of the tested compounds could be used to predict their ability to inhibit lipid oxidation in complex food systems.

MATERIALS AND METHODS

Materials. Fresh ground beef (15% fat) and corn oil were purchased from a local grocery store. Brij 35, ferulic acid, and rosmarinic acid were attained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Coumaric acid, propyl gallate, gallic acid, ascorbic acid, 2-thiobarbituric acid (TBA), ferrous sulfate, barium chloride, imidizole, ammonium thiocyanate, hexanal, ethylenediammetetraacetic acid (EDTA), BHT, fluorescein sodium salt, 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH), DPPH•, and α-tocopherol were acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO). Trichloroacetic acid (TCA) and *tert*-butylhydroquinone (TBHQ) were obtained from Acros Organics (Pittsburgh, PA). Sodium acetate, sodium phosphate dibasic and monobasic, hydrochloric acid, other reagent grade chemicals, test tubes, gas chromatography (GC) vials, seals, and septa were obtained from Fisher Scientific (Pittsburgh, PA).

Methods. *Free Radical Scavenging Assays.* The free radical scavenging activity of selected polar compounds (ascorbic acid, ferulic acid, gallic acid, propyl gallate, and coumaric acid) (**Figure 1**) was determined using a modified ORAC assay (*13*). First, a 75 mM phosphate buffer (pH 7.0) solution containing 100 μM EDTA and 300 mM AAPH was prepared and kept on ice. A separate fluorescein solution (50 nM) in 75 mM phosphate buffer (pH 7.0) was prepared immediately before each experiment. Stock solutions of the test compounds (500 μM) were prepared in the 75 mM phosphate (pH 7.0) buffer. For each experiment, 2.7 mL of the fluorescein solution was added to a capped glass test tube (13 mm × 100 mm) and held at 37 °C in a Forma Scientific 2095 water bath (Marietta, OH) for 15 min. Then, 0.1 mL of the test compound solution (final concentration of 5 μM) was added followed by 0.2 mL of the AAPH solution. Analyses were performed in a Hitachi F2000 fluorescence spectrophotometer (San Jose, CA) containing a heating/stirring unit, where the sample was kept at a controlled temperature of 37 °C, mixed at 50% speed in the dark. The excitation wavelength was 493 nm, and emission was 515 nm. Fluorescence was recorded every minute for 40 min, and the fluorescence relative to the initial time (F/F_0) was calculated from the fluorescence decay curve. Trolox was used as a reference, and data are expressed as μmol of Trolox equivalents (TE) per g of sample.

The free radical scavenging activity of the selected nonpolar compounds (BHT, TBHQ, rosmarinic acid, and α-tocopherol) (**Figure 2**) was determined using the DPPH• free radical method. Stock solutions of the test compounds (3.84 mmol/L) were prepared in methanol and were added to a methanolic DPPH• solution to make the final DPPH• concentration 0.06 mmol/L. Loss of DPPH• 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 median effective concentration of the test compound needed to decrease the DPPH• concentration by 50% was calculated and expressed as the EC_{50} (*14*, *15*).

Lipid Oxidation in Cooked Ground Beef. Raw ground beef was mixed for 2−3 min in a Hobart N-50 mixer (Troy, OH) to obtain a homogeneous sample, and then, 50 g of beef was weighed into 150 mL beakers. Test compounds (50 or 200 μL, respectively, of a 50 mM stock solution in methanol) were mixed into the ground beef by hand to achieve a final AO concentration of 0.05 or 0.2 mmol/kg muscle. The control sample contained only methanol. Next, 10 g of the raw ground beef samples was placed into test tubes (16 mm × 125 mm) and cooked in a water bath (NESlab GP-200; Thermo Fisher Scientific; Waltham, MA set at 90 °C), until an internal temperature of 77 °C was reached. The cooked beef was then immediately cooled in cold tap water and transferred to a refrigerator for 20 min. Cooled, cooked beef samples were removed from the test tubes, crumbled, and mixed by hand to obtain a homogeneous consistency. The cooked beef was placed in plastic sample bags and stored in a refrigerator (4−8 °C) in the dark and sampled periodically over 96 h of storage.

