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Antioxidant Activity of Vegetables and Their Blends in Iron-Catalyzed Model Systems

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Abstract

Vegetables are known to contain high amounts of natural antioxidants such as ascorbate, α-tocopherol, β-carotene, and flavinoids. The antioxidant activities of several vegetables including broccoli, carrot, green pepper, spinach and tomato, and their blends were investigated using various iron-catalyzed lipid peroxidation systems. In linoleic acid micelles, carrot and spinach significantly inhibited lipid peroxidation by 29.0% and 35.8%, respectively ($p<0.05$). Blends of two, three, or four vegetables including spinach increased the inhibitory effect on lipid peroxidation, mainly due to high level of antioxidants in spinach. In beef homogenates, tomato significantly inhibited lipid peroxidation by 19.9% ($p<0.05$), whereas spinach and broccoli significantly stimulated lipid peroxidation by 67.3% and 11.5%, respectively ($p<0.05$). In the presence of 100 μM ferrous ions, all vegetables inhibited degradation of deoxyribose by 43.6~77.6% ($p<0.05$). In the presence of 100 μM ferric ions, broccoli and spinach stimulated deoxyribose degradation by 39.8% and 55.8%, respectively. These results indicate that the antioxidant activity of vegetables varied with the different model systems and depended on the provided environment such as iron content and substrates. The activity of the various combinations (blends) of vegetables was strongly related to that of the individual vegetable.

Key words: linoleic acid micelle, beef homogenate, vegetables, lipid peroxidation

INTRODUCTION

Lipid peroxidation has received much attention in food science, nutrition, and clinical medicine because its products are related to food deterioration, cytotoxicity, and a number of pathological reactions involved in the etiology of degenerative diseases (1). Lipid peroxidation plays a major role in deterioration of restructured and precooked food products (2,3). The peroxidative deterioration of lipid produces toxic compounds, and changes the flavor, color, texture, and nutritive value of foods (4). Oxidation of muscle lipids involves the peroxidation of polyunsaturated fatty acids which are present in the cell membranes of muscle foods (5).

Dried and precooked meat products offer convenience and extended shelf life at room temperature. However, meats are iron rich and also provide copious lipid substrates for lipid peroxidation. Transition metals such as Fe (II) and Cu (II), and heme compounds in muscle foods initiate lipid peroxidation by producing reactive oxygen species and also enhance lipid peroxidation by decomposing the preformed lipid peroxides (6-9). To counteract these effects, meat products have been manufactured by grinding and mixing with vegetables such as potato, tomato, carrot, green pepper, or onion. The resulting products contain additional nutrients such as fiber, carotenoids, flavonoids, and vitamin C that meat products lack. These nutrients in vegetables may also act as antioxidants, thereby preventing the warmed-over flavor of meat products and extending shelf-life.

Natural antioxidants in numerous vegetables such as broccoli, carrot, green pepper, spinach, and tomato commonly include ascorbic acid, α-tocopherol, β-carotene, and flavonoids. The natural antioxidants may function as a reducing agent (ascorbic acid), oxygen radical scavenger (α-tocopherol and flavonoids), quencher of singlet oxygen (β-carotene and lycopene), and chelator of metals (phytic acid) (10-13). Ascorbate, a prooxidant, also produces free radicals by reducing transition metals or molecular oxygen (14).

The purpose of this study was to investigate the antioxidant activities of vegetables and their blends on lipid peroxidation using linoleic acid micelles as a pure model system and beef homogenates as a complex model system. In addition, the hydroxyl radical scavenging activities of vegetables were examined using a deoxyribose model system.

