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Antioxidant Activity in Common Beans (*Phaseolus vulgaris* L.)[§]

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Beans were pearled to evaluate the feasibility of increasing antioxidant activity and phenolic antioxidants. Phenolics were concentrated mostly in the hull fraction at about 56 mg of catechin equivalents per gram of sample. The methanolic extracts of the pearled bean samples were screened for antioxidant potential using the β-carotene−linoleate and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) in vitro model systems. The pearled material, also referred to as milled samples, exhibited antioxidant activity that correlated with phenolic content and inhibited DPPH significantly in a dose-dependent manner. Phenolics and antioxidant activities were also examined in chromatographic fractions of methanolic extracts of manually obtained hulls that represented a model used previously to ascertain antimutagenic activity. Fractions extracted with ethyl acetate/acetone and acetone displayed antioxidant activity, which implies potent free radical scavenging activity with antimutagenic activity.

KEYWORDS: Phenolics; antioxidant activity; β-carotene; DPPH; pearling; dry milling; beans

INTRODUCTION

Dry beans, a staple food in many Latin American countries, are receiving increasing attention as a functional food. Consumption of dry beans has been linked to reduced risk of diabetes and obesity (*1*) because of the markedly alternating effects on blood sugar and insulin response and thereby their potential use for prevention and control of diabetes (*2*). The inhibitory role of dry beans in reduction of coronary heart disease has also been noted (*3*) and confirmed recently (*4*). The study by Hughes et al. (*5*) provides experimental data to support existing epidemiological research linking high levels of dry bean consumption with reduced colon cancer risk. It is becoming clear that beans, as a source of dietary fiber, regulate gastric emptying and thus the rate of digestion and absorption, prolong the postprandial presence of intestinally derived lipoprotein, and augment the gastrointestinal response in humans (*6*).

The physiological effects of dry bean consumption may be due to the presence of abundant phytochemicals including polyphenolics, which possess both anticarcinogenic and antioxidant properties. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases. Bean extracts, especially from the hulls, are known to possess antioxidant activity. Thus, the crude methanolic extract of peas showed strong antioxidant activity due to the presence of pH-dependent pigments (*7*, *8*). A freeze-dried navy bean hull extract evaluated in storage studies with soy and sunflower oils showed stronger antioxidant activity than a BHA−BHT mixture (9). Mung bean hulls possess antioxidant activity, which has reducing power, scavenge DPPH radicals, and inhibit lipid peroxidation and nonlipid oxidative damage (*10*, *11*).

Our previous studies (*12*, *13*) showed that bean hulls contained large amounts of phenolic compounds, and their methanolic extract exhibited substantial antimutagenic activity against *Salmonella typhimurium* and aflatoxin B_1. This antimutagenic activity that involves formation of complexes between phenolic compounds and mutagens may apparently be mediated by the scavenging activity of the phenolics. Therefore, one of the objectives of this study was to evaluate the antioxidant activity of the methanolic extracts of bean hulls that served as a model previously to ascertain antimutagenic activities (*12*, *13*). Since the hull is rich in phenolics, a simple pearling method was used to obtain hulls and other bean fractions for subsequent assessment of their phenolics and antioxidant activities. Information on antioxidant activity will increase the understanding of the function of bean products in the diet to reduce chronic diseases.

MATERIALS AND METHODS

Dry beans (*Phaseolus vulgaris* cv. Flor de Mayo FM-38, red testa), grown and harvested in 2001, were kindly provided by Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP, Mexico). This cultivar was used in our previous studies (*12*, *13*) and was selected because it is preferred for consumption in the central region of Mexico.

