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Lipid Peroxidation by "Free" Iron Ions and Myoglobin as Affected by Dietary Antioxidants in Simulated Gastric Fluids

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Grilled red turkey muscle (Doner Kabab) is a real "fast food" containing ~200 μM hydroperoxides, homogenized in simulated gastric fluid and oxidized more rapidly at pH 3.0 than at pH 5.0, after 180 min, producing 1200 and 600 μM hydroperoxides, respectively. The effects of "free" iron ions and metmyoglobin, two potential catalyzers of lipid peroxidation in muscle foods, were evaluated for linoleic acid peroxidation at pH 3.0 of simulated gastric fluid. The prooxidant effects of free iron ions on linoleic acid peroxidation in simulated gastric fluid was evaluated in the presence of ascorbic acid. At low concentrations of ascorbic acid, the effects were prooxidative, which was reversed at high concentrations. In the presence of metmyoglobin, ascorbic acid with or without free iron enhanced the antioxidative effect. Lipid peroxidation by an iron−ascorbic acid system was inhibited totally by 250−500 μM catechin at pH 3.0. The catechin antioxidant effect was determined also in the iron−ascorbic acid system containing metmyoglobin. In this system, catechin totally inhibited lipid peroxidation at a concentration 20-fold lower than without metmyoglobin. The ability of catechin to inhibit lipid peroxidation was also determined at a low pH with β-carotene as a sensitive target molecule for oxidation. The results show that a significant protection was achieved only with almost 100-fold higher antioxidant concentration. Polyphenols from different groups were determined for the antioxidant activity at pH 3.0. The results show a high antioxidant activity of polyphenols with orthodihydroxylated groups at the B ring, unsaturation, and the presence of a 4-oxo group in the heterocyclic ring, as demonstrated by quercetin.

KEYWORDS: Lipid peroxidation; iron ions; myoglobin; dietary antioxidants; gastric fluid; red meat; frying oil; foods

INTRODUCTION

Peroxidation in foods is one of the major degrative processes responsible for losses in food quality. In addition to the potential implication of lipid peroxidation for changes in flavor, color, and texture, the oxidation of unsaturated lipids results in a significant generation of cytotoxic and genotoxic compounds (*1*−*10*). Furthermore, the free radicals generated by the process of lipid peroxidation not only generate cytotoxic compounds but also cooxidize vitamins such as vitamin A and carotenoids, vitamin E, and vitamin C, affecting the nutritional quality of the food.

Transition metals such "free" iron or copper ions, with their labile electrons, are well-suited to catalyze redox reactions. Hemeproteins, in general, and myoglobin and hemoglobin, in particular, are implicated in lipid peroxidation of foods and in vivo biological systems (*2*, *4*). Foods contain not only endogenous catalysts but also dietary antioxidants; the overall results of oxidation and byproducts formation in the systems depend on the concentrations and activities of all of these constituents in the medium.

Most recently, we found that human gastric fluid (HGF) may be an excellent medium for enhancing the oxidation of lipids and other dietary constituents (*5*). We believe that the stomach acts as a bioreactor in which many molecules interact and that the oxidation of high fat and cholesterol-rich foods could be enhanced by endogenous metal catalysts. The overall results of these reactions, however, will depend not only on the activity of endogenous catalysts and oxidizing compounds but also on the presence of dietary antioxidants, mostly polyphenolic compounds, which could generally affect lipid peroxidation.

Several studies found that oxidized lipids in the diet are a source of plasma hydroperoxides and oxidized lipids in chylomicrons are a source of animal and human serum (*6*, *7*). Chylomicrons containing dietary oxidized lipids may be a metabolic product involved in the injury to the arterial wall and may constitute a potential link between postprandial lipaemia and atherogenesis (*8*−*10*).

The aim of this study was to better understand the interactions between free metal catalysts, myoglobin (hemeproteins), and dietary antioxidants affecting lipid peroxidation in simulated gastric fluid (SGF) at pH 3.0. We hypothesize that these reactions in the stomach would have an impact on our health.

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MATERIALS AND METHODS

Metmyoglobin (metMb, from horse skeletal muscle), soybean lipoxygenase (type I-B), β-carotene, linoleic acid, Tween 20, butylated hydroxytoluene, catechin, pepsin (A, from porcine stomach mucosa), ferrous ammonium sulfate, xylenol orange, and triphenylphosphine (TPP) were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium chloride, hydrogen peroxide (30%), and L-(+)-ascorbic acid (AA) were obtained from Merck (Darmstadt, Germany). Ferric chloride (Fe) was obtained from Riedel-de-Haen (Hannover, Germany). Sodium borohydride was from BDH (Poole, Dorset, England). Solvents were all high-performance liquid chroamtography grade (J. T. Baker, Phillipsburg, NJ). Soybean oil, red wine (Israeli Cabernet Sauvignon), and grilled turkey meat (shawarma) were bought at commercial stores in Israel. HGF was collected with permission from fasting healthy volunteers during regular gastric endoscopic tests and kept at −80 °C pending use. SGF was freshly prepared according to the U.S. Pharmacopoeia (*11*); it contained 0.2% NaCl, 0.32% pepsin, and 10 mM HCl (pH 2.0) or less for other pH values.