MATERIALS AND METHODS

Materials and reagents

Linoleic acid, deoxyribose, 2-thiobarbituric acid (TBA), Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], HEPES (N-2-hydroxyethyl piperazine N′-2-2-ethansulfonic acid), butylated hydroxytoluene (BHT), sucrose, and tetraethoxypropane (TEP) were purchased from Sigma Chemical Company (St. Louis, MO). Trichloroacetic acid (TCA) and sodium hydroxide were obtained from EM Science (Cherry

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Hill, NJ). Hydrogen peroxide, pyrophosphate, nitric acid, ammonium acetate, ascorbic acid, and sodium chloride were obtained from Mallinckrodt Inc. (Paris, KY). Broccoli, carrot, green pepper, spinach, and tomato were purchased from Smith's Food and Drug Co., Logan, UT and homogenized for 1 min at level 2 in 0.15 M NaCl with a polytron homogenizer (Omni 5000 Interantional Co., Waterburg, CT). The pH of the homogenates was adjusted to 7.0.

Linoleic acid micelle preparation

Linoleic acid micelle was prepared by diluting 500 mg linoleic acid in 20 ml of chloroform. The chloroform solution was dried under vacuum and resuspended in 100 ml of degassed, argon-saturated 0.15 M NaCl. The suspension was then sonicated with 10 pulses of 30 s under argon. The lipid preparations were stored at 4°C under an argon atmosphere. Since most buffers trap hydroxyl radicals or interfere with iron conversions, reactions were carried out in unbuffered condition. The pH of 0.15 M NaCl was carefully adjusted to 7.0 immediately prior to use.

Homogenate preparation

Beef round muscle (select grade) and vegetables were purchased from Smith's Food and Drug Co., Logan, UT. External fat was removed, and the muscle was chopped into small pieces using a stainless steel knife. The chopped beef muscle and vegetables including broccoli, carrot, green pepper, spinach, and tomato were homogenized for 1 min at level 2 in 50 mM HEPES buffer or 0.15 M NaCl (pH 7.0) with a polytron homogenizer (Omni 5000 International Co., Waterburg, CT). The pH of the homogenate was adjusted to 7.0 with 0.1 N NaOH by using a pH meter (Orion Research Incorporated Co., Boston, MA). The incubation mixture included 9 ml of 20% (w/v) beef homogenate and 1 ml of distilled deionized water or 1 ml of 20% vegetable homogenates or their blends (by equal ratio) and was incubated for 2 hours at 37°C. After incubation the homogenate was used for the thiobarbituric acid (TBA) test.

Chemical analysis

Lipid peroxidation in linoleic acid micelles

The thiobarbituric acid reactive substances (TBARS) assay in linoleic acid micelles was performed using a modified spectrophotometric method (15). After incubation of the reaction mixture (final volume, 1 ml) which contained 0.8 ml of linoleic acid micelles (5 mg/ml) in 0.15 M NaCl, 0.1 ml of 20% vegetable homogenates in 0.15 M NaCl, and 0.1 ml of 1 mM iron salts for 60 min at 37°C, 50 μl of 1% BHT was added to the mixture to stop the reaction and to prevent spurious lipid peroxidation during heating. Two milliliters of a TBA-TCA stock solution (1% TBA in 0.05 N NaOH plus 2.8% TCA in deionized water) were added to the reaction mixture. The mixture was then heated for 10 min in a boiling water-bath to develop the pink chromogen of TBA-reactive substances and cooled with tap water. Two milliliters of 1-butanol were added to the mixture and vortexed well. Absorbance of the supernatant was read at 532 nm with a UV 2100 U spectrophotometer (Shimadzu Co., Kyoto, Japan). TBARS were calculated from a standard curve of malondialdehyde, a breakdown product of TEP.

Lipid peroxidation in beef homogenates

TBARS assay for beef homogenate was performed as described by Buege and Aust (15). The reaction mixture contained 1.0 ml of incubation mixture and 2.0 ml of 15% TCA-0.375% TBA-0.025 N HCl stock solution. The mixture was heated for 10 min in a boiling water bath to develop the pink color, cooled with tap water, and centrifuged at 1,500 × g for 15 min. TBARS were quantitated with a modified method to rectify for the interference of soluble sugars (16). Absorbance of sucrose was read at 532 nm and 440 nm. TBARS was corrected with the amount of sucrose in the sample mixture.