Thiobarbituric acid reactive substances (TBARS) were measured using a modified method of Srinivasan and Xiong (*16*). A buffer solution containing 50 mM dibasic sodium phosphate, 0.1% EDTA, and 0.1% propyl gallate was prepared and kept cold (4−8 °C). A 30% TCA solution and a 0.02 M TBA solution were also prepared and kept cold (4−8 °C). Cooked ground beef (2 g) was added to 16 mm × 100 mm glass test tubes, and the weights were recorded. Blanks contained 2 g of deionized water. The cold buffer solution (8 mL) was added to each cooked beef sample followed by homogenization for 20 s with a Tekmar Tissumizer (Cincinnati, OH). TCA solution (2 mL) was then added, and the tubes were capped and centrifuged at 2000*g* for 5 min in a Fisher Scientific Centrific Centrifuge 225A. A 2 mL aliquot from the upper supernatant layer was added to a glass 16 mm × 125 mm screw cap test tube and mixed with 2 mL of TBA solution. The tubes were capped and vortexed for 5 s. The samples were incubated in a boiling water bath (NESlab GP-200; Thermo Fisher Scientific) for 15 min. The samples were cooled in an ice water bath for 1 min and transferred to a refrigerator to cool for 30 min. Absorbance was measured at 533 nm using a Thermo Spectronic Genesys 20 Spectrophotometer, and TBARS were expressed as mg TBARS/kg muscle using the molar extinction coefficient of the malondialdehyde−TBA complex (1.56×10^5 M^{-1} cm^{-1}) and the weight of each sample.

Lipid Oxidation in Oil-in-Water Emulsions. An oil-in-water emulsion consisting of 5 wt % corn oil, 0.5 wt % Brij 35 (a nonionic surfactant),

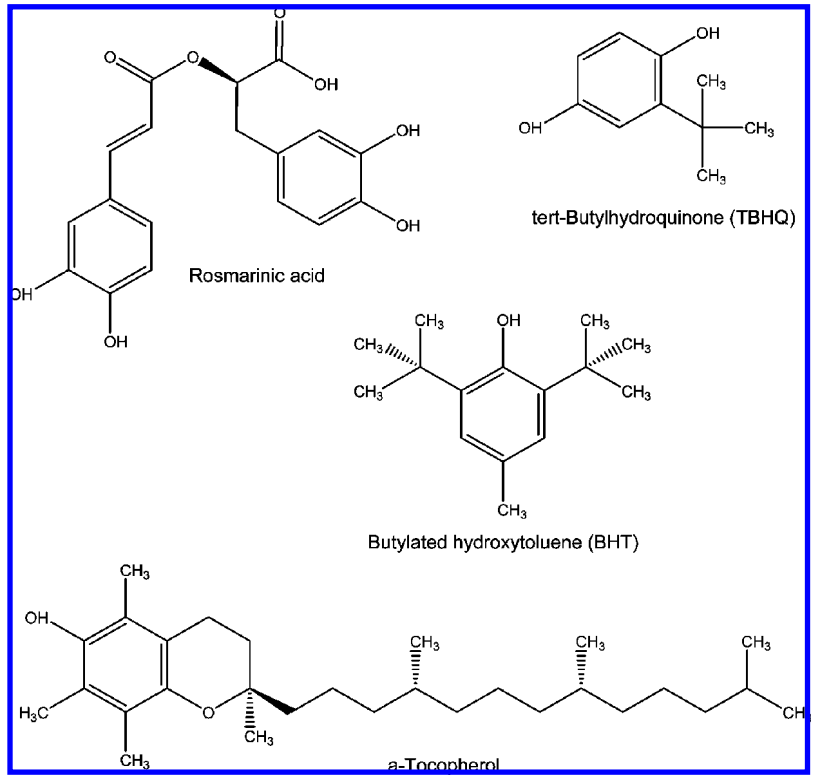

Figure 2. Structures of nonpolar test compounds.