Hydroperoxides Measurement in Linoleic Acid Emulsions. Spectroscopic Measurement. Hydroperoxides were determined by means of the ferrous ion oxidation−xylenol orange (FOX2) method (*12*), including spectral analysis at 560 nm with a H_2O_2 standard curve. The reaction mixtures contained 2.7 mM linoleic acid, 0.14% (v/v) Tween 20, and 0.14% (v/v) ethanol as emulsifiers, in various solutions: 0.01 M acetate buffer (pH 7.0−3.0), HCl solutions (pH 3.0−1.5), SGF, and HGF. The catalysts (metMb, Fe-AA, or metMb-Fe-AA) and the antioxidants (red wine or catechin) were added to the reaction mixtures, as indicated in the Results section. Catechin was dissolved in 10% ethanol in water; thus, the ethanol concentration in the reactions was 1−2%. The reaction tubes were incubated in triplicate in a shaking bath at 37 °C for 180 min. Samples of 25 or 50 μL were taken from the tubes during the incubation and added to 475 or 950 μL, respectively, of FOX2 reagent. Before each experiment, an old stock solution of linoleic acid was mixed with fresh solution in order to maintain an accurately consistent initial level of hydroperoxides.

It had been confirmed in preliminary experiments that the presence of metMb, Fe, AA, wine, or catechin themselves and the concentrations used did not interfere with the FOX2 assay. Under the experimental conditions, the optical absorbance of the red wine pigments at 560 nm was up to 0.04 O.D.; therefore, the absorbance of control treatments in acidic methanol solution (as in the FOX2 reagent but without the xylenol orange) was deducted from the absorbance of the parallel treatments.

Kinetics Measurement on Linoleic Acid Peroxidation. The determination of rapid changes of diene concentrations due to linoleic acid oxidation by metMb was also evaluated by a rapid kinetic accessory (SFA-12, Hi-TECH Scientific, Salisbury, United Kingdom) connected to aHP8452A UV−vis daiode array spectrophotometer.

Hydroperoxides Measurement in Turkey Muscle Tissue. Grilled turkey meat, in the form of small slices, was divided into portions and kept at −80 °C pending use in the experiment. This muscle tissue was ground with three volumes of liquid for 1 min in a laboratory blender (Waring, New Hartford, CT) and adjusted to pH 3.0. The liquid was comprised of SGF with red wine or ethanol solution or water as controls. The wine was diluted with 12% ethanol solution, so that the ethanol content (final concentration 6%) was the same in all of the treatments, except for the water control. The meat−liquid mixture in each treatment was divided among several tubes and incubated in a shaking bath at 37 °C for 180 min. At five time points, the hydroperoxides in the samples were extracted in methanol under slow stirring for 15 min and filtered through no. 1442 filter paper (Whatman, England). One hundred microliter samples of the filtered solution were subjected to the FOX2 assay, which included TPP reagent controls, to prevent potential interference from ferric ions and the red wine pigments.

***β*-Carotene Measurement.** The preparation of β-carotene stock solution in water was as published previously (*5*). In brief, β-carotene (25 mg) and Tween 20 (0.9 mL) were solubilized in chloroform (25 mL). The solution (1 mL) was evaporated to dryness and solubilized by H_2O (10 mL) to a stock solution. The control reaction mixture contained 2.7 mM linoleic acid, 14 μM β-carotene, 0.18% (v/v) Tween 20, and 0.14% (v/v) ethanol, dissolved in SGF (pH 3.0, final). The peroxidation was catalyzed by the addition of metMb (20 μM), Fe^{3+} (10 μM), and AA (100 μM) with or without catechin (2.5 mM). The reaction tubes were incubated in duplicate in a shaking bath at 37 °C for 90 min. At four time points, the reaction was stopped by mixing with one volume of hexane and one volume of ethanol, and the mixture was left for another 5 min for phase separation. The β-carotene was extracted with the hexane upper phase and determined by spectral analysis at 460 nm. The extinction coefficient of β-carotene in hexane at 460 nm (for 1%, $A = 2550$) was used for calculation of the carotenoid concentration.

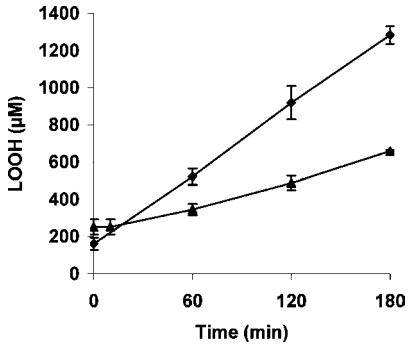


Figure 1. Lipid peroxidation of grilled red turkey muscle (Donor Kabab) as affected by pH. The pH was adjusted by SGF and HCl to pH 5.0 or 3.0 and incubated at 37 °C. The hydroperoxides were measured by the FOX 2 method. Data are means ± SD ($n = 3$). Heated muscle tissue pH 3.0 (♦); heated muscle tissue pH 5.0 (▲).