Hydroxyl radical scavenging activity

The deoxyribose degradation as an indication of hydroxyl radical scavenging activity was determined by TBARS formation (17). The reaction mixtures containing 0.8 ml of 10 mM deoxyribose in 10 mM phosphate buffered saline (pH 7.0), 0.1 ml of 20% vegetable homogenates in 0.15 M NaCl (pH 7.0), and 0.1 ml of 1.0 mM ferrous sulfate or 1.0 mM ferric citrate were incubated for 30 min at 37°C. The ferric citrate was used after standing for 2 hr at room temperature. The following steps were the same as described in the linoleic acid system.

Antioxidant measurement

Ascorbic acid was determined by a modified version of AOAC (18). After grinding and filtering the sample, 2 ml of the sample was taken and 5 ml of metaphosphoric acid-acetic acid solution was immediately added to prevent further ascorbic acid degradation. The samples were then passed through a 0.45 μm Millipore filter and the filtrates were titrated with the 2,6-dichloroindophenol standard solution. Carotene was determined using the method of AOAC (18) after the sample was bulked and macerated with a blender (Braun Multipractic MC 100, Braun Co., Lynnfield, MA). Carotene content was determined by reference to a standard curve constructed using β-carotene (Sigma Chemical Co., St. Louis) in the range of 0~12 mg/100 g. After the weighed sample was ground with powdered Na_2SO_4 and extracted with Soxhlet extraction thimbles using absolute alcohol as a rinsing liquid, α-tocopherol was determined with the AOAC method (18).

Total iron measurement

The total iron concentration was determined in wet-ashed samples by using ferrozine (13). Each homogenized sample (1.0 ml) was digested with 5 ml of concentrated nitric acid and 0.2 ml of 30% hydrogen peroxide on a hot plate until it formed a white ash. The ash was dissolved in 0.2 ml of 1.0 N HCl and diluted with 0.8 ml deionized water. One milliliter of 1.0% ascorbic acid was added, and the tubes were vortexed. After 20 min, 1 ml of 10% ammonium acetate buffer and 1 ml of 1 mM ferrozine color reagent were added, and the mixture was mixed well. The mixture was allowed to stand at room temperature for 45 min before the absorbance of each sample was determined at 562 nm.

Nonheme iron masurement

The tissue nonheme iron was extracted with 1.0 ml of 25% trichloroacetic acid and 1.0 ml of 4% pyrophosphate (19). These were added to 1.0 ml of 20% tissue homogenate and boiled in a water-bath for 20 min. The mixture was then centrifuged at 2,500×g for 10 min, and the supernatant was carefully saved in a 10 ml volumetric flask. The extraction step was performed three times on the same sample. The collected supernatant was brought to a total volume of 10 ml with distilled deionized water. One milliliter of the supernatant was used for determining nonheme iron using the ferrozine method described above.

Statistical analysis

Data were analyzed using the SAS (20) program on duplicate samples with three replications. If the overall F-test was significant, the least significant difference procedure was used to determine significant differences at the level of $p<0.05$ between the means of the treatment groups.

RESULTS AND DISCUSSION

Iron and antioxidant content

The iron content of beef and vegetables was determined (Table 1). Total iron content of beef round muscle was 2.43 mg/100 g wet weight, and nonheme iron content was 0.74 mg/100 g wet weight. Nonheme iron was 30.5% of the total iron. The total iron in vegetables was as follows: 1.73, 0.76, 0.67, 5.34, and 0.51 mg/100 g wet weight for broccoli, carrot, green pepper, spinach, and tomato, respectively. The nonheme iron was expected to be equal to the amount of total iron because of negligible amount of heme iron in vegetables.

Table 1 also shows antioxidant content of five different vegetables. Broccoli contains a high content of ascorbic acid and carotene; carrots contain the highest content of carotene; green pepper has the highest content of ascorbic acid and α-tocopherol; spinach has appreciable amounts of all three antioxidants; and tomato has low antioxidant content compared with the other vegetables. These results were comparable to other reported data (21). Although flavonoid contents in these vegetables were not measured, flavonoids also affect the antioxidant activity of vegetables by scavenging oxygen radicals (22). Ascorbic acid is used in many food systems, sometimes in combination with phenolic antioxidants, to increase stability (23). At least two mechanisms, which regenerate the primary antioxidant or inactivate pro-oxidant metals, are involved in its antioxidant action (10). Ascorbic acid inhibits heme catalyzed lipid peroxidation whereas it accelerates nonheme iron catalytic lipid peroxidation (24).

