Determination of Antioxidant Properties of Aroma Extracts from Various Beans

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Aroma extracts from fresh soybeans, mung beans, kidney beans, and azuki beans were prepared using simultaneous steam distillation and solvent extraction (SDE) under mild conditions (55 °C and 95 mmHg). Extracts were examined for antioxidative activities in two different assays. The aroma extracts isolated from all beans inhibited the oxidation of hexanal for nearly one month at a level of 250 μL/mL. Mung bean and soybean extracts inhibited malonaldehyde (MA) formation from cod-liver oil by 86% and 88%, respectively, at the 250 μL/mL level. Azuki and kidney bean extracts inhibited MA formation from cod-liver oil by 76% and 53%, respectively, at the 250 μL/mL level. The antioxidative activities of mung bean and soybean extracts were comparable with that of the natural antioxidant, α-tocopherol (vitamin E).

Keywords: *Aroma chemicals; bean extracts; natural antioxidants*

INTRODUCTION

Naturally occurring antioxidants, such as vitamin C and vitamin E, as well as phenolic compounds, possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases, aging, and brain dysfunction (Yagi et al., 1993; Pietta et al., 1998). Many aroma constituents found in plants have been found to possess some medicinal activities, including antioxidant properties (Kramer, 1985; Farag et al., 1989). In fact, the leaves and flowers of plants containing numerous aroma chemicals have been widely used in both folk medicine and aroma therapies (Vickers, 1996). Until recently, aroma chemicals have been investigated from the viewpoint of flavor and fragrance chemistry. However, some medicinal activities of aroma chemicals, such as their antioxidative activities, have been discovered through using essential oils in aromatherapy (Tisserand, 1988; Hoffmann, 1987).

Beans have been a staple part of diets for thousands of years and remain the main source of protein in many countries today. They are high in complex carbohydrates, protein, and fiber, yet are extremely low in fat. Beans also contain bioactive chemicals, such as antioxidants, which inhibit the diseases mentioned above (White and Xing, 1997). Many natural antioxidants are found in the seeds and the nuts of plants (Namiki, 1990). Several studies reported that bean constituents possessed antioxidative activities in various model systems (Duh et al., 1997; Tsuda et al., 1993). Beans also contain many volatile components (Buttery et al., 1975) which may possess some antioxidative activity. Recently, volatile aroma chemicals found in brewed coffee were reported to possess antioxidant potentials comparable to those of vitamins C and E (Singhara et al., 1998).

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In the present study, aroma extracts from fresh soybeans, mung beans, kidney beans, and azuki beans were examined for their antioxidant activity in two different assays.

MATERIALS AND METHODS

Beans. Fresh soybeans [*Glycine max* (L.) Merr.], mung beans [*Vigna radiata* (L.) R. Wilczek], kidney beans [*Phaseolus vulgaris* L.], and azuki beans [*Vigna angulariz* (Willd.) Ohwi & Ohashi] were purchased from a local market.

Chemicals. Hexanal, hexanoic acid, undecane, carbon tetrachloride, *N*-methylhydrazine (NMH), 2-methylpyrazine, sodium dodecyl sulfate (SDS), ferrous chloride, and α-tocopherol (vitamin E) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cod-liver oil (approximately 70% ω-3 fatty acid methyl esters), butylated hydroxytoluene (BHT), trizma hydrochloride, and trizma base were bought from Sigma Chemical Co. (St. Louis, MO). Hydrogen peroxide and ethyl acetate were bought from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). The standard stock solution of 2-methylpyrazine was prepared by adding 10 mg of 2-methylpyrazine to 1 mL of dichloromethane and was stored at 5 °C. Authentic 1-methylpyrazole (1-MP) was synthesized from malonaldehyde (MA) and *N*-methylhydrazine according to the method developed previously (Umano et al., 1988).