and 5 mM sodium acetate/imidazole buffer (pH 7) was prepared by dissolving Brij 35 in the buffer and then combining the aqueous phase with the oil. A coarse emulsion was made by homogenizing the lipid and aqueous phases for 2 min using a two-speed hand-held homogenizer (Biospec Products, Inc.; Bartlesville, OK) at the highest speed setting. The coarse emulsion was then passed three times through an APV two-stage high-pressure valve homogenizer (APV Americas, Wilmington, MA) at 3000 psi. The final mean droplet diameter of the emulsion (d_{43}) was 0.38 ± 0.1 μm, as determined by laser light scattering (Mastersizer MSS; Malvern Instruments, Westborough, MA). The emulsion was separated into equal amounts, and test compounds (50 mM stock solution in methanol) were added to achieve a final antioxidant concentration of 50 μM. The control contained only methanol. One milliliter of emulsion was pipetted into 10 mL headspace vials and stored (37 °C) in the dark and sampled periodically over for 24 days of storage.

A method adapted from Nuchi et al. (*17*) was utilized to determine lipid hydroperoxides in oil-in-water emulsion. Emulsion (0.3 mL) was added to a mixture of 1.5 mL of isooctane/2-propanol (3:1 v/v) and vortexed (10 s, 3 times), and the organic solvent phase was isolated by centrifugation at 1000*g* for 2 min. The organic solvent phase (200 μL) was added to 2.8 mL of methanol/1-butanol (2:1), followed by 15 μL of 3.94 M ammonium thiocyanate and 15 μL of ferrous iron solution (prepared by adding equal amounts of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). After 20 min of incubation at room temperature, the absorbance was measured at 510 nm using an Ultrospec 3000 pro UV−vis spectrophotometer (Cambridge, England). Hydroperoxide concentrations were determined using a standard curve prepared from cumene hydroperoxide.

For headspace hexanal analysis, emulsion (1 mL) was placed into 10 mL headspace vials and sealed with poly(tetrafluoroethylene) butyl rubber septa. Headspace hexanal was determined using a Shimadzu 17A gas chromatograph equipped with a Hewlett-Packard 19395A headspace sampler (*15*). The headspace conditions were as follows: incubation time, 15 min; sample temperature, 55 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1 min; and sample run time, 9 min. The volatile headspace components were separated isothermally at 65 °C on a HP methyl silicone (DB-5) 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 250 °C. Concentrations were determined using a standard curve made from hexanal.

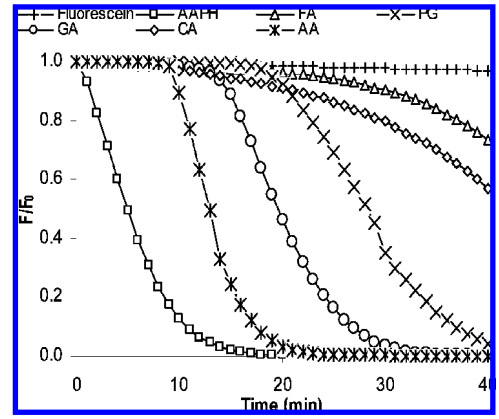

Figure 3. Changes in the relative fluorescence intensity of 45 nM fluorescein (λ_{EM}, 493 nm; λ_{EX}, 515 nm) in the presence of 20 mM AAPH and 5 μM ferulic acid (FA), propyl gallate (PG), gallic acid (GA), coumaric acid (CA), or ascorbic acid (AA) at 37 °C.

Statistics. ORAC and DPPH$^{\bullet}$ measurements were performed once on duplicate samples. TBARS, lipid hydroperoxides, and headspace hexanal measurements were performed once on triplicate samples. Differences between means were determined with the least-squares means procedure at $p < 0.05$ (*18*).

Results. *Free Radical Scavenging Capacity Assays.* The free radical scavenging capacity of polar compounds as determined by the ORAC assay was ferulic acid > coumaric acid > propyl gallate > gallic acid > ascorbic acid (**Figure 3** and **Table 1**). The ORAC value of the compounds, which is represented by area under the fluorescence decay curve (AUC), is expressed as μmol of TEs. Other researchers have also studied the free radical scavenging activity of several of these compounds using the ORAC assay. In these studies, Nenadis and others (*19*) found gallic acid to have a higher ORAC value than ascorbic acid. Gomez-Ruiz et al. (*20*) found that ferulic acid was more active than

Table 1. ORAC Values of Selected Compounds Expressed as μmol of TEs/mL[a]

test compound	$ORAC_{FL}$
ferulic acid	13.75 ± 0.23 a
coumaric acid	12.18 ± 0.15 b
propyl gallate	10.75 ± 0.16 c
gallic acid	8.22 ± 0.08 d
ascorbic acid	5.15 ± 0.11 e

[a] A higher ORAC value represents greater free radical scavenging capacity. Letters indicate a significant difference ($p \leq 0.05$) between means.

