Inhibition of Soybean Oil Oxidation by Extracts of Dry Beans *(Phaseolus vulgaris)*

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ABSTRACT: Polyphenolic compounds were extracted from pinto, kidney, white (Great Northern), pink, and black beans by hot methanol extraction and added to soybean oil. Oil oxidation was assayed by thiobarbituric acid-reactive substances (TBARS). All bean polyphenolics extracts exhibited similar antioxidant capacity in delaying the onset of iron-catalyzed oxidation better than butylated hydroxyanisole (BHA), propyl gallate (PG), and ascorbic acid. Bean polyphenolic compounds were stable after heating to 50°C and maintained their antioxidant potential after 3 mon at 4°C. Bean extracts effectively inhibited iron-catalyzed oxidation of soybean oil, probably by chelating metal ions, because no antioxidant effect was observed when soybean oil was oxidized without an iron catalyst. *JAOCS 74,* 1025–1030 (1997).

KEY WORDS: Antioxidants, chelators, flavonoids, legumes, oxidation, pinto beans, polyphenols, vegetable oil.

A better understanding of the importance of antioxidants in health and disease has prompted increased research of antioxidant functioning in foods (1–3). Polyphenolic compounds occur naturally in most plants shown to possess antioxidant activity. Dry beans *(Phaseolus vulgaris)* contain significant quantities of polyphenolic compounds but are typically a poor source of known antioxidants, such as ascorbic acid, α-tocopherol, and β-carotene. Polyphenolic compounds with antioxidant properties, such as flavonoids, phenolic acids, and lignans (4), are found in a wide range of plants, including spices, fruits, vegetables, and legumes (5). Extracts from spices, including caraway, clove, cumin, rosemary, nutmeg, parsley, sage, and thyme, have been shown to inhibit the oxidation of vegetable oils effectively (6–8), while extracts from soybeans (9), navy beans (10), wild rice (11), and grapes (12) have also been reported to possess *in vitro* antioxidant activity.

Beans *(P. vulgaris)* contain considerable amounts of phenolic compounds (13–15) that possess varying degrees of antioxidant activity. Deshpande and Cheryan (16) determined the total phenolic content of 10 cultivars of dry beans and reported values for total phenolics that ranged from 25–153 mg catechin equivalents/100 g dry beans. Limited information is available on *in vitro* antioxidant activity of dry beans; however, Onyeneho and Hettiarachchy (10) observed that navy bean hull extract was more protective against oil oxidation than butylated hydroxyanisole-butylated hydroxytoluene (BHA-BHT). Similarly, Tsuda *et al.* (17,18) reported that extracts from white-seed-coated pea beans *(P. vulgaris)* were as effective as BHA or α-tocopherol at inhibiting vegetable oil oxidation. The objective of this research was to evaluate *in vitro* the antioxidant potential of extracts from five cultivars of dry beans *(P. vulgaris)* in comparison with BHA, propylgallate (PG), and ascorbic acid.

MATERIALS AND METHODS

Materials. Five cultivars of beans, pinto, kidney, Great Northern, pink, and black beans, were obtained from a local grocery store and stored at room temperature and humidity until use. BHA, PG, chlorogenic acid, Folin and Ciocalteu's Phenol Reagent, and 98% 2-thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO). Malonaldehyde bis(diethyl acetal) (98%) was obtained from Aldrich Chemical Co. (St. Louis, MO). All other chemicals were reagent-grade. Purified deionized type I water from a MilliQ Plus System (Millipore Co., Bedford, MA) was used.

Preparation of bean extracts. Approximately one cup of dry beans (185 ± 2 g) was rinsed with water and cooked in three cups (690 mL) water in a pressure cooker at 15 psi (1 atm) for 20 min. Cooked beans and the remaining liquid were poured into a borosilicate baking pan and dried for 14 h in a convection oven at 60°C. Dried beans were cooled and ground to pass a mesh 25 sieve (710 nm), then stored at 4°C in an airtight container.