Determination of Red Wine Polyphenols. The polyphenols content of the wine was determined with Folin−Ciocalteau reagent and calculated as catechin equivalents (*13*).

Statistical Analysis. Results (means ± SD) are expressed as percentage or weight or molar basis. Statistical significance was assayed using one-way analysis of variance, followed by a ranking procedure using Student−Newman−Keuls test (SAS Software, SAS Institute Inc., Cary, NC). Results are the means of triplicates, and in the figures, each error bar (I) denotes the standard deviations.

RESULTS

Lipid peroxidation in red muscle tissue is catalyzed by an iron redox cycle formed by ferrous ions and AA and metMb (*2*). Grilled red turkey muscle (Shawarma, Doner Kabab), a real food containing ∼200 μM hydroperoxides, was ground for 1 min with three parts of SGF adjusted to pH 3.0 or 5.0 at 37 °C for 180 min. The results show a rapid lipid peroxidation at pH 3.0, which was 2.5-fold higher than at pH 5.0, and an increase after 180 min by 1000 and 400 μM hydroperoxides, respectively (**Figure 1**). The effect of free iron ions on lipid peroxidation of linoleic acid at SGF was evaluated at pH 3.0, in the presence of 200 μM AA (**Figure 2**). The increase of iron concentration enhanced lipid peroxidation. AA alone enhanced the accumulation of hydroperoxides from 100 to 400 μM, most probably because of impurities of free metal ions (**Figure 2**).

At a low concentration of AA, the effect is prooxidative, which is reversed at a high concentration. The interaction between iron ions and AA and metMb, two catalyzers of lipid peroxidation in muscle foods, is presented also in **Figure 3A**,**B**. MetMb, which catalyzed hydroperoxide decomposition, at 20 μM acts in the beginning of the incubation to decrease hydroperoxides; however, after a short antioxidative effect, its catalysis induces the propagation of linoleate peroxidation. In **Figure 3A**, iron catalyzed lipid peroxidation in the presence of AA (100 μM), and the couple worked prooxidatively. In the presence of metMb, AA with or without free iron ions enhances

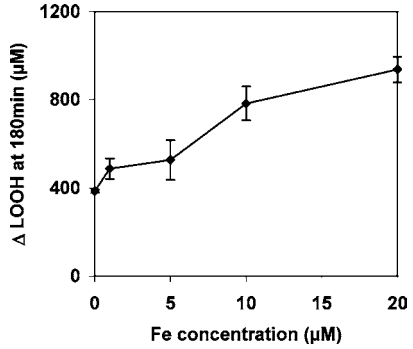


Figure 2. Effect of free iron ions on lipid peroxidation at pH 3.0. The system contained SGF at pH 3.0 (adjusted with HCl), linoleic acid (2.7 mM), linoleic hydroperoxides (100 μM), Tween 20 (0.18%), ethanol (0.14%), $FeCl_3$, and AA (200 μM). Data are means ± SD ($n = 3$). Linoleic acid peroxidation in the presence of $FeCl_3$ at different concentrations and AA (♦).

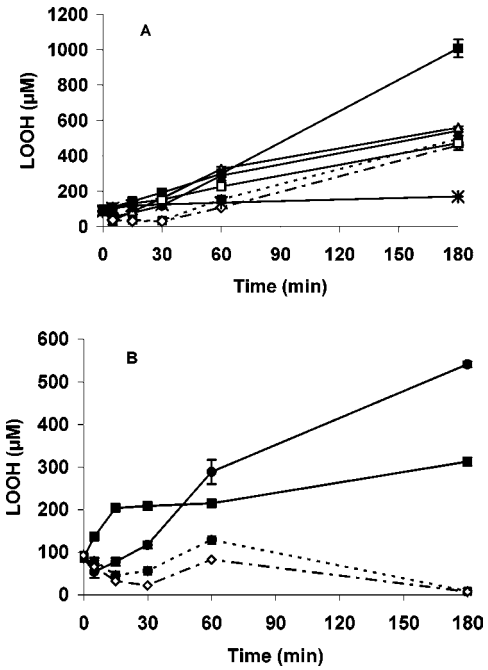


Figure 3. Effect of free iron ions on lipid peroxidation as affected by AA in the presence of metMb. The system contained SGF at pH 3.0 (adjusted with HCl), linoleic acid (2.7 mM), linoleic hydroperoxides (100 μM), Tween 20 (0.18%), ethanol (0.14%), $FeCl_3$ (10 μM), metMb (20 μM), and AA [100 μM (**A**) and 2000 μM (**B**)], incubated at 37 °C. Data are means ± SD ($n = 3$). Fe (*); AA (□); Fe + AA (■); metMb (●); Fe + metMb (Δ); AA + metMb (◇); and Fe + AA + metMb (+).

the antioxidative effect of metMb. This effect is more pronounced at a high AA concentration of 2000 μM (**Figure 3B**).