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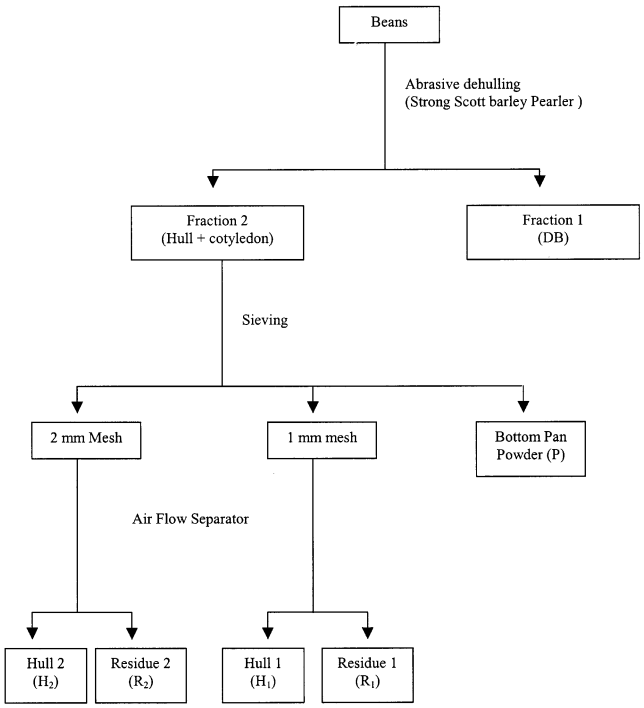

Figure 1. Flow diagram for the separation process of bean hulls.

Chemicals. Butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), (+)-catechin, β-carotene, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were from Sigma Chemical Co. (Toronto, ON, Canada). α,α′-Azodiisobutyramidine dihydrochloride (ADIBA) and linoleic acid were from Fluka (Sigma-Aldrich Canada Ltd.). Twin 20 was obtained from BDH Chemicals (Toronto, ON, Canada).

Sample Processing and Extractions. Six milling products were prepared from dry beans (*Phaseolus vulgaris* cv. FM38) as described in **Figure 1**. A Strong−Scott barley pearler was employed for abrasion, and manual sieving with 2- and 1-mm screens yielded three fractions: material retained on a 10-mesh U.S. standard, 2-mm sieve; material retained on an 18-mesh U.S. standard, 1-mm sieve; and material that passed through the 1-mm sieve. The hull and residue fractions were obtained by air separation in a laboratory aspirator (Cuthbert Co. Ltd., Winnipeg, MB, Canada) by adjusting air flow through the cylinder. A sample of beans was also dehulled manually after tempering in water to loosen the hulls, followed by lyophilization. All samples except the material that passed through the 1-mm sieve were ground to a fine powder in a small coffee mill.

Samples (0.2 g) were extracted with methanol (10 mL), 2 h in the dark, using a magnetic stirrer at room temperature, and centrifuged at 13000*g* for 10 min. The supernatant recovered was stored in the dark at −20 °C until analysis.

Total Phenolic Assay. The total phenolic content of methanolic bean extracts was determined by following the procedure of Mazza et al. (*14*), adapted for use with microplates. Briefly, the method consisted of mixing 10 μL of sample with 240 μL of a solution of 2% HCl in 75% ethanol in a 96-well microtitration flat-bottom plate (Corning). The absorbance of the solution was monitored at 280 nm with a spectrophotometer (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA) to measure total phenolics. Catechin dissolved in methanol at concentrations ranging from 0 to 200 mg/L was used as a standard. Phenolic content was expressed as milligrams equivalent of (+)-catechin per gram of sample.

Fractionation of Methanolic Extract. An aliquot (0.38 g) of the methanolic extract of manually obtained hull fraction (MH) was applied to a chromatographic column (3.4 × 50 cm) packed with silica gel (F_{254}, 0.063−0.20 mm; 70−230 mesh ASTM; Fluka) and eluted using solvents (100 mL each) of increasing polarity, starting with chloroform, a mixture of chloroform/ethyl acetate (1:1 v/v), ethyl acetate, ethyl acetate/acetone (1:1, v/v), acetone, acetone/methanol (1:1 v/v), methanol, and finally a mixture of methanol/water (1:1 v/v). Eight fractions (100 mL each) were collected; solvent was removed under vacuum using a rotary evaporator at 40 °C. The residues were weighed, solubilized in 2.5 mL of methanol, and stored in the dark at −20 °C until analysis.