To extract polyphenolic compounds, ground beans (2.00 ± 0.05 g) were placed in a 250-mL Erlenmeyer flask with 50 mL 80% methanol. The flask was capped with foil and heated for 5 min with stirring on a Thermolyn Nuova II heating plate (Dubuque, IA) at 64°C. Contents were filtered through #1 filter paper (Whatman Inc., Hillsboro, OR) into a 100-mL volumetric flask. The bean residue was extracted again by adding an additional 40 mL 80% methanol and heating on a hot plate at 64°C for an additional 10 min. The second extract was also filtered through #1 filter paper into the volumetric flask. The

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flask was filled to volume with 80% methanol. Methanolic bean extracts were prepared in duplicate for each bean variety and stored at 4°C.

Determination of total polyphenolic compound content. Total polyphenolic compound content in bean extracts was determined by the procedure of Weurman and Swain (19). Methanolic extract (0.5 mL) was added to 5 mL purified water and vortexed, then 1 mL of Folin and Ciocalteu's Phenol Reagent was added and vortexed. After 5 min, 1 mL saturated sodium carbonate solution was added and vortexed again. The color was allowed to develop for 1 h, and the absorbance was read at 640 nm in a DU640 Spectrophotometer (Beckman Inc., Fullerton, CA). A standard curve was prepared at the same time with chlorogenic acid at concentrations ranging from 0–100 µg. The quantity of total polyphenolics in the sample was calculated as chlorogenic acid equivalents by using the standard curve.

Oil oxidation with and without iron catalyst. All beakers used in the oil oxidation study had been presoaked in 0.4% wt/vol EDTA overnight, rinsed thoroughly with deionized water, and dried. Soybean oil purchased from a local grocery store was mixed thoroughly, and 100.00 ± 0.05 g of oil was weighed into each beaker. The soybean oil contained 93.7 mg α-tocopherol and 0.02 mg iron. Equal amounts of $FeCl_3 \cdot 6H_2O$ were added to each beaker as a catalyst. Each beaker, containing oil and catalyst, had one of the following test solutions added: BHA, PG or ascorbic acid in 80% methanol, or bean polyphenolic extract in 80% methanol. The materials in each beaker were mixed, and all beakers were stored uncovered in a 50°C convection oven for up to 20 d in the dark, while the progress of soybean oil oxidation was followed. When evaluating the oxidation of soybean oil in the absence of a catalyst, the same procedure was followed, except that the $FeCl_3 \cdot 6H_2O$ catalyst was not added. Duplicate samples of soybean oil were oxidized each time.

Thiobarbituric acid (TBA) assay. The TBA assay was modified from the procedure of Kim and LaBella (20). Oil samples (1.00 ± 0.01 g) were weighed into test tubes; to each test tube, 4 mL 50 mM potassium hydrogen phthalate (pH 3.5) was added and vortexed, followed by an addition of 2 mL 1% TBA in 0.05 N NaOH. The test tube was vortexed, capped with aluminum foil and heated in a Dri-bath heating block (Thermolyn, Dubuque, IA) for 30 min at 100°C. The tube was allowed to cool to room temperature, the aqueous bottom layer was drawn off with a Pasteur pipette, and absorbance was determined at 532 nm in a Beckman DU640 Spectrophotometer. The TBA assay was conducted in duplicate for each sample. A standard curve was prepared at the same time with malonaldehyde bis(diethyl acetal) at concentrations ranging from 10–40 nanomoles (21). The quantity of thiobarbituric acid-reactive substances (TBARS) in each sample was calculated as nanomoles of malonaldehyde bis(diethyl acetal) by means of the standard curve.