Table 2. Free Radical Scavenging Activity of Nonpolar Antioxidants as Determined by the DPPH• Assay[a]

antioxidant	EC_{50}
rosmarinic acid	0.2 ± 0.02 a
BHT	0.29 ± 0.01 b
TBHQ	0.31 ± 0.03 b
α-tocopherol	0.38 ± 0.03 c

[a] Letters indicate a significant difference ($p \leq 0.05$) between means.

coumaric acid, while Davalos et al. (*21*) found that ferulic and coumaric acids had similar radical scavenging activities.

DPPH• produces a nonpolar free radical; therefore, it was used to evaluate the free radical scavenging activity of the nonpolar compounds in methanol. The DPPH• assay results indicate that the order of free radical scavenging activity of the nonpolar compounds was rosmarinic acid > BHT ≥ TBHQ > α-tocopherol (**Table 2**). Sun and Ho (*22*) tested various compounds using the DPPH• assay and also found that BHT and TBHQ (0.1−1.0 mg/mL) had similar free radical scavenging activities. Conversely, Devi and Arumughan (*23*) found TBHQ to have a higher free radical scavenging capacity than BHT. Chen and Ho (*24*) looked at numerous compounds and determined that rosmarinic acid had a higher free radical scavenging capacity than α-tocopherol and BHT; however, they found α-tocopherol to be a more effective free radical scavenger than BHT.

Inhibition of Lipid Oxidation in Foods. The ability of the selected compounds to inhibit lipid oxidation in a complex food was tested in cooked ground beef and oil-in-water emulsions. Cooked ground beef was chosen because it is extremely susceptible to lipid oxidation and because it represents a heterogeneous food with different lipid phases (e.g., phospholipid membranes and neutral lipids). With the exception of propyl gallate, all polar antioxidants tested at 0.05 mmol/kg beef had no effect on lipid oxidation; therefore, the same group of antioxidants was investigated at a concentration of 0.2 mmol/kg beef. Propyl gallate and gallic acid were the most effective polar compounds in inhibiting TBARS formation in the cooked beef. After 72 h of storage, propyl gallate and gallic acid inhibited TBARS formation 92 and 63%, respectively. Coumaric acid and ferulic acid (0.2 mmol/kg beef) had little effect on reducing lipid oxidation as compared to the control until 72 h ($p \leq 0.05$), where they inhibited TBARS formation 13 and 12%, respectively. Ascorbic acid (0.2 mmol/kg beef) was prooxidative after 72 h ($p \leq 0.05$), increasing TBARS formation 10% as compared to the control (**Figure 4**). Propyl gallate (0.2 mmol/kg beef) has also been found to inhibit lipid oxidation in cooked restructured beef steaks (*25*) as well as cooked ground beef, lamb, and pork at a concentration of 0.2 mmol/kg meat (*26*). Ramanathan and Das (*27*) also found that ascorbic acid (0.17 and 1.14 mmol/kg meat) acts as a prooxidant in ground fish.

In cooked ground beef containing nonpolar compounds, only TBHQ was able to inhibit TBARS formation, while BHT, rosmarinic acid, and α-tocopherol were not effective when added at a concentration of 0.05 mmol/kg beef. Consequently, this group of nonpolar compounds was investigated again at a higher concentration (0.2 mmol/kg beef). TBHQ (0.2 mmol/kg beef) was the most effective nonpolar antioxidant tested, inhibiting TBARS formation 85% after 72 h of storage. BHT, rosmarinic acid, and α-tocopherol inhibited TBARS formation but less effectively than TBHQ. BHT and rosmarinic acid inhibited TBARS formation in a similar manner and were slightly more effective ($p \leq$ 0.05) than α-tocopherol (**Figure 5**). TBHQ has been found to inhibit lipid oxidation in cooked beef, lamb, and pork at concentrations of 0.2 and 1.2 mmol/kg muscle, respectively (*26*, *28*), and in cooked herring at a concentration of 1.2 mmol/kg muscle (*29*). Higher concentrations of BHT than used in this study were reported to inhibit lipid oxidation in cooked ground pork when added at concentrations of 0.14 and 0.45 (*28*) and 1.0 mmol/kg muscle in cooked ground beef (*30*). α-Tocopherol has been reported to be a weak antioxidant in cooked ground beef (*31*) and cooked ground pork (*32*).