The effect of AA concentration in the presence of 10 μM free iron ions with or without metMb (20 μM) on the accumulation of hydroperoxides is demonstrated in **Figure 4**. The effect of catechin, a well-known natural antioxidant, was determined in the linoleate peroxidation system by free iron−AA, at pH 3.0 of SGF. Lipid peroxidation was inhibited totally in the iron−AA system by 250−500 μM catechin (**Figure 5A**). A catechin antioxidant effect was determined also in the system containing metMb (20 μM) and iron−AA (10−100 μM) as catalyzers. Catechin in this system inhibited totally the peroxidation at 25 μM, a 20-fold lower concentration than in the system without metMb. Moreover, in this system, 25 μM catechin helps to not only prevent propagation, but in coupling

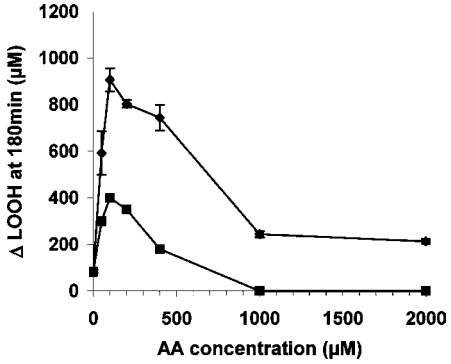


Figure 4. Effect of AA concentration on lipid peroxidation by free iron ions (10 μM) with or without metMb (20 μM) at pH 3.0. The system contained compounds as described at **Figure 3**. Fe (◆); Fe + metMb (■).

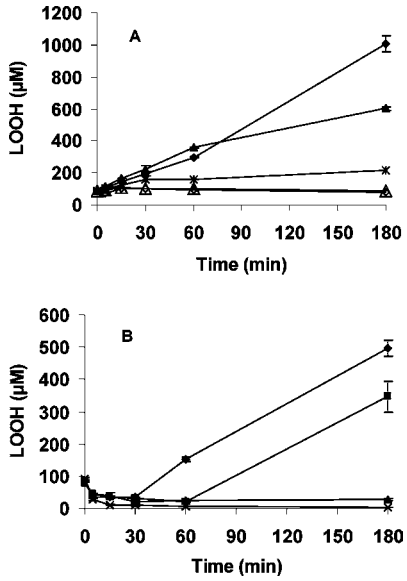


Figure 5. Inhibition of lipid peroxidation by the polyphenol catechin in a system catalyzed by free iron ions and AA with or without metMb at pH 3.0. The system contained SGF at pH 3.0 (adjusted with HCl), linoleic acid (2.7 mM), linoleic hydroperoxides (100 μM), Tween 20 (0.18%), ethanol (0.14%), iron ions ($FeCl_3$, 10 μM), AA (100 μM), and metMb (20 μM) incubated at 37 °C. Data are means ± SD ($n = 3$). (**A**) Without metMb: control no catechin (◆); catechin (10 μM) (▲); catechin (50 μM) (*); catechin (250 μM) (○); and catechin (500 μM) (Δ). (**B**) With metMb: control no catechin (◆); catechin (10 μM) (■); catechin (25 μM) (▲); and catechin (50 μM) (*).

with metMb, they work to break down the hydroperoxides to zero (**Figure 5B**). As determination of the hydroperoxide concentration is not a very sensitive method to determine the free radical scavenging properties of an antioxidant, we have also determined that the ability of catechin to inhibit lipid peroxidation, at low pH, was also determined with β-carotene as a sensitive target molecule for oxidation. The results show that a significant protection (when the concentration of the hydroperoxides was 13 μM) was achieved only by a high concentration of red wine polyphenols (2.5 mM catechin equivalent) (**Figure 6**).

The antioxidation by polyphenols at pH 3.0 is affected not only by the presence of catalyzers such as free iron and metMb but also by the concentration of the hydroperoxides (**Figure 7A**,**B**). Red wine polyphenols at a concentration of 1.0 and 2.5 mM inhibit β-carotene oxidation much better than catechin at the same concentrations.

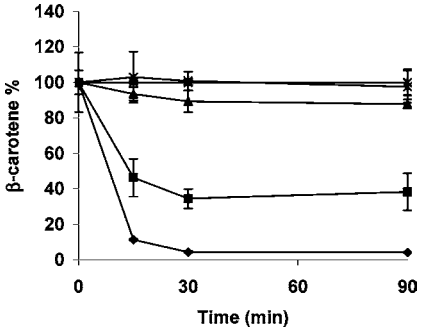


Figure 6. Inhibition of β-carotene cooxidation by red wine polyphenols. The system contains SGF at pH 3.0 (adjusted with HCl), linoleic acid (2.7 mM), linoleic hydroperoxides (13 μM), Tween 20 (0.18%), ethanol (0.14%), iron ions ($FeCl_3$, 10 μM), AA (100 μM), and metMb (20 μM). Data are means ± SD ($n = 3$). Control, only β-carotene + linoleic acid (□); all the system without wine (♦); red wine (500 μM equivalent catechin polyphenols) (■); red wine (1 mM) (▲); and red wine (2.5 mM) (*).