Statistical analysis. Statistical analysis was performed by conducting an analysis of variance (ANOVA) and a least significant difference test where appropriate. Statistically significant differences were determined at a level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Polyphenolic compound content of bean cultivars. Relative concentration of bean polyphenolic compounds varied from highest to lowest as follows: kidney ≅ pink ≅ pinto > black ≫ Great Northern (Table 1). However, no consistent relationship was observed between bean polyphenolic compound content and antioxidant capacity, with polyphenolic extracts from each of the bean cultivars demonstrating an approximately equal capacity to inhibit soybean oil oxidation when present at concentrations of 1 to 2 ppm (Fig. 1). Equal volumes of bean extract were used in this experiment. Because the Great Northern beans contain approximately one-half of the polyphenolics of the other beans, data were collected on the antioxidant capacity of 1 ppm of Great Northern polyphenolics and 2 ppm of the other beans. Similar results were reported by Lee *et al.* (22) who observed little correlation between the phenolic content of red peppers and their antioxidant activity.

Iron-catalyzed oxidation of soybean oil. Methanol extracts of the five bean cultivars examined in this study were effective in inhibiting iron-catalyzed oxidation of soybean oil at 50°C when compared to the control (Fig. 1). Rapid oxidative deterioration of the control oil began after approximately 7 d, but rapid deterioration did not begin until after 11 days for the soybean oil treated with bean extracts (Fig. 1). Thus, bean extracts were able to extend the induction period (IP), or the time during which an oil can effectively resist oxidation (23), by approximately 4 d (Fig. 1).

BHA was present at greater quantitites than the bean extracts (100–200×) but showed no significant difference in inhibiting oxidation compared to the control (Fig. 1). The ineffectiveness of BHA at inhibiting oxidation in an oil system was also observed by Augustin and Berry (24) with palm olein stored at room temperature or 60°C with 200 ppm BHA. The ineffectiveness of BHA may be explained by the observations of Kikugawa *et al.* (25) who reported that BHA is readily degraded by both heat and visible light and loses much of its antioxidant capacity when exposed to heat or light for more than 4 d. The breakdown products of BHA degrada-

TABLE 1
Polyphenolic Compound Content of Five Cultivars of Dry Beans *(Phaseolus vulgaris)*, Calculated as Chlorogenic Acid Equivalents (mg/g dry weight)

Cultivar	µg/mL ± SE[a]	mg/g bean, dry wt
Kidney	112.36 ± 2.54	5.61
Pink	107.15 ± 2.44	5.35
Pinto	93.93 ± 2.14	4.68
Black	77.51 ± 1.45	3.87
Great Northern	47.20 ± 0.41	2.35

[a]Polyphenolic content calculated as chlorogenic acid equivalents, with standard error.