***β*-Carotene Bleaching Method.** The antioxidant activity of isolated fractions was evaluated according to the β-carotene−linoleate model system (*15*) based on the procedures of Marco (*16*) and Velioglu et al. (*17*). Aliquots (20 μL) of each extract or fraction or BHT (1000 μM) and 200 μL of the β-carotene solution were added to a well in a 96-well flat-bottom microtitration plate (ICN Biomedicals Inc., Aurora, OH). The sample mixture was diluted by transferring 30 μL to another plate containing air-sparged distilled water (210 μL). Dilutions were done in triplicate since the β-carotene bleaching reaction was subject to noticeable variations. ADIBA (20 μL, 0.3 M) was added to each well to initiate the reaction. Absorbance readings were recorded in an MRX plate reader (Spectramax Plus 384) using a 450-nm filter at 0 min and at intervals of 10−90 min. Plates were kept in the dark at ambient temperature between readings. Antioxidant activity was calculated by four different methods described previously (*17*, *18*). For the first method, the log of the absorbance was plotted against time, as a kinetic curve, and the slope was expressed as the AOX value. The second method of calculation, based on first-order kinetics, was conducted as described by Al-Saikhan et al. (*19*):

$$\ln(a/b)/5 = \text{sample degradation rate}$$

where ln is the natural log, a is the initial absorbance at 450 nm and at time 0, b is the absorbance at 450 nm and at 10, 20, and 30 min, and 5 represents the time, in minutes. Antioxidant activity (AA) was also calculated as % inhibition relative to the control using the following relationship:

$$\text{AA} = (R_{\text{control}} - R_{\text{sample}}) \times 100/R_{\text{control}} \quad (1)$$

where R_{control} and R_{sample} are the degradation rates of β-carotene in reactant mix without and with sample extract, respectively. The AA values for different times were averaged to give one AA value for the sample.

The third method of expression, based on the oxidation ratio (ORR), was calculated using the equation proposed by Marinova et al. (*20*):

$$\text{ORR} = R_{\text{sample}}/R_{\text{control}} \quad (2)$$

where R_{sample} and R_{control} are the same as in eq 1.

In the fourth method, the antioxidant activity coefficient (AAC) was calculated according to Mallet et al. (*21*):

$$\text{AAC} = (A_{\text{sample 90}} - A_{\text{control 90}}) \times 1000/(A_{\text{control 0}} - A_{\text{control 90}}) \quad (3)$$

where $A_{\text{sample 90}}$ is the absorbance of the sample at $t = 90$ min, and $A_{\text{control 0}}$ is the absorbance of the control at $t = 0$ min.

DPPH Method. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), also known as 1,1-diphenyl-2-picrylhydrazyl or α,α-diphenyl-β-picrylhydrazyl, is a free radical used for assessing antioxidant activity. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorbance at 515 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substance. Determination of antioxidant activity by the DPPH method (*15*) was adapted for use with microplates. Briefly, a solution of DPPH (150 μM) was prepared in 80% methanol instead of 100%. Using 80% methanol had the advantage of a faster reaction rate for some compounds such as BHT and lower evaporative losses. Samples or standard was added to a well in a 96-well flat-bottom visible light plate containing 200 μL of DPPH solution. Samples were prepared in triplicate for each of four concentration used (0−500 μM). The plate was then covered and left in the dark at room temperature (~20 °C). After 30, 60, 75, 90, and 120 min, absorbance at 520 nm was measured in a spectrophotometer (Spectramax Plus 384).