Tocopherols, which have been known for many years to have antioxidant properties, are widely distributed in vegetable oils. The stability of animal fats has occasionally been improved by adding a small quantity of crude or lightly refined vegetable oil. Cort (25) found tocopherol to be more effective in animal fats than in vegetable oils. Dugan and Kraybill (26) reported that α-tocopherol at concentration of 0.05% had an optimum effect in lard. When α-tocopherol was used in lard with BHA at 0.01%, increasing α-tocopherol concentrations from 0.01% to 0.05% to 0.1% progressively decreased the stability of the lard. Thus, the pro-oxidant effect of α-tocopherol is apparent when its higher concentrations were used with other antioxidants in an oxidizing fat system.

Carotenoids from vegetables have been presumed to be antioxidants since they impart improved stability to some foods. β-Carotene is the major carotenoid precursor of vitamin A. The role of β-carotene as a quencher of singlet oxygen appears to offer some explanation for its short-time effect in systems which may be exposed to light or other promoters of singlet oxygen (12). Lycopene, which is found at levels up to 90% of total carotenoids, has also been known to act as a singlet oxygen quencher (27).

Antioxidant activity in linoleic acid micelles

The antioxidant activity of vegetables (20%, pH 7.0) including broccoli (B), carrot (C), green pepper (G), spinach (S), and tomato (T) and their blends was investigated using an iron-catalyzing linoleic acid peroxidation system (Table 2). Carrots and spinach significantly inhibited iron-catalyzed lipid peroxidation in linoleic acid micelles ($p<0.05$). The percent inhibition was 29.9 and 35.8% for carrot and spinach, respectively. Green pepper and tomato slightly stimulated lipid peroxidation in the linoleic acid micelles, but were not significantly different from the control. In combinations of two vegetables, C+S, G+S, and S+T significantly inhibited lipid peroxidation by 35.0, 30.2, and 20.5%, respectively ($p<0.05$). The inhibitory effects of the combinations might be mainly due to that of spinach. The combinations of three vegetables, B+C+S, B+C+T, B+S+

Table 1. Iron and antioxidant content (per 100 g) in beef and fresh vegetables

Foods	Total iron (mg)	Nonheme iron (mg)	Ascorbic acid (mg)	α-Tocopherol (μg)	β-Carotene (μg)
Beef	2.43±0.02	0.74±0.04	ND	620	-
Broccoli	1.73±0.01	ND	114.0	480	1,100
Carrot	0.76±0.03	ND	0.007	680	8,200
Green pepper	0.67±0.03	ND	139.0	2,850	240
Spinach	5.34±0.07	ND	52.0	1,800	3,800
Tomato	0.51±0.02	ND	24.2	800	620 (3100)[1,2]

[1]Data from Heinonen et al. (33), [2]Lycopene (μg/100 g fresh product)

ND: Not determined.

Table 2. Effect of vegetables and their blends on lipid peroxidation in linoleic acid micelles