Isolation of Aroma Chemicals by Simultaneous Steam Distillation and Solvent Extraction (SDE). Soybeans, mung beans, kidney beans, or azuki beans (200 g) were placed separately into 3-L round-bottom flasks with 1 L of deionized water, and the steam distillate was obtained at 55 °C and 95 mmHg. Steam distillate was extracted with 100 mL of carbon tetrachloride simultaneously for 3 h using a modified Likens-Nickerson apparatus (Schultz et al., 1977). The extracts were dried over anhydrous sodium sulfate for 12 h. The sodium sulfate was filtered out, and the solvent was removed using a rotary flash evaporator. The distillation was stopped when the volume of extract was reduced to approximately 5 mL. The extract was transferred into a vial, the distillation flask was washed with a minimum amount of carbon tetrachloride, and the washings were added to the vial. The solvent was further removed under a purified nitrogen stream until the total volume was reduced to exactly 2.5 mL. These concentrates were defined as the total aroma extract for each type of bean tested and were used to determine antioxidant activities in both the Lipid/MA assay and Aldehyde/Carboxylic Acid assay.

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Measurement of Total Aroma Compounds in Extracts. Solvents from extracts obtained by SDE were removed under a purified nitrogen stream until the total volume of concentrate was reduced to approximately 100 μL. The mass of each concentrate was measured using an analytical balance. Each concentrate was then analyzed by gas chromatography (GC) equipped with a flame ionization detector (FID) to determine the percent total peak-area of aroma compounds and solvent. The total mass of aroma chemicals was calculated by multiplying the percent representing the total peak-area of aroma compounds with the mass of each extract. Each experiment was repeated three times. The detector response to solvent was determined to be linear over a range of 0.2 to 1.0 μL injected with a R^2 value of 0.99.

Aldehyde/Carboxylic Acid Assay. Antioxidative activity of the samples obtained by SDE was tested using their inhibitory effect toward oxidation of aldehyde to carboxylic acid (Macku and Shibamoto, 1991). Test samples (100 μL, 200 μL, and 500 μL) were added to a 2-mL dichloromethane solution of hexanal (3 mg/mL) containing 0.2 mg/mL of undecane as a GC internal standard. The oxidation of the sample solution was initiated by heating at 60 °C for 10 min in a sealed vial, and the vial was then stored at room temperature. The headspace of each vial was purged with pure air (1.5 L/min, 3 s) every 24 h for the first 10 days. The decrease in hexanal was monitored at 5-day intervals. Standards of BHT and α-tocopherol were also examined for their antioxidative activity using the same methodology.

Lipid/MA Assay. Antioxidative activity of extracts was determined by analyzing MA formed from cod-liver oil upon oxidation after derivatizing to 1-MP with NMH (Tamura et al., 1991). An aqueous solution (5 mL) containing 10 μL of cod-liver oil, 0.25 mmol of trizma buffer (pH 7.4), 5 μmol of ferrous chloride, 10 μmol of hydrogen peroxide, 0.75 mmol of potassium chloride, and 0.2% of surfactant SDS was incubated with various amounts of the aroma extracts for 18 h at 37 °C in a 20-mL test tube. The oxidation of samples was stopped by adding 50 μL of a 4% BHT solution (Ichinose et al., 1989). The sample tubes were covered with aluminum foil during incubation to avoid any influence of light on the lipid peroxidation. Known antioxidants, α-tocopherol and BHT, were used to compare antioxidant activity to that of the aroma extracts tested. NMH (30 μL) was added to the above oxidized cod-liver-oil solutions, and the solutions were stirred for 1 h at room temperature. Each reaction solution was extracted with 10 mL of dichloromethane using a liquid−liquid continuous extractor for 3 h. The solution was saturated with NaCl prior to extraction to prevent the formation of an emulsion. The extract was adjusted to exactly 10 mL by adding dichloromethane and 20 μL of a 2-methylpyrazine solution as a GC internal standard. To avoid damage to the nitogen−phosphorus detector (NPD) the solvent (dichloromethane) in the adjusted solution was removed under a purified nitrogen stream, and then the volume was adjusted to exactly 10 mL with ethyl acetate. The solution was analyzed for 1-MP by a GC with a NPD.