A plot of $A_{520\text{ nm}}$ versus concentration was made for each time interval. The sample concentration with initial absorbance closest to that of the blank (DPPH + solvent) was chosen for final calculation of

Table 1. Yield of Milled Samples from *Phaseolus vulgaris* cv. FM-38

sample	yield (%)[a]
dehulled beans (DB)	63.42 a
hull 2 (H_2)	4.53 c
residue 2 (R_2)	4.07 c
hull 1 (H_1)	2.85 c
residue 1 (R_1)	5.12 c
powder (P)	18.20 b

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level.

antiradical activity (ARA), defined by the following equation from Burda and Oleszek (*22*):

$$\text{ARA} = 100 \times (1 - \text{absorbance of sample/absorbance of control}) \quad (4)$$

TEAC. The Trolox equivalent antioxidant capacity (TEAC) assay, based on the reaction of DPPH with Trolox, was used to compare the radical scavenging activity of a compound to that of Trolox, a water-soluble vitamin E analogue (*23*, *24*). Sample or standard (100 μM BHT or (+)-catechin, 22 μL) was added to a well in a 96-well flat-bottom visible light plate containing 200 μL of DPPH solution. The plate was then covered and left in the dark at room temperature (~20 °C). Trolox solutions in the 0−800 μM concentration ranges were employed for calibration. The decrease in absorbance at 520 nm 30 min after addition of a compound was used for calculating the TEAC. The TEAC value, an indicator of the antioxidant capacity of the sample relative to Trolox on a molar basis, was calculated as follows (*23*):

$$\text{TEAC}_{\text{sample}} = \Delta A_{\text{sample}}/(\text{slope [sample]}) \quad (5)$$

where ΔA_{sample} is the decrease in absorbance of the sample over 30 min, and [sample] is the concentration of the sample in micromolar (based on catechin equivalent).

Data were subjected to analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, and Pearson correlation according to SAS methods (*25*).

RESULTS AND DISCUSSION

Processing of beans yielded two highly purified hull fractions, H_2 and H_1, and a residue fraction R_2 that contained hull with small amounts of cotyledon (**Table 1**). The yield of the residue fraction R_1 was similar to that obtained by abrasive dehulling of kidney beans (~5%) (*26*). The combined yield of hull (H_1 + H_2) obtained from the pearling process (7.4%) and manually (about 8%) was similar to those reported previously (*12*). The yield of dehulled beans at 63% was similar to those of *Phaseolus aureaus* obtained by home-scale and traditional commercial methods (*27*). The proportion of hulls and cotyledons obtained manually from the beans were 8 and 90%, respectively.

Total phenolic contents of hull, whole, and dehulled beans ranged from 2.2 to 78.2 mg of catechin equivalents per gram of sample, and from 0.6 to 6.3 mg of catechin equivalents expressed per gram of beans (**Table 2**). Our results for total phenolics of bean samples are in good agreement with those reported previously (*12*, *28*−*30*). The highest concentration of phenolics was obtained for MH, followed by H_2 and H_1 fractions. The manually separated hull (MH) displayed 37-fold greater phenolic content than the whole bean flour (WBF) and dehulled beans (DB). The similar contents of phenolics for H_2 and R_2 suggest that air separation was inefficient in classifying these fractions and reflects their similar yields (**Table 1**). As expected, dehulled beans obtained either manually or by pearling had the lowest phenolic concentration. Phenolic content of whole bean flour (2.09 mg/g) was within the range reported for common beans (*31*).

Table 2. Phenolic Content in Methanolic Extracts of Milled Bean Samples

	concentration[a]	
sample[b]	mg/g sample[c]	mg/g beans[d]
H_2	56.31 b	2.55 b
R_2	55.30 b	2.25 bc
H_1	57.04 b	1.63 d
MH	78.24 a	6.28 a
DB	2.08 d	1.32 d
P	7.52 c	1.37 d
R_1	11.61 c	0.59 e
WBF	2.09 d	2.09 c
MDB	2.22 d	2.04 c

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level. [b] H_2, hull 2; R_2, residue 2; H_1, hull 1; MH, manually removed hull; DB, dehulled beans; P, powder; R_1, residue 1; WBF, whole bean flour; MDB, manually dehulled beans. [c] Concentration of phenolics expressed as milligram equivalents of (+)-catechin per gram of sample. [d] Concentration of phenolics expressed as milligram equivalents of (+)-catechin per gram of beans.