Vegetables	TBARS[1)] (nmole/ml linoleic acid)	Inhibition (-) or Stimulation (+) (%)
Linoleic acid, Control	12.95±1.34	-
Broccoli (B)	12.40±1.19	-4.2
Carrot (C)	9.19±0.57	-29.0*
Green pepper (G)	13.55±1.23	+4.6
Spinach (S)	8.32±0.61	-35.8*
Tomato (T)	13.36±1.28	+3.2
B+C	11.64±1.32	-10.1
B+G	15.19±1.81	+17.3
B+S	11.51±1.13	-11.1
B+T	14.35±1.25	+10.8
C+G	14.28±1.18	+10.3
C+S	8.42±0.78	-35.0*
C+T	11.78±1.11	-9.0
G+S	9.04±0.70	-30.2*
G+T	14.41±1.36	+11.3
S+T	10.29±0.62	-20.5*
B+C+G	14.25±1.39	+9.7
B+C+S	8.01±0.50	-38.1*
B+C+T	10.30±0.76	-20.5*
B+G+S	11.98±1.37	-7.5
B+G+T	15.09±2.45	+16.5
B+S+T	9.02±1.14	-30.3*
C+G+S	9.83±0.90	-24.1*
C+G+T	11.50±1.28	-11.2
G+S+T	9.72±0.62	-24.9*
B+C+G+S	10.20±0.68	-21.2*
B+C+G+T	12.41±0.89	-4.2
B+G+S+T	11.01±1.24	-15.0
C+G+S+T	11.04±1.30	-14.7
B+C+G+S+T	10.77±0.65	-16.8*

[1)]Data represent the mean±SD of three determinations.
*Significantly ($p<0.05$) different from the control value.

T, C+G+S, and G+S+T showed significant inhibitions on lipid peroxidation by 38.1, 20.5, 30.3, 24.1, and 24.9%, respectively. The inhibitory effects of these combinations seemed to be due to the inhibitory activity of spinach and carrot. Combinations of four or five vegetables showed inhibitory effects on lipid peroxidation in linoleic acid micelles but their inhibitory effects were not greater than those of the above three combinations.

Both ferrous and ferric ions were used for the initiation and stimulation of lipid peroxidation in the linoleic acid micelle system. Although the mechanism of iron-catalyzed lipid peroxidation is still unresolved, the rate of peroxidation appears to be maximal when the ratio of ferrous to ferric ion is one to one (6,7). Aust and colleagues demonstrated that there is also an essential requirement for ferric ions in order for ferrous ions to stimulate lipid peroxidation maximally (6-8). They proposed a Fe(II)-O^2-Fe(III) complex as an initiating species (8). The inhibitory effects of carrot and spinach may be due to high concentrations of β-carotene and α-tocopherol (12). β-Carotene may result in decreased oxygen species which can initiate and stimulate lipid peroxidation. Palozza and Krinsky (28) reported that β-carotene suppressed microsomal lipid peroxidation and that the oxidation was strongly inhibited with combination of α-tocopherol. The high content of ascorbic acid in green pepper might also contribute to the weak stimulatory effect on lipid peroxidation because ascorbic acid, as a reducing agent, can stimulate lipid peroxidation by reaction with ferric ion. Also, α-tocopherol can act as a prooxidant by reducing ferric ion in an aqueous lipid dispersion (29). Regardless of the high iron content of spinach, it significantly inhibited lipid peroxidation in this model system. Therefore, the iron content of vegetables might not affect lipid peroxidation. This result may be due to the model system which includes 50 μM Fe (II) and 50 μM Fe (III).

Antioxidant activity in beef homogenate

As compared with the linoleic acid micelle system, the beef homogenate system is complex. Beef muscles contain a great number of compounds (prooxidants and antioxidants) which may affect lipid peroxidation. Beef and vegetable homogenate mixtures (9 : 1) incubated for 2 hrs were used to determine the formation of TBARS. As shown in Table 3, tomato signifi-

Table 3. Effect of vegetables and their blends on lipid peroxidation in beef muscle homogenates in 50 mM HEPES buffer