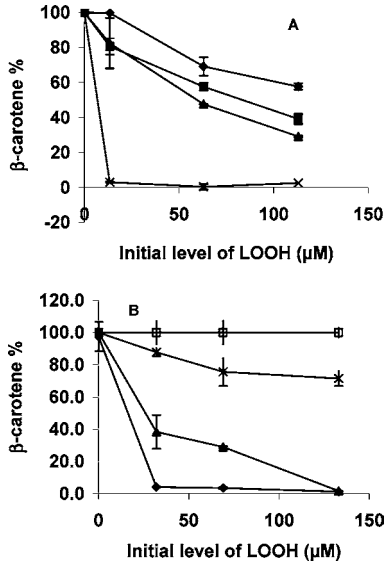


Figure 7. Inhibition of β-carotene cooxidation by polyphenols in a system of lipid peroxidation catalyzed by iron–AA and metMb as affected by hydroperoxide concentrations at pH 3.0. The system contains SGF at pH 3.0 (adjusted with HCl), linoleic acid (2.7 mM), linoleic hydroperoxides (between 13 and 140 μM), Tween 20 (0.18%), ethanol (0.14%), metMb (20 μM), AA (100 μM), and iron ions (10 μM). Data are means ± SD ($n = 3$). (**A**) Catechin: control, no catechin (×); catechin (0.5 mM) (▲); catechin (1 mM) (■); and catechin (2.5 mM) (◆). (**B**) Red wine (polyphenols as equivalent of catechin): control, no red wine (◆); 0.5 mM (△); 1 mM (×); and 2.5 mM (□).

The activity of various groups of polyphenols as antioxidants was determined at pH 3.0 in a linoleic acid (2.7 mM) system containing 100 μM hydroperoxides and in the presence of metMb (10 μM) and antioxidants (100 μM). The antioxidant index was calculated from the equation Δ[LOOH] catechin / Δ[LOOH] tested compound = antioxidant units (AU), after reaction for 10 and 180 min (**Tables 1−3**). Catechin, an abundant compound in red wine, was chosen to be the reference, with an antioxidant index of 1.

Phenolic compounds from different groups containing hydroxyls on ring B at positions 3′ and 4′ were tested. The results show that quercetin (flavonol), luteolin (flavone), and eriodictol (flavanone) were the most active compounds. The flavonol structure seems to be very important for the antioxidant activity at low pH (**Table 2**).

The anthocyanidin, malvidin, was found to act better than the anthocyanin, malvidin-3-glucoside, which is the main color compound in red wine. The red wine polyphenols antioxidant index activity (100 μM catechin equivalent) resembled this of catechin and acted better than the white wine polyphenols at the same concentrations.

DISCUSSION

Muscle foods contain large amounts of endogenous catalysts, such as free iron ions (5−20 μM) and myoglobin (100−250 μM), which accelerate lipid peroxidation (*2*, *14*−*16*). Myoglobin can act prooxidatively or antioxidatively, depending on the concentration of H_2O_2, hydroperoxides, and reducing agents and their compartmentalization in muscle cells (*17*−*19*). Most recently, we hypothesized that the stomach works as a bioreactor in which many chemicals interact and that the oxidation of high fat and cholesterol-rich foods could be enhanced by endogenous metal catalysts from these foods. We demonstrated that the HGF is an excellent medium for enhancing the oxidation of lipids and other dietary constituents (*5*). In this study, we show indeed that lipid peroxidation of a real fast food (turkey Doner Kebab, shawarma) is significantly more rapidly oxidized in SGF at pH 3.0 than at pH 5.0. As lipid peroxidation in muscle tissues is catalyzed by free iron ions and redox cycled and affected by myoglobin (*1*, *15*, *18*, *20*), we have determined the effect of SGF at low pH on these reactions.