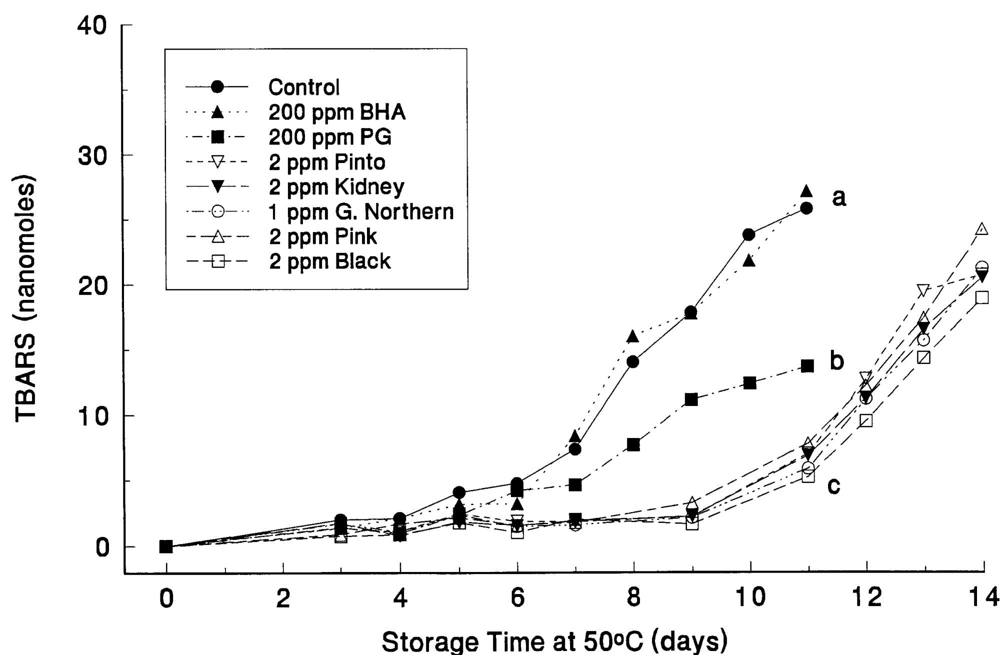

FIG. 1. Inhibition of 5 ppm Fe-catalyzed soybean oil oxidation by butylated hydroxyanisole, propyl gallate, and bean extracts. Treatments at 11 d with letters in common are not significantly different ($P \leq 0.05$). TBARS, thiobarbituric acid-reactive substances.

tion retain some antioxidant activity, but are generally less effective than intact BHA (25). In this experiment, PG was also present in greater quantities (100–200×) than bean polyphenols and inhibited soybean oil oxidation to an extent that was intermediate between BHA and bean extracts (Fig. 1). Augustin and Berry (24) reported that PG at 200 ppm effectively retarded oxidative deterioration of palm olein.

Beans with white seed coats typically have lower polyphenolic compound content than beans with colored seed coats (14), and the white-seed-coated Great Northern beans in this study were expected to have significantly lower antioxidant capacity than the colored seeds. However, no significant differences in antioxidant capacity were noted among the five bean cultivars (Fig. 1). These results are in contrast with the results of Tsuda *et al.* (17) who reported measuring no antioxidative activity in ethanolic extracts from pea beans *(P. vulgaris)* with a white seed coat. However, the authors' results are in agreement with Onyeneho and Hettiarachchy (10) who also reported considerable antioxidant activity in ethanol extracts from navy beans with white seed coats.

The ability of bean extracts to inhibit soybean oil oxidation in a dose-dependent manner was evaluated (Fig. 2). Extracts of both black and kidney beans produced significant ($P \leq 0.05$) reductions in soybean oil oxidation, compared to the control, after 7 d, and a consistent dose-dependent relationship was observed. However, no significant differences were noted between the antioxidative potential of black and kidney bean extracts at either a 1-, 9-, or 12-ppm concentration. In this experiment, methanol without any antioxidant was also added to the oil to demonstrate that the inhibition of soybean oil oxidation was not due to the presence of methanol (Fig. 2).

The antioxidant capacities of equal (10 ppm) quantities of pinto bean extract, PG, and ascorbic acid were evaluated. Little deterioration of the iron-catalyzed oil treated with pinto extract was observed after 7 d (Fig. 3). Oils with pinto extract and ascorbic acid were significantly different from the control, while oxidative inhibition by PG was not significantly ($P \leq 0.05$) different from the control.

A comparison of the capacity of pinto bean extract, PG, and ascorbic acid to inhibit oil oxidation in the absence of an iron catalyst at 50°C was conducted. Without an iron catalyst, the oils with either bean extract or ascorbic acid oxidized at a rate similar to that of the control oil (Fig. 3), and only PG effectively inhibited oxidation ($P \leq 0.05$).

To evaluate the stability of bean polyphenolics, the antioxidant potential of a fresh methanolic pinto bean extract was compared with that of an extract that had been stored for 3 mon at 4°C. Two concentrations (1 and 10 ppm) of pinto extract, used to verify the existence of a dose-dependent response, showed significant differences ($P \leq 0.05$) by concentration but not by age of extract (Fig. 4).