Vegetables	TBARS[1)] (nmole/g beef)	Inhibition (-) or Stimulation (+) (%)
Beef, Control	16.77±1.46	-
Broccoli (B)	18.70±2.75	+11.5
Carrot (C)	15.40±1.39	-8.2
Green pepper (G)	15.36±1.30	-8.4
Spinach (S)	28.05±3.25	+67.3*
Tomato (T)	13.44±1.26	-19.9*
B+C	18.45±1.86	+10.0
B+G	17.06±1.48	+1.7
B+S	39.23±5.52	+133.9*
B+T	17.60±1.82	+4.9
C+G	16.31±1.57	-2.7
C+S	26.73±3.12	+59.4*
C+T	16.68±1.90	-0.5
G+S	31.54±4.43	+88.1*
G+T	13.08±1.21	-22.0*
S+T	30.49±2.95	+81.8*
B+C+G	16.20±1.74	-3.4
B+C+S	27.14±3.62	+61.8*
B+C+T	14.35±1.54	-14.4
B+G+S	29.75±3.85	+77.4*
B+G+T	13.91±1.16	-17.1*
B+S+T	32.52±4.72	+93.9*
C+G+S	16.65±1.84	-0.7
C+G+T	10.48±1.40	-37.5*
G+S+T	19.79±2.28	+18.1
B+C+G+S	18.10±2.03	+7.9
B+C+G+T	11.43±1.15	-31.8*
B+G+S+T	17.98±1.60	+6.1
C+G+S+T	12.05±1.06	-28.1*
B+C+G+S+T	15.30±1.25	-8.8

[1)]Data represent the mean±SD of three determinations.
*Significantly ($p<0.05$) different from the control value.

cantly inhibited the formation of TBARS in beef homogenates ($p<0.05$), whereas spinach significantly stimulated TBARS formation. The antioxidant activity of tomato might be responsible for its major carotenoid, lycopene, which supercedes β-carotene in singlet oxygen quenching efficiency in biological sytems, and low content of nonheme iron (27). The percent inhibition of carrot, green pepper, and tomato were 8.2, 8.4 and 19.9%, respectively. The percent stimulation of broccoli and spinach were 11.5 and 67.3%, respectively. In combinations of two vegetables, B+S showed the strongest stimulatory effect on the TBARS formation (133.9%). C+S, G+S and S+T also showed a strong stimulatory effect on lipid peroxidation in beef homogenates ($p<0.05$). While, G+T inhibited lipid peroxidation by 22.0% ($p<0.05$). In combinations of three vegetables, the stimulatory effects were lower than in combination of two vegetables, whereas the inhibitory effects were higher. B+G+T and C+G+T had significant inhibitions on lipid peroxidation in beef homogenates by 17.1 and 37.5%, respectively, and their inhibitory effects appeared to be additive to the inhibitory effect of each vegetable by itself. In combinations of four vegetables, B+C+G+T and C+G+S+T significantly inhibited TBARS formation in beef homogenates ($p<0.05$). With an increasing number of vegetables in the combinations, there was a stronger inhibitory effects in this model system. These results can be applied for making new meat products, meat stew-sticks which contain a blend of meats and vegetables. Addition of vegetables to meats products can not only provide a nutritionally balanced diet needed for healthy human but may also counteract detrimental effects of meat by supplying antioxidants, fiber and flavonoids. In addition, the antioxidants of vegetables in a meat stew-stick may result in preventing a warmed-over flavor and increasing shelf life.

Although both nonheme iron and heme iron can catalyze lipid peroxidation, nonheme iron is known to play a more important role in lipid peroxidation (9). Muscle tissue contains a considerable amount of iron bound to proteins. Myoglobin is the predominant hemeprotein in muscle. Broccoli and spinach had high contents of nonheme iron and ascorbic acid. These characteristics of broccoli and spinach may be related to the stimulatory effect on the formation of TBARS in beef homogenate. The addition of iron to beef homogenates increased lipid peroxidation (30). Therefore, the nonheme iron of broccoli and spinach may have an iron-additive effect in beef homogenates. In addition, ascorbic acid can reduce ferric ion in beef to ferrous ion which will catalyze the production of hydroxyl radicals via the Fenton reaction, thereby resulting in the initiation and stimulation of lipid peroxidation (31).