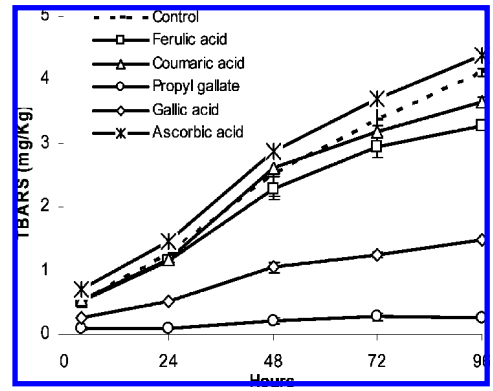

Figure 4. Formation of TBARS in cooked ground beef containing ferulic acid (FA), propyl gallate (PG), gallic acid (GA), coumaric acid (CA), or ascorbic acid (AA) (0.2 mmol/kg beef) during storage at 4 °C for 96 h. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

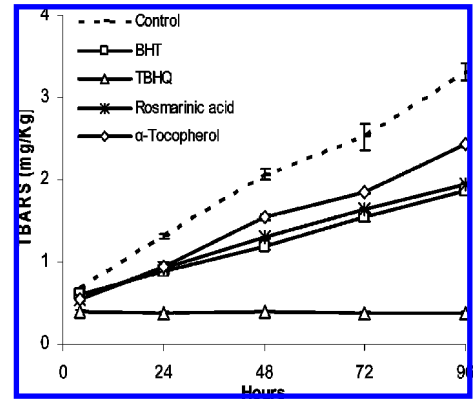

Figure 5. Formation of TBARS in cooked ground beef containing BHT, TBHQ, rosmarinic acid, or α-tocopherol (0.2 mmol/kg beef) during storage at 4 °C for 96 h. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

The ability of the selected compounds to inhibit lipid oxidation in foods was also tested in a model corn oil-in-water emulsion. Emulsion was selected because it is a heterogeneous, multiphase food system where antioxidants partition into the lipid, water, and lipid−water interface phases. Propyl gallate, gallic acid, and ferulic acid (50 μM) prolong the formation of lipid hydroperoxides and hexanal in the model emulsion (**Figure 6**). The order of effectiveness was propyl gallate > gallic acid > ferulic acid. Chang and co-workers (*33*) found that propyl gallate and gallic acid (200 μM) could inhibit lipid oxidation in stripped corn oil-in-water emulsions. Stockmann and others (*34*) found that propyl gallate but not gallic acid could inhibit lipid oxidation in a stripped corn oil-in-water emulsions oxidation at 1 μM. On the contrary, Huang and Frankel (*35*) reported that in stripped corn oil-in-water emulsions, both gallic acid and propyl gallate (5 and 20 μM) accelerated the formation of lipid hydroperoxide and hexanal. Nenadis et al. (*19*) reported that ferulic acid (150 μM) inhibited lipid hydroperoxide formation in a Tween 20 stabilized triolein oil-in-water emulsion. In our study, coumaric and ascorbic acids (50 μM) had no effect on lipid hydroperoxides but increased hexanal formation as compared to the control. Sorensen et al. (*36*) reported that coumaric acid (61 μM) was