Linoleate peroxidation at low pH is affected by the concentration of free iron and AA very similar with what is known for neutral pH. In the presence of free iron ions, AA at a low concentration acts prooxidatively and at high concentrations, it acts antioxidatively (*1*, *21*). The antioxidative effect in the presence of a high concentration of AA is enhanced by metMb, which seems to act in this system as a pseudo-peroxidase (*19*, *20*), using AA as the electron donor. The ferrous ions break down the hydroperoxides in the linoleate system to free radicals by the following reactions:

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\bullet} + HO^{-} \quad (1)$$

$$Fe^{3+} + A(OH)_2 \rightarrow Fe^{2+} + A(OH)O^{\bullet} \quad (2)$$

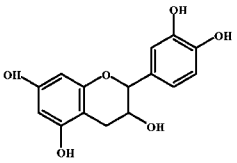
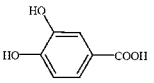
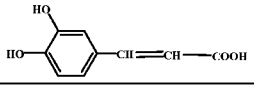
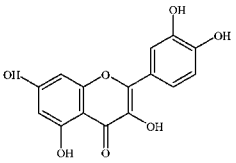
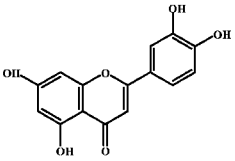
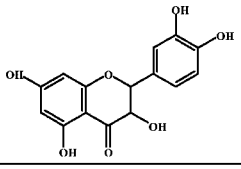
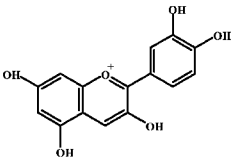
where LOOH is linoleate hydroperoxide, $A(OH)_2$ is AA, and $A(OH)O^{\bullet}$ is ascorbyl radical.

The ferric ion generated is reduced by AA to ferrous form by reaction 2. This reaction seems to be affected by pH. It might be expected that the pH dependence of iron reduction by AA is closely related to the acid dissociation of the hydroxyls, since the dissociated form at a high pH has a stronger electron-donating property than the undissociated form (*22*, *23*).

Nevertheless, lipid peroxidation of the linoleic acid containing hydroperoxides (100 μM) by the iron−AA system was more rapid at pH 3.0 than at pH 5.0. This is most probably because the reduction of iron is not the limiting rate factor (*5*).

In general, via the metal redox cycle, a low AA concentration is prone to work prooxidatively and at high concentrations, and it will tend to be an antioxidant, via scavenging of lipid free radicals (*23*). Lipid peroxidation by the iron redox cycle at low pH was inhibited by catechin, a well-known dietary polyphenol present in many edible plant foods. Catechin significantly inhibits lipid peroxidation by the iron redox cycle but only at high concentrations. Lipid peroxidation by the iron redox cycle at low pH contained metMb (20 μM) and was inhibited by a 20-fold lower concentration of catechin. In these reactions,

Table 1. Antioxidant Activity at pH 3.0 of Various Polyphenols with an Orthodiphenol Structure at the B Ring

Group	Compound		Antioxidant index at 10min	Antioxidant index at 180min
Flavanols	Catechin		1.0	1.0
Benzoic acids	Protocatechuic acid		1.2	0.1
Cinnamic acids	Caffeic acid		1.7	2.5
Flavonols	Quercetin		7.0	5.5
Flavones	Luteolin		4.2	6.1
Flavanones	Eriodictol		1.6	5.7
Anthocyanins	Cyanidin		1.6	1.1

catechin acts mostly to reduce oxidized metMb, keeping metMb to work as an efficient hydroperoxidase and as a free radical scavenger.

Ferrous ions and metMb break down hydroperoxides to lipid free radicals. These free radicals could propagate linoleic acid peroxidation or cooxidize other molecules. During these reactions, it is very important to determine the potential of the free radicals to damage other molecules. Determination of hydroperoxide accumulation could be done in the same reaction not to evaluate the potential damage to other molecules by free radicals. This is because in the same reactions the ratio between the breakdown and the accumulation of hydroperoxides could remain almost the same. β-Carotene cooxidation could provide such a sensitive target for oxidation, which will better identify the potential damage affected by free radicals.

β-Carotene at a concentration of 14 μM was incubated in the presence of linoleic acid and hydroperoxides at different concentrations, and the reaction was catalyzed by free iron and metMb (**Figures 6** and **7**). The results show that the inhibition of β-carotene cooxidation was achieved with a relatively very high concentration of catechin or red wine polyphenols, almost 100-fold higher than that concentration, which prevents accumulation of lipid hydroperoxides (**Figure 7**).

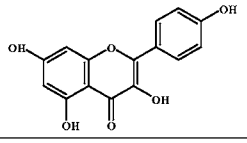
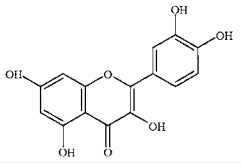
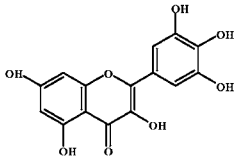
These results were achieved most probably because of the high hydrophobicity of β-carotene and its high potential to donate electrons from the double bonds. The results emphasize the importance of determining antioxidation by several methods and especially to use a method, which simulates the potential of the antioxidant to prevent the damage affected by free radicals. The inhibition of β-carotene oxidation was also dependent on the initial hydroperoxide concentration. Increasing the initial hydroperoxide concentration in the system decreased the potential of antioxidants to inhibit the oxidation. It was found that red wine antioxidants containing a large spectra of polyphenols act better to protect β-carotene oxidation than catechin alone. The high antioxidant activity of the polyphenols in red wine could be attributed to the synergistic effects of a mixture of polyphenol compounds to work as antioxidants (*24*). Several very active antioxidants may preferentially interact with the lipid free radicals. The resulting oxidized phenolics ($PhO^{\bullet}$) are reduced and possibly regenerated by less active phenolics (ArOH) by the following reactions:

$$ROO^{\bullet} + PhOH \rightarrow ROOH + PhO^{\bullet} \quad (3)$$

$$PhO^{\bullet} + ArOH \rightarrow PhOH + ArO^{\bullet} \quad (4)$$

Table 2. Antioxidant Activity at pH 3.0 of Various Polyphenols with Different Amounts of Hydroxyls at the B Ring