Antioxidant effects on degradation of deoxyribose

In the presence of iron, the protective effect of vegetables against deoxyribose degradation was investigated by determining the formation of TBARS (Table 4). When beef homogenate was added to the reaction mixture containing deoxyribose and ferrous sulfate, it inhibited TBARS formation from deoxyribose degradation. The inhibitory effect of beef homogenate on deoxyribose degradation might be due to carnosine or histidine, an inherent antioxidant found in skeletal muscle which can chelate transition metals such as iron and copper. All vegetables significantly inhibited the degradation of deoxyribose catalyzed by ferrous ion between the range of 43.6~77.6%. Green pepper was most effective on the inhibition of the ferrous ion-catalyzed degradation of deoxyribose. When ferric citrate was used for the induction of deoxyribose damage, carrot and tomato inhibited the degradation of deoxyribose by 23.0 and 33.6%, respectively ($p<0.05$). Broccoli and spinach stimulated the degradation of deoxyribose by 39.8 and 55.8%, respectively ($p<0.05$). The effects of vegetables on TBARS formation from deoxyribose degradation were similar to those in beef homogenate system. The stimulation of broccoli and spinach on TBARS formation may be related to the content of ascorbic acid, which can facilitate the reduction of ferric to ferrous ion. In contrast, in the presence of ferrous ion, ascorbic acid may inhibit the degradation of deoxyribose by retaining the ferrous ion in the aqueous solution. Moreover, the high iron content in broccoli and spinach might also be associated with the increased TBARS formation from deoxyribose degradation. Addition of low concentrations of ferrous sulfate or ferric citrate to deoxyribose caused degradation of the sugar into a malondialdehyde-like compound, which forms a chromogen with TBA. Hydroxyl radicals are known to be responsible for the breakdown of deoxyribose (17). Ferrous ion in an aqueous solution is slowly oxidized to form superoxide anions, resulting in the rapid formation of hydrogen peroxides by dismutation at a neutral pH. The ferrous ions interact with hydrogen peroxides to form hydroxyl radicals via the Fenton reaction. Ferric citrate can also cause damage to deoxyribose after standing for several hours at room temperature (32). In our study, ferric citrate solution standing for two hours at room temperature may not allow complete autoreduction of ferric

Table 4. Effect of vegetables on formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts

Beef and vegetables	Absorbance at 532 nm[1)]	Inhibition (-) or stimulation (+) (%)
Beef	0.062±0.013	-
Ferrous sulfate (100 μM)	0.401±0.057	-
Beef	0.210±0.021	-47.6*
Broccoli	0.193±0.024	-51.9*
Carrot	0.226±0.028	-43.6*
Green pepper	0.090±0.016	-77.6*
Spinach	0.219±0.035	-45.4*
Tomato	0.126±0.013	-68.6*
Ferric citrate (100 μM)	0.113±0.013	-
Ascorbate (100 μM)	0.238±0.044	+110.6*
Broccoli	0.158±0.014	+39.8*
Carrot	0.087±0.010	-23.0*
Green pepper	0.102±0.012	-9.7
Spinach	0.176±0.016	+55.8*
Tomato	0.075±0.009	-33.6*

[1)]Data represent the mean±SD of three determinations.
*Significantly ($p<0.05$) different from the control value.

to ferrous ion, thereby resulting in lower TBARS formation for the control. Hemeproteins such as myoglobin and hemoglobin can also catalyze the deoxyribose degradation (13). Without addition of iron, beef homogenate itself which contain both heme and nonheme iron, catalyzed the degradation of deoxyribose in our study. The extent of the deoxyribose damage, however, was much lower compared with addition of ferrous sulfate.

Natural antioxidants such as ascorbic acid, α-tocopherol, β-carotene, and flavonoids are found in numerous vegetables including broccoli, carrot, green pepper, spinach, and tomato. In this study, the antioxidant activities of vegetables and their blends on lipid peroxidation and degradation of deoxyribose were different from various systems. The results indicate that the antioxidant activity of vegetables depends on the provided environment such as iron content (or iron redox state) and substrates. The antioxidant activity of individual vegetables affected that of the combinations of several vegetables. Some combinations of vegetables had an additive inhibitory effect on lipid peroxidation, whereas some counteracted a stimulatory effect of individual vegetable on lipid peroxidation. The results of this study may give insight about which vegetables to choose, how to blend and the amounts of vegetables and meats to mix to produce a nutritionally balanced food product from red meats and vegetables.

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