Instrumental Analysis. The quantitative analysis of hexanal and 1-MP was conducted according to an internal standard method (Ettre, 1967). A Hewlett-Packard (HP) model 5890 GC, equipped with a 30-m × 0.25-mm i.d. (d_f = 0.25 μm) DB-1 bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and a FID, was used for analysis of hexanal and determination of total aroma compounds in extracts. The linear velocity of the helium carrier gas was 30 cm/sec at a split ratio of 20:1. The injector and the detector temperatures were 300 °C and 280 °C, respectively. The oven temperature was programmed from 40 °C to 180 °C at 4 °C/min and held for 10 min.

A HP model 6890 GC, equipped with a 30-m × 0.25-mm i.d. (d_f = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and a FID, was used for analysis of determination of total aroma compounds in the extracts. The linear velocity of the helium carrier gas was 30 cm/sec at a split ratio of 20:1. The injector and the detector temperatures were each 250 °C. The oven temperature was programmed from 50 °C to 180 °C at 3 °C/min and held for 20 min.

A HP model 6890 GC, equipped with a 30-m × 0.25-mm i.d. (d_f = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (J & W Scientific, Folsom, CA) and a NPD, was used for analysis of 1-MP.

RESULTS AND DISCUSSION

The yields of total aroma chemicals (relative to the fresh beans) for mung beans, soybeans, kidney beans, and azuki beans were 0.0023, 0.0033, 0.0015, and 0.0012% (w/w), respectively, from SDE. The masses of total aroma chemicals from mung beans, soybeans, kidney beans, and azuki beans were 4.7 ± 0.1, 6.6 ± 0.2, 3.1 ± 0.2, and 2.4 ± 0.6 mg, respectively. The values are mean ± standard deviation ($n = 3$).

The aldehyde/carboxylic acid conversion assay (Macku and Shibamoto, 1991) is a relatively simple assay that can easily be used to gauge the antioxidant potential of a chemical or a group of chemicals in an organic system.

Figures 1a−d show the relative amounts of remaining hexanal in samples containing various amounts of extracts from mung beans (a), soybeans (b), kidney beans (c), and azuki beans (d) in the aldehyde/carboxylic acid assay over a period of 45 days. The values are mean ± standard deviation ($n = 3$). All extracts exhibited dose-dependent inhibitory activity. In all experiments, the hexanal in a control was completely oxidized to hexanoic acid after 30 days. Over 45 days, the sample containing 500 μL of carbon tetrachloride contained almost the same amount of hexanal as did the control, suggesting that a trace amount of carbon tetrachloride, which may remain in the aroma extracts, did not significantly influence the antioxidant activities of the aroma extracts. At a concentration of 250 μL/mL, mung beans (Figure 1a, ca. total aroma chemicals = 940 μg) and soybeans (Figure 1b, ca. total aroma chemicals = 1300 μg) inhibited the hexanal oxidation by 100% for more than 45 days. At a 100 μL/mL level, mung beans (ca. total aroma chemicals = 380 μg), and soybeans (ca. total aroma chemicals = 520 μg) inhibited hexanal oxidation by 70% for 45 days. At a 250 μL/mL level, kidney beans (Figure 1c) and azuki beans (Figure 1d) inhibited hexanal oxidation by 90% and 80%, respectively, for a period of 43 days.

Figure 2 shows the percent of hexanal remaining in samples containing different amounts of extracts from beans and 50 μg/mL of BHT or α-tocopherol at the end of the storage period of 45 days. The values are mean ± standard deviation ($n = 3$). At a level of 250 μL/mL, the inhibitory activity of extracts from mung beans and soybeans was comparable to that of α-tocopherol or BHT. Both α-tocopherol and BHT inhibit hexanal oxidation by 100% at 50 μg/mL.

The lipid/MA assay is specific for the determination of MA in samples (Umano et al., 1988). MA formed upon the oxidation of lipids is derivatized to 1-MP and is subsequently determined by a GC equipped with a NPD (Umano et al., 1988; Nishiyama et al., 1994; Ogata et al., 1996).