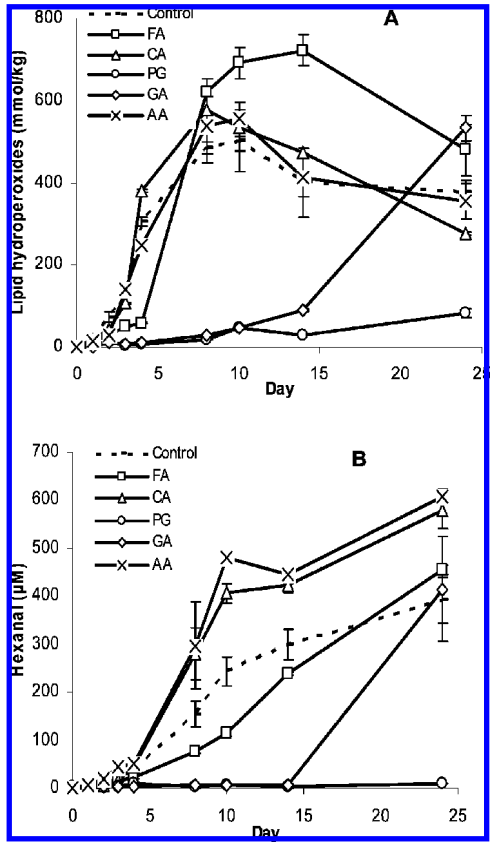

Figure 6. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in a corn oil-in-water emulsion containing 50 μM ferulic acid (FA), propyl gallate (PG), gallic acid (GA), coumaric acid (CA), or ascorbic acid (AA) during storage at 55 °C in the dark for 24 days. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

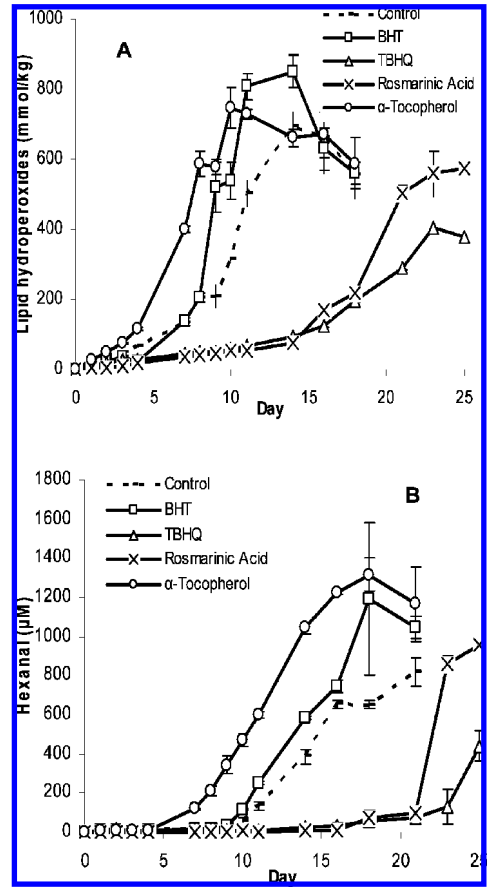

Figure 7. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in a corn oil-in-water emulsion containing 50 μM BHT, TBHQ, rosmarinic acid, or α-tocopherol during storage at 55 °C in the dark for 24 days. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

found to have no effect on hydroperoxide levels in fish oil-in-water emulsions. Mahoney and Graf (*37*) and Porter (*38*) reported that ascorbic acid (8 μM) was prooxidative with regard to the oxidation of 160 μM arachidonic acid suspended in Tris buffer.

In the model corn oil-in-water emulsions containing nonpolar compounds, TBHQ and rosmarinic acid (50 μM) were able to inhibit both lipid hydroperoxide and hexanal formation, with TBHQ being more effective than rosmarinic acid. In oil-in-water emulsions containing stripped corn oil, 200 μM TBHQ was effective at inhibiting lipid hydroperoxides (*33*). Li and others (*39*) found that 20 μM TBHQ inhibited lipid hydroperoxide formation in a stripped soybean oil-in-water emulsion. Rosmarinic acid (8 μM) was reported to exhibit slight antioxidant activity but was prooxidantive at 14 μM when the oxidation of stripped corn oil-in-water emulsions was monitored by lipid hydroperoxides and hexanal (*40*). In this study, BHT and α-tocopherol (50 μM) increased lipid hydroperoxides and hexanal formation as compared to the control (**Figure 7**). Li and others (*39*) found that BHT (200 μM) inhibited lipid hydroperoxide formation in a stripped soybean oil emulsion. Cillard and Cillard (*41*) reported that in systems containing linoleic acid dispersed with Tween 20, α-tocopherol was prooxidative at high levels (50 mM) and antioxidative at low amounts (25 μM), while Frankel and co-workers (*40*) found α-tocopherol (23 uM) to be effective at inhibiting hydroperoxide and hexanal formation in stripped corn oil-in-water emulsions.