A. Flavonols

Compound	OH		Antioxidant index at 10min	Antioxidant index at 180min
Kaempferol	1		3.5	6.3
Quercetin	2		7.0	5.5
Myricetin	3		4.9	6.2

B. Flavones

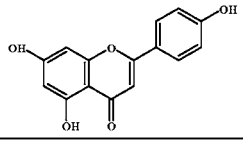
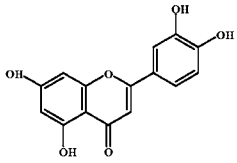
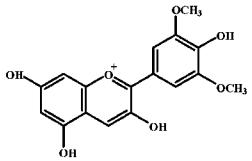
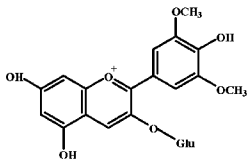
Apigenin	1		0.7	0.03 *
Luteolin	2		4.2	6.1

Table 3. Antioxidant Activity at pH 3.0 of an Anthocyanidin, Anthocyanin, Red Wine, and White Wine Polyphenols

Group	Compound		Antioxidant index at 10min	Antioxidant index at 180min
Anthocyanidins	Malvidin		2.8	1.7
Anthocyanins	Malvidin-3-g		1.8	0.1
Red wine	Polyphenols		1.6	0.9
White wine	Polyphenols		0.9	0.7

Surprisingly, it has been found that reaction 4 is almost 300 times more reactive than reaction 3. The phenoxyl radical is roughly 300 times as reactive as a peroxyl radical in abstracting phenolic hydrogen atoms (*25*). These results could explain the synergistic effects of the polyphenols from the red wine.

Flavonoids have been shown to be very potent antioxidants by many researchers (*26*−*30*), but most of these studies were performed at neutral pH. Our results showed that the reactivity of the polyphenols is dependent on the structure and the hydroxyls groups, which are allocated around the heterocyclic and aromatic B ring. On the basis of these results, we found that polyphenols such as flavonones (eriodictyol), flavone (luteolin), and flavonol (quercetin) containing an orthodihydroxyl structure are highly active against linoleic acid peroxidation by metMb at low pH. The double bond between carbons 2 and 3 in flavone and flavonol increases the antioxidative activity. Our results on the reactivity of polyphenolics at low pH resemble these at neutral pH for the ortodihydroxyl and the 2−3 double bond structures (*28*−*30*) but not for the amount of the hydroxyls around the B ring. Kaempferol (3′-OH) was found to work very similarly with myricetin (3′,4′,5′-OH). We do not have an explanation for these results. Low pH decreases the rate of hydrogen displacement from polyphenolic compounds and therefore decreases their antioxidant effects (*22*). Most recently, decreasing the pH was found to have lower effects on the antioxidant activity of procyanidins than catechin (*31*).

In conclusion, the low pH of the gastric fluid medium increased lipid peroxidation of red meat. Free iron ions catalyzed lipid peroxidation at low pH. This effect is enhanced by an electron donor molecule such as AA, and this reaction is efficient at the low pH of the gastric fluid medium. MetMb and other endogenous food catalyzers could work at low concentrations to accelerate lipid peroxidation but at high concentrations to inhibit lipid peroxidation. This effect is supported especially in the presence of electron donor molecules such as AA, catechin, or other polyphenols. The marker molecule to be used for identification of oxidation or antioxidation in biological systems is very important, to determine the potential injury from free radicals. It was found that β-carotene, a molecule containing 11 double bonds, which needs an input of very low energy for oxidation, could be a very sensitive marker for evaluation of oxidation in biological systems.

It seems that cooked red meat and heated frying oils are prone to more lipid peroxidation in the gastric medium. Lipid peroxidation in gastric medium could be very efficiently inhibited by the antioxidant network of reducing compounds found in foods such as AA, glutathione, and especially polyphenols in mixtures. They most probably work in the stomach during the meal as the antioxidant network provided by vegetables, fruits, and beverages such as red wine, tea, cacao, or coffee.

LITERATURE CITED

(1) Kanner, J.; German, J. B.; Kinsella, J. E. Initiation of lipid oxidation in biological systems. *CRC Crit. Rev. Food Sci. Nutr.* **1987**, *25*, 317−362.

(2) Kanner, J. Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **1994**, *36*, 169−189.