Figure 3 shows the results of the lipid/MA assay in the presence of extracts from mung beans, soybeans, kidney beans, and azuki beans. The values are mean ± standard deviation ($n = 3$). The amount of MA formed from 10 μL of cod-liver oil alone was 1864 ± 27 nmol under the conditions used in the experiment. All ex-

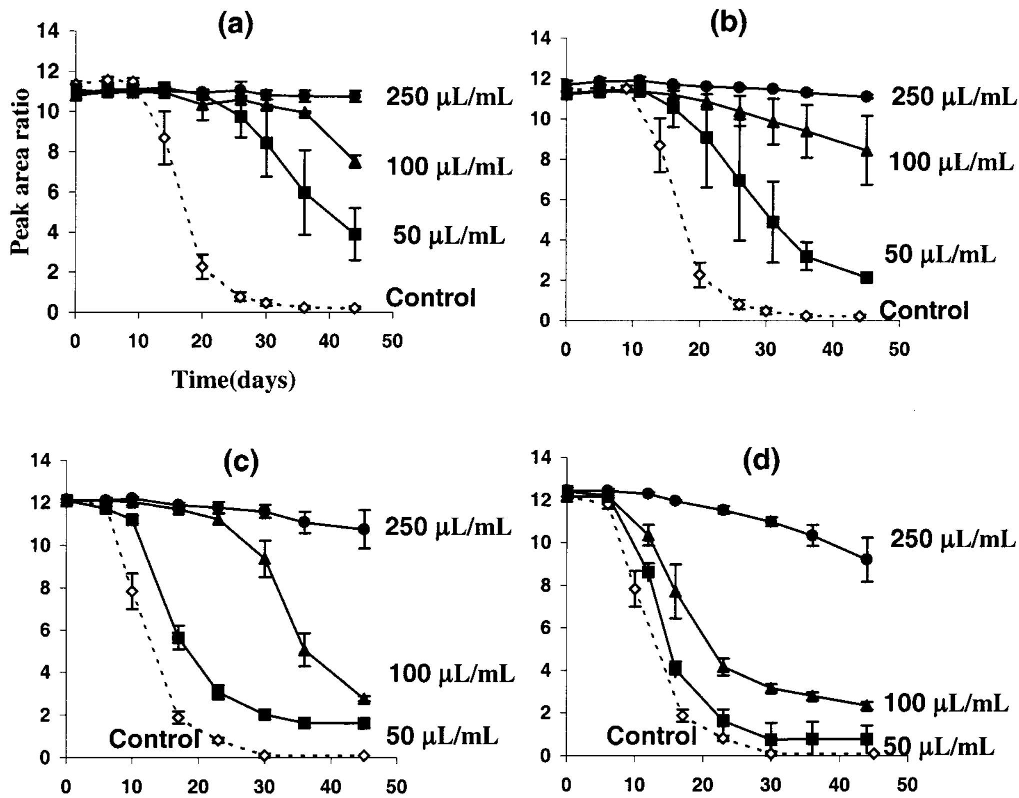

Figure 1. Relative amounts of remaining hexanal in samples with aroma extracts isolated from mung beans (a), soybeans (b), kidney beans (c), and azuki beans (d).

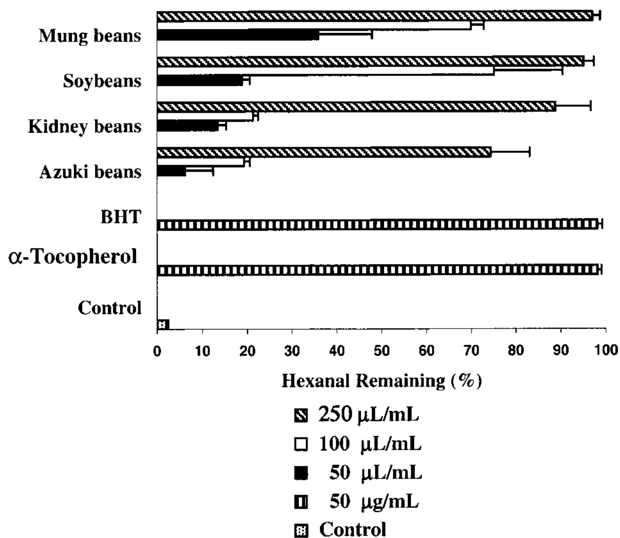

Figure 2. Percent of hexanal remaining in samples containing different amounts of extracts from beans and 50 μg/mL of BHT or α-tocopherol at the end of a storage period of 45 days.

tracts exhibited a dose-dependent activity which was similar to the results obtained by the aldehyde/carboxylic acid assay. The inhibitory effect of carbon tetrachloride (100 μL/mL), the solvent in each extract, was only 3%. Among the beans tested, mung beans and soybeans exhibited the most potent antioxidative activities.