DISCUSSION

Results from the free radical scavenging assays were not able to consistently predict which compounds were the most effective antioxidants in either cooked ground beef or corn oil-in-water emulsion. The most effective free radical scavengers were ferulic acid as determined by the ORAC (**Figure 3** and **Table 1**) and rosmarinic acid as determined by the DPPH$^{\bullet}$ assay (**Table 2**). In the cooked ground beef, ferulic was not able to inhibit lipid oxidation, and rosmarinic acid was moderately effective (**Figures 4** and **5**). Conversely, propyl gallate and TBHQ, which were found to be intermediate free radical scavengers (**Figure 3** and **Tables 1** and **2**), were effective at inhibiting lipid oxidation in the cooked ground beef (**Figures 4** and **5**). Ascorbic acid increased TBARS formation in cooked beef (**Figure 4**) even though the ORAC assay indicated that it could scavenge free radicals (**Figure 3** and **Table 1**).

In the oil-in-water emulsions, rosmarinic acid, TBHQ, gallic acid, and propyl gallate were able to decrease lipid oxidation (**Figures 6** and **7**). Of these compounds, only rosmarinic acid was a strong free radical scavenger, while the others had intermediate radical scavenging capacity. Ferulic acid (a strong free radical scavenger as determined by ORAC; **Figure 3** and **Table 1**) was less effective than gallic acid and propyl gallate, while coumaric acid, ascorbic acid, BHT, and α-tocopherol were prooxidative as they increased hydroperoxide and hexanal formation in oil-in-water emulsion (**Figures 6** and **7**).

There are several possible explanations why the ORAC and DPPH$^{\bullet}$ assays were inconsistent in predicting the ability of compounds to inhibit lipid oxidation in cooked beef and corn oil-in-water emulsions. Some compounds have the ability to inhibit lipid oxidation through mechanisms in addition to free radical scavenging. Iron is a major prooxidant in both cooked

muscle foods and oil-in-water emulsions (*1*−*3*, *42*, *43*). Some phenolic compounds are able to chelate iron, while others like ferulic acid, which do not have a galloyl moiety, do not bind iron (*44*). Lack of chelating activity could help explain why compounds like ferulic and coumaric acid, which are good free radical scavengers, did not inhibit lipid oxidation in cooked, ground beef and oil-in-water emulsions as effectively as compounds such as propyl gallate and TBHQ.

Another possible reason why free radical scavenging activity did not consistently relate to inhibition of lipid oxidation in cooked ground beef and oil-in-water emulsions could be due to the ability of some compounds to participate in redox reactions with iron resulting in the formation of ferrous ions, which are stronger prooxidants than their oxidized counterpart, ferric ions (*43*, *45*, *46*). Ascorbic acid is very effective at reducing ferric to ferrous ions (*37*, *45*), which could help explain why it promoted lipid oxidation in cooked ground beef and oil-in-water emulsions (**Figures 4** and **6**), even though it is capable of scavenging free radicals (**Figure 3**). The ability of phenolics such as gallic acid to reduce iron (*46*) could also have decreased their antioxidant activity in foods.

The effectiveness of a compound at inactivating free radicals can also be dependent on its physical location in a food (e.g., water phase, lipid droplet, membrane phospholipids, or adipose lipid). The sites at which compounds partition can dictate whether it is present at the location where free radicals are promoting oxidation (*11*). Lipid oxidation in muscle foods primarily occurs in cellular membranes (*2*, *42*, *45*), and in oil-in-water emulsions, it occurs in the lipid droplet or at the lipid−water interface (*6*). In addition, the location of antioxidants in emulsions can be influenced by surfactants (*6*). Therefore, if a compound were to preferentially partition at these locations, they might inhibit lipid oxidation more effectively. Because assays such as ORAC and DPPH$^{\bullet}$ do not contain any lipids, they are unable to determine how the physical location of a compounds influences its antioxidant activity; this could explain why the free radical scavenging activity of a compound did not relate to its ability to inhibit lipid oxidation in foods.