Oxidation of soybean oil without an iron catalyst. An iron catalyst is commonly used to accelerate oxidative reactions for evaluating the effectiveness of antioxidants. To control for the influence of the iron catalyst, additional experiments were conducted by using soybean oil without added iron catalyst. In the absence of an iron catalyst, bean extracts showed no significant differences in oil oxidation when compared to the control (Fig. 3). Similar results were reported by Shimada

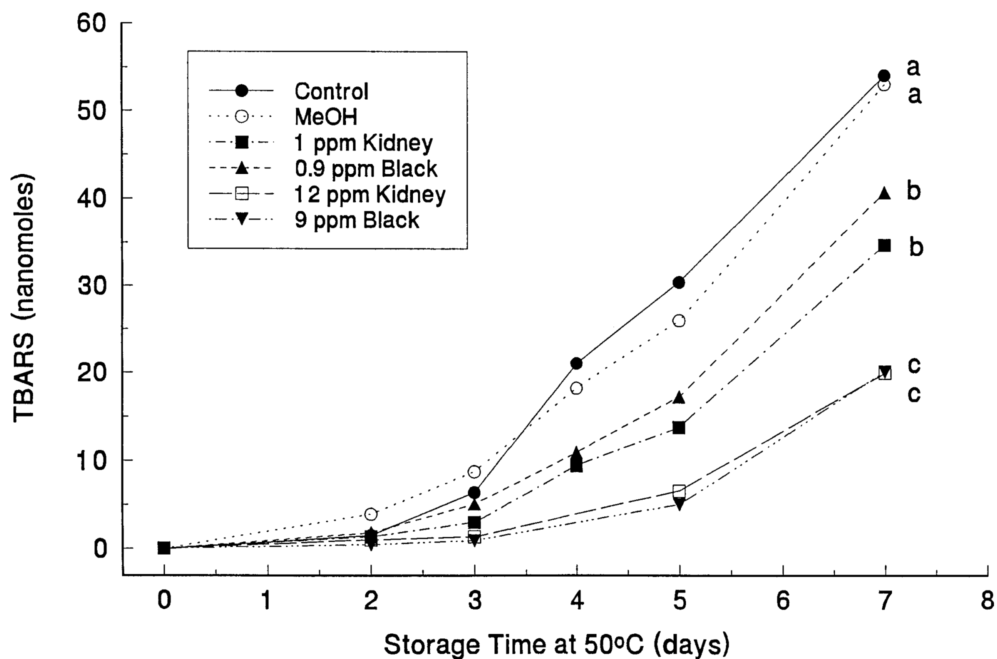

FIG. 2. Dose-dependent inhibition of 10 ppm Fe-catalyzed soybean oil oxidation by extracts of black and kidney beans. Treatments at 7 d with letters in common are not significantly different ($P \leq 0.05$). See Figure 1 for abbreviation.