Figure 4 shows the inhibitory effects (%) of the bean extracts along with those of α-tocopherol and BHT toward MA formation from cod-liver oil at a level of 120 μg/mL. The values are mean ± standard deviation (n = 3). The aroma extract of mung beans, which exhibited the greatest activity among the beans tested, showed inhibitory activity similar to α-tocopherol. Mung bean extract and α-tocopherol inhibited MA formation by 86% and 96%, respectively, at the level of 120 μg/mL. The aroma extract of soybeans inhibited MA formation by 83%. BHT inhibited MA formation by 97% at the level of 120 μg/mL. The aroma extracts of kidney beans and azuki beans also inhibited MA formation by 76% and 58%, respectively, at the level of 120 μg/mL.

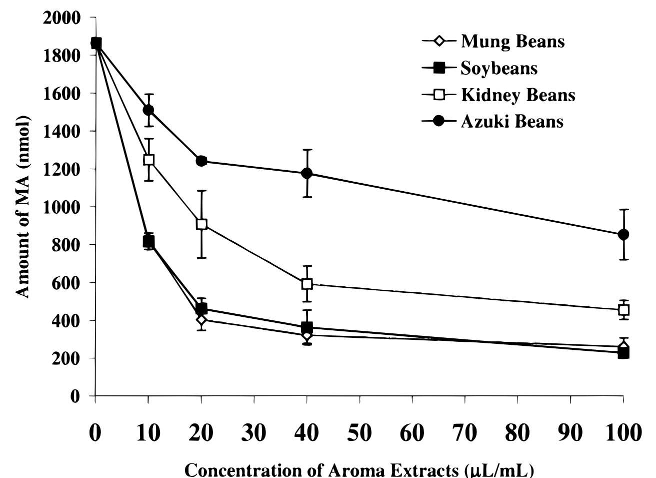

Figure 3. Amounts of MA formed from cod-liver oil in the presence of various amounts of bean extracts.

CONCLUSION

Aroma extracts from four different types of beans exhibited antioxidative activities. The antioxidant activities of these aroma extracts, as well as BHT and α-tocopherol, were consistent in both the aldehyde/carboxylic acid assay and the lipid/MA assay. Aroma extracts are composed of a complex mixture of chemicals

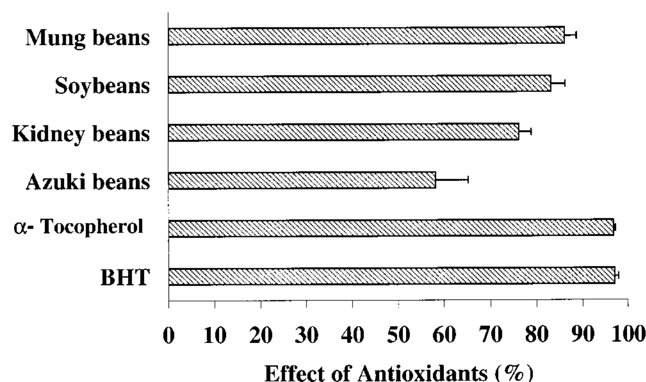

Figure 4. Inhibitory effects (%) of the bean extracts, α-tocopherol, and BHT toward MA formation from cod-liver oil at a level of 120 μg/mL. Values are measured as % formation of MA.

in which not all compounds can be expected to have an antioxidant potential. However, the results of the present study suggest that some aroma chemicals in beans are antioxidants. The results indicate that the antioxidative activities of aroma extracts from beans are not as potent as those of the known antioxidants, BHT and α-tocopherol. However, there is a wide variety of aroma chemicals present in natural plants. Therefore, the total activity may be comparable to those of known antioxidants. Because humans are exposed to reactive oxygen species 24 hours a day, it is extremely important that antioxidant supplements be taken constantly and consistently. Exposure to these aroma chemicals may help to prevent oxidative damages, which are a factor in many diseases. Further identification of the aroma chemicals contained in the aroma extracts of the beans tested is currently underway.