Table 3. Phenolic Content of Fractions Obtained from Chromatographic Separation of Manually Removed Hull (MH)[a]

			concentration[a]	
fraction[b]	yield (%)[a]	relative phenolic content (% of total)[a]	mg/g sample[c]	μg/g beans[d]
CH	3.6 g	2.5 e	55.4 f	11.0 e
CH/EA	3.9 e	2.9 e	63.5 f	15.0 e
EA	0.6 h	6.2 d	135.4 d	4.6 g
EA/AT	1.3 g	5.2 d	113.0 e	8.8 f
AT	5.6 c	27.0 b	586.7 b	208.0 b
AT/ME	44.0 b	35.5 a	772.4 a	2073.0 a
ME	5.7 d	19.8 c	430.7 c	148.0 c
ME/H_2O	34.8 a	0.8 f	18.1 g	56.2 d

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level. [b] CH, chloroform; CH/EA, chloroform/ethyl acetate (1:1, v/v); EA, ethyl acetate; EA/AT, ethyl acetate/acetone (1:1, v/v); AT, acetone; AT/ME, acetone/methanol (1:1, v/v); ME, methanol; ME/H_2O, methanol/water (1:1, v/v). [c] Concentration of phenolics expressed as milligram equivalents of (+)-catechin per gram of sample. [d] Concentration of phenolics expressed as microgram equivalents of (+)-catechin per gram of beans.

Methanolic extract of hulls obtained manually (MH) was further separated into eight different fractions with chloroform (CH), ethyl acetate/chloroform (EA/CH), ethyl acetate (EA), ethyl acetate/acetone (EA/AT), acetone (AT), acetone/methanol (AT/ME), and methanol/water (ME/H_2O) (**Table 3**) The acetone/methanol and aqueous methanol extracts averaged over 6 times the yields of acetone or methanol alone. Aqueous methanol (50%) is known to be the most efficient solvent for extraction of flavonoids in freeze-dried vegetables and fruits (*32*). The AT, AT/ME, and ME extracts had 7.5, 9.9, and 5.5 times the phenolic contents, respectively, of the crude extract of the manually hulled fraction. The amount of components extracted by solvents decreased in the following order: AT/ME > ME/H_2O > AT = ME > EA/CH > CH > EA/AT > EA. The yield of extract from bean hulls increased with increasing polarity of the solvents, as reported previously for peanut hulls (*33*) and buckwheat seed components (*34*). Polar solvents extracted phenolics with different efficiency, where the most polar solvents, AT and ME and their mixtures, extracted the maximum amount of phenolic compounds (587, 431, and 772 mg of catechin equivalent per gram, respectively) (**Table 3**). With other

Table 4. Antioxidant and Antiradical Activities of Methanolic Extracts from Samples of Beans (*Phaseolus vulgaris* cv. FM-38)

	antioxidant activity, β-carotene method[a]				antiradical activity, DPPH method[a]	
sample[b]	AOX[c]	AA[d]	ORR[e]	AAC[f]	ARA[g]	TEAC[h]
BHT	0.79	87.2 a	0.13 e	1192.9 a	14.5 d	1.2 d
catechin	3.70	55.9 b	0.43 d	426.4 b	47.9 a	3.1 a
H_2	6.21	31.9 d	0.68 b	277.2 d	30.7 b	2.4 b
R_2	6.30	31.6 d	0.67 b	147.9 e	28.3 c	2.1 c
H_1	8.47	16.6 e	0.83 a	15.0 f	27.5 c	2.1 c
MH	5.38	44.1 c	0.56 c	333.3 c	32.6 b	2.4 b

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level. $n = 3$. [b] BHT and catechin were measured at final concentrations of 1000 μM. [c] Antioxidant value. [d] Antioxidant activity. [e] Oxidation rate ratio. [f] Antioxidant activity coefficient. [g] Antiradical activity. [h] Trolox equivalent antioxidant capacity.

solvents and solvent mixtures, such as EA, EA/AT, EA/CH, CH, and ME/H_2O, the contributions of phenolics were 135, 113, 64, 55, and 18 mg of catechin equivalent per gram, respectively. That AT/ME extracted the maximum amount of phenolic compounds was not surprising since both solvents (acetone and methanol) are known to be efficient in extracting procyanidins from grape seed phenolics (*35*).