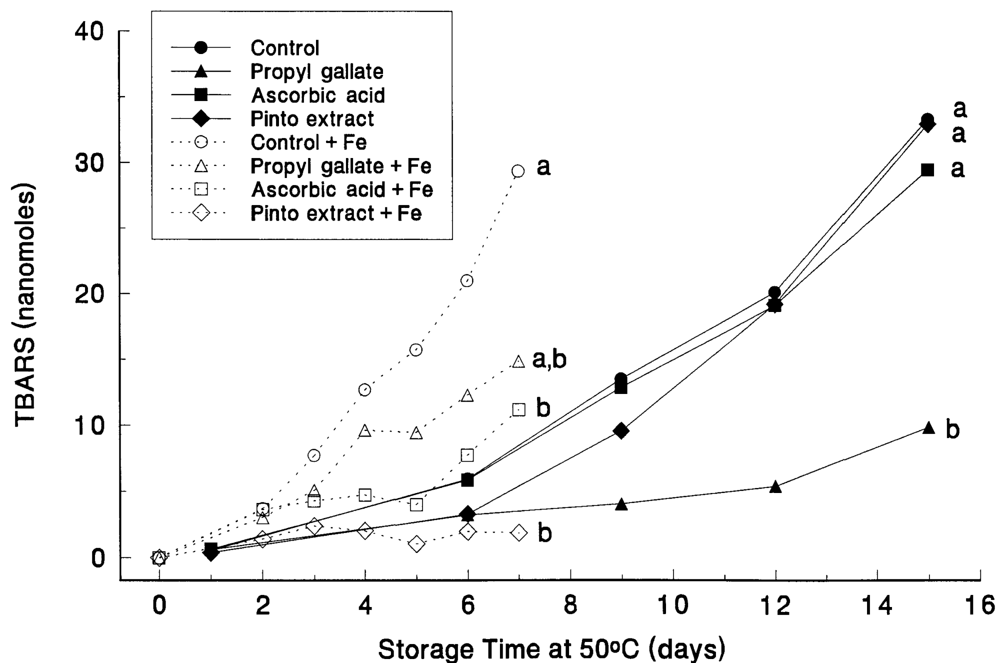

FIG. 3. Comparison of the antioxidant capacity of equal (10 ppm) quantities of pinto polyphenolics, propyl gallate, and ascorbic acid in soybean oil with and without iron catalyst. Treatments at 7 and 15 days with letters in common are not significantly different ($P \leq 0.05$). See Figure 1 for abbreviation.

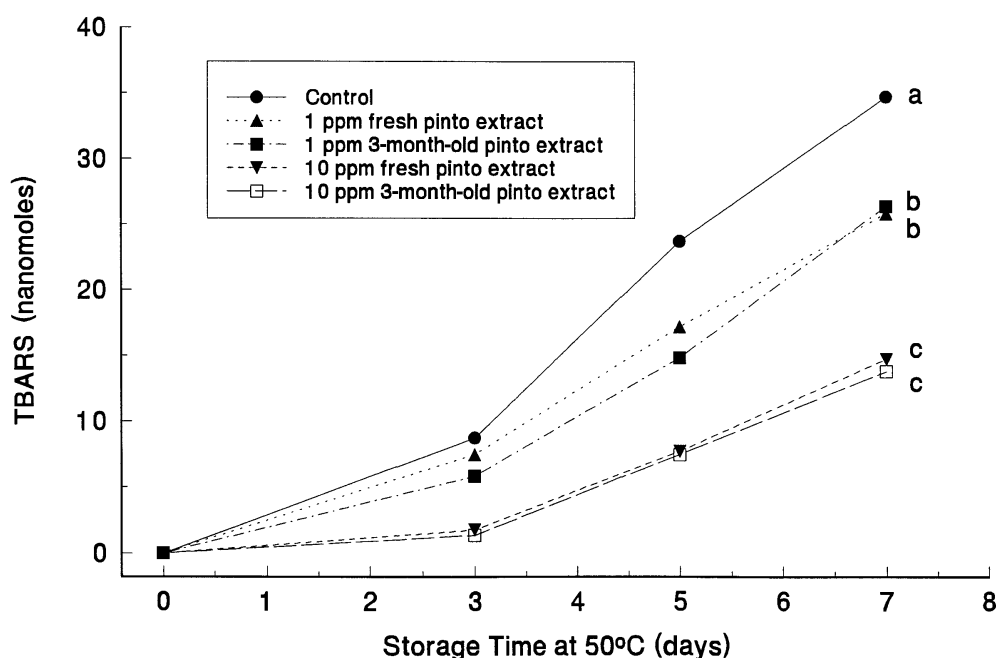

FIG. 4. Comparison of the antioxidant capacity of fresh and three-month-old extracts of pinto bean polyphenolics. Letters indicate differences among treatments at the same storage time (day 7) analyzed by analysis of variance and least significant difference test with $P \leq 0.05$. See Figure 1 for abbreviation.

et al. (26) who showed that xanthan effectively inhibited iron-catalyzed oxidation of soybean oil but did not inhibit oxidation when an iron catalyst was absent. They concluded that xanthan's antioxidant capacity resulted from its ability to chelate iron and make the iron unavailable as a catalyst for oxidative reactions.

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