LITERATURE CITED

Buttery, R.; Seifert, R. M.; Ling, L. Characterization of some volatile constituents of dry red beans. *J. Agric. Food Chem.* **1975**, *23*, 516−519.

Duh, P. D.; Yen, W. J.; Du, P. C.; Yen, G. C. Antioxidant activity of mung bean hulls. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1059−1063.

Ettre, L. S. Interpretation of analytical results. In *The Practice of Gas Chromatography*; Ettre, L. S., Zlatkis, A., Eds.; Interscience Publishers: New York, 1967; pp 402−440.

Farag, R. S.; Badei, A. Z. M. A.; Hewedi, F. M.; El-Baroty, G. S. A. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chem. Soc.* **1989**, *66*, 792−799.

Hoffmann, D. Aromatherapy. In *The Herbal Handbook*; Healing Arts Press: Rochester, VT, 1987.

Ichinose, T.; Miller, M. G.; Shibamoto, T. Gas chromatographic analysis of free and bound malonaldehyde in rat liver homogenates. *Lipids* **1989**, *42*, 1527−1532.

Kramer, R. E. Antioxidants in clove. *J. Am. Oil Chem. Soc.* **1985**, *62*, 111−113.

Macku, C.; Shibamoto, T. Volatile antioxidants produced from heated corn oil/glycine model system. *J. Agric. Food Chem.* **1991**, *39*, 1990−1993.

Namiki, M. Antioxidants/antimutagens in food. *Crit. Rev. Food Sci. Nutr.* **1990**, *29*, 273−300.

Nishiyama, T.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. Formation and inhibition of genotoxic glyoxal and malonaldehyde from phospholipids and fish liver oil upon lipid peroxidation. *J. Agric. Food Chem.* **1994**, *42*, 1728−1731.

Ogata, J.; Hagiwara, Y.; Hagiwara, H.; Shibamoto, T. Inhibition of malonaldehyde formation by antioxidants from ω-3 polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **1996**, *73*, 653−656.

Pietta, P.; Simonetti, P.; Mauri, P. Antioxidant activity of selected medicinal plants. *J. Agric. Food Chem.* **1998**, *46*, 4487−4490.

Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **1977**, *25*, 446−449.

Singhara, A.; Macku, C.; Shibamoto, T. Antioxidative activity of brewed coffee extracts. In *Functional Foods for Disease Prevention II: Medicinal Plants and Other Foods*; Shibamoto, T., Terao, J., Osawa, T., Eds.; ACS Symp. Ser. 701; American Chemical Society: Washington, DC, 1998; pp 101−109.

Tamura, H.; Kitta, K.; Shibamoto, T. Formation of reactive aldehydes from fatty acids in an Fe^{2+}/H_2O_2 oxidation system. *J. Agric. Food Chem.* **1991**, *39*, 439−442.

Tisserand, M. *Aromatherapy for Women;* Healing Arts Press: Rochester, VT, 1988; p 87.

Tsuda, T.; Osawa, T.; Nakayama, T.; Kawakishi, S.; Ohshima, T. Antioxidant activity of pea bean (*Phaseolus vulgaris* L.) extract. *J. Am. Oil Chem. Soc.* **1993**, *70*, 909−913.

Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of free malonaldehyde in photoirradiated corn oil and beef fat via a pyrazole derivative. *Lipids* **1988**, *23*, 811−814.

Vickers, A. *Massage and Aromatherapy*; Chapman & Hall: London, 1996; pp 127−167.

White, P. J.; Xing, Y. Antioxidants from cereals and legumes. In *Natural Antioxidants: Chemistry, Health Effects, and Applications*; Shahidi, F., Ed.; AOAC Press: Champaign, IL, 1997; pp 25−63.

Yagi, K., Ed. *Active Oxygens, Lipid Peroxides, and Antioxidants*; CRC Press: New York, 1993.

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