The ability of a compound to inhibit lipid oxidation could also be influenced by its interactions with prooxidants or other antioxidants. One example of this type of relationship is the ability of ascorbic acid to regenerate oxidized α-tocopherol to reactivate α-tocopherol in biological membranes (*38*, *47*). Another example of multiple compounds inhibiting lipid oxidation better than single compounds is when the compounds partition into different phases where they inhibit different oxidation pathways. For example, a water-soluble compound could inactivate hydroxyl radicals generated from hydrogen peroxide, while a cell membrane-soluble compound could inactivate peroxyl radicals generated from phospholipid hydroperoxides. Finally, combinations of free radical scavengers and chelators could be more effective than individual compounds since a metal chelator could decrease metal-promoted free radical generation, thus decreasing the oxidation of free radical scavengers so that they are effective for longer periods of time. Antioxidant interactions could help explain why the effectiveness of antioxidants can be very different in refined oils vs oils stripped of their antioxidants.

Finally, the ability of a compound to inhibit lipid oxidation can be concentration-dependent. Concentration-dependent reactivity can be due to the ability of compounds to participate in more than one reaction. For example, a compound that can reduce a metal to make it more prooxidative can often also donate an electron to inactivate a free radical. In situations such as this, a compound could act as a prooxidant at a low concentration where metal reduction is prevalent but be an antioxidant at high concentrations where there are sufficient electrons to inactivate numerous free radicals, including those produce by the prooxidative metals. This is the case for compounds that are strong reducing agents such as ascorbic acid (*45*). For weaker reducing agents, low concentrations may not cause significant metal reduction but can still result in free radical inactivation. However, if the concentration of the antioxidant increases, metal reduction could become significant, thus diminishing the activity of the antioxidant. Similar scenarios could also be envisioned for compounds that can inactivate free radicals and chelate metals since chelation can often increase the water solubility of a metal, making it more prooxidative. Finally, the effectiveness of some antioxidants can increase with increasing concentration. However, this only occurs up to certain concentrations where further addition of the antioxidant does not further decrease lipid oxidation. Therefore, if an antioxidant like tocopherol is added to an oil that has been stripped of its naturally occurring tocopherols, the tocopherol is found to be antioxidative, while if the same amount of tocopherol is added to unstripped oil, the tocopherol is ineffective.

While the free radical scavenging activity did not consistently relate to antioxidant activity, it is interesting to note that the antioxidant polar paradox hypothesis (e.g., nonpolar antioxidants are most effective in oil-in-water emulsions) was also not able to consistently predict the ability of a compound to inhibit lipid oxidation in the oil-in-water emulsions. For example, compounds such as BHT and α-tocopherol, which have essentially no water solubility (*48*), did not inhibit lipid oxidation in the corn oil-in-water emulsions, while gallic acid, of which 70% partitions into the aqueous phase of a 10% corn oil-in-water emulsions (*34*, *48*), was an effective antioxidant. This suggests that while hypotheses such as the antioxidant polar paradox are helpful in understanding how antioxidants behave in model food systems, it may be very difficult to develop a system that allows for the accurate prediction of a compound's antioxidant effectiveness in all foods.

CONCLUSIONS

Free radical scavenging assays such as ORAC and DPPH$^{\bullet}$ were not able to consistently predict the ability of compounds to inhibit lipid oxidation in cooked ground beef. The lack of correlation between free radical scavenging and antioxidant activity in a complex food is likely due to the multitude of factors that can impact the ability of a compound to inhibit lipid oxidation. The major drawback of the free radical scavenging assays is that they do not measure the ability of a compound to chelate metals, partition into lipids where oxidation is prevalent, or interact with other antioxidants and prooxidants (e.g., metals) in a food product. Therefore, while simple one-dimensional free radical scavenging assays can be helpful in evaluating the antioxidant mechanisms of a compound, the data from these assays should not be used to imply that compounds with high free radical scavenging capacities are good antioxidants in food systems.

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