(3) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, 1998; pp 187−225.

(4) Baron, C. P.; Andersen, H. J. Myoglobin induced lipid oxidation. A review. *J. Agric. Food Chem.* **2002**, *50*, 3887−3897.

(5) Kanner, J.; Lapidot, T. The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biol. Med.* **2001**, *31*, 1388−1395.

(6) Staprans, I.; Hardman, D. A.; Pan, X. M.; Feingold, K. R. Effect of oxidized lipids in the diet on oxidized lipid levels in postprandial serum chylomicrons of diabetic patients. *Diabetes Care* **1999**, *22*, 300−306.

(7) Ursini, F.; Zamburlini, A.; Cazzolato, G.; Maiorino, M.; Bon, G. B.; Sevanian, A. Postprandial plasma lipid hydroperoxides: a possible link between diet and atherosclerosis. *Free Radical Biol. Med.* **1998**, *25*, 250−252.

(8) Proctor, S. D.; Pabla, C. K.; Mamo, J. C. Arterial intimal retention of pro-atherogenic lipoproteins in insulin deficient rabbits and rats. *Atherosclerosis* **2000**, *149*, 315−322.

(9) Williams, M. J.; Sutherland, W. H.; McCormick, M. P.; de Jong, S. A.; Walker, R. J.; Wilkins, G. T. Impaired endothelial function following a meal rich in used cooking fat. *J. Am. Coll. Cardiol.* **1999**, *33*, 1050−1055.

(10) Cohn, J. C. Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr. Opin. Lipidol.* **2002**, *13*, 19−24.

(11) The United States Pharmacopoeia, Inc., Rockville, MD, 2000.

(12) Nourooz-Zadeh, J. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxides in plasma. *Methods Enzymol.* **1999**, *300*, 58−62.

(13) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic and phosphotungestic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−148

(14) Decker, E. A.; Hultin, H. O. Lipid oxidation in muscle foods via redox iron. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 33−54.

(15) Monahan, F. J.; Crackel, R. L.; Gray, J. I.; Buckley, D. J.; Morrissey, P. A. Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. *Meat Sci.* **1993**, *34*, 95−106.

(16) Kanner, J.; Harel, S. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. *Arch. Biochem. Biophys.* **1985**, *237*, 314−321.

(17) Kanner, J.; Salan, M. I.; Harel, S.; Shegalovitch, I. Lipid peroxidation of muscle food, the role of the cytosolic fraction. *J. Agric. Food Chem.* **1991**, *39*, 242−246.

(18) Gorelik, S.; Kanner, J. Oxymyoglobin oxidation and membranal lipid peroxidation initiated by iron redox cycle. *J. Agric. Food Chem.* **2001**, *49*, 5939−5944.

(19) Kanner, J. Mechanism of nonenzymic lipid peroxidation in muscle foods. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 55−73.

(20) Harel, S.; Kanner, J. Haemoglobin and myoglobin as inhibitors of hydroxyl radical generation in a model system of "iron redox" cycle. *Free Radical Res. Commun.* **1989**, *6*, 1−10.

(21) Kumamoto, M.; Sonda, T.; Nagayama, K.; Tabata, M. Effects of pH and metal ions on antioxidative activities of catechins. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 126−132.

(22) Lemanska, K.; Szymusiak, H.; Tyrankowska, B.; Zielinski, R.; Soffers, A. E. M. F.; Rietjens, I. M. C. M. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biol. Med.* **2001**, *31*, 869−881.

(23) Kanner, J.; Mendel, H.; Budowski, P. Prooxidant and antioxidant effects of ascorbic acid and metal salts in a beta-carotene-linoleate model system. *J. Food Sci.* **1977**, *42*, 60−64.

(24) Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grape and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.

(25) Foti, M.; Ingold, K. V.; Lusztyk, J. The surprisingly high reactivity of phenoxyl radicals. *J. Am. Chem. Soc.* **1994**, *116*, 9440−9447.

(26) Lapidot, T.; Harel, S.; Granit, R.; Kanner, J. Bioavailability of red wine anthocyanins as detected in human urine. *J. Agric. Food Chem.* **1998**, *46*, 4297−4302.
(27) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.
(28) Van Acker, S. A. B. E.; Van den Berg, D. J.; Tromp, M. N. J. L.; Griffioen, D. H.; Van Bennekom, W. P.; Van der Vijgh, W. J. F.; Bast, A. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biol. Med.* **1996**, *20*, 331−342.
(29) Bors, W.; Heller, W.; Saran, M. C. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343−355.
(30) Rice-Evans, C. A.; Miller, N. J. Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packers, L., Eds.; Marcel Dekker: New York, 1998; pp 199−219.
(31) Ursini, F.; Rapuzzi, I.; Toniolo, R.; Tubaro, F.; Bontempelli, G. Characterization of the antioxidant effect of procyanidins. *Methods Enzymol.* **2001**, *335*, 338−350.

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