The β-carotene bleaching method was used to evaluate antioxidant activity in bean samples. Samples with high phenolic content were adjusted to 1000 μM (as μM catechin equivalents), while DB, P, WBF, and MDB, fractions with lower phenolic content, were tested at 150 μM catechin equivalent. BHT and (+)-catechin were used as standards at a concentration of 1000 μM. The measure of antioxidant activity by the β-carotene bleaching test showed that only samples MH, H_2, R_2, and H_1 (**Table 4**) exhibited antioxidant activity, indicating that it was concentrated near the surface of the seed. The other samples (DB, P, R_1, WBF, and MDB) showed little or no antioxidant activity, probably due to low phenolic content (<12 mg/g of sample). There was a trend toward reduced antioxidant activity with decreasing particle size. Antioxidant activity indices, AOX (antioxidant activity), AA (% inhibition relative to control), ORR (ratio), and AAC (antioxidant activity coefficient), for bean samples followed similar trends. Their values varied from highest to lowest as follows: MH > H_2 = R_2 > H_1 (**Table 4**). The extract from MH was more efficient than those from H_2, R_2, and H_1, and at 290 mg/L concentrations the inhibition of β-carotene bleaching was equivalent to 23.7 mg/L of BHT. Extracts from H_2 exhibited significantly higher AAC values than R_2, despite their similar AOX, AA, and ORR values. As expected, antioxidant activity of extracts reflected their phenolic content. The inhibition of β-carotene bleaching by bean extracts (**Figure 2**) showed that antioxidant activity of milled samples was lower than those of the standards BHT and catechin. Phenolic content, expressed as milligrams per gram of sample, showed significant correlation with AA, ORR, and AAC (r = 0.762, 0.762, and 0.702, respectively, with $p < 0.0001$ for AA and ORR, and $p < 0.05$ for AAC). When phenolic content was measured as milligrams equivalent of (+)-catechin per gram of beans, it showed strong correlation with AA and ORR, with $r > 0.91$ ($p < 0.0001$), and AAC, with $r = 0.825$ ($p < 0.005$).

The free radical scavenging activity of methanolic extract from the bean samples was also tested by measuring their ability to quench the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). This assay provides stoichiometric information with respect to the number of electrons taken up by the tested compounds in the presence of the stable free radical. The

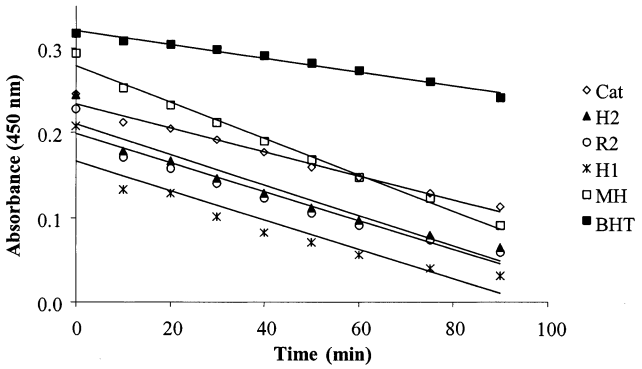

Figure 2. Antioxidant activity of methanolic extracts from milled samples of beans (*Phaseolus vulgaris* cv. FM-38) assayed by the β-carotene bleaching method (BHT and catechin at 1000 μM concentration were used as reference). H_2, hull 2; R_2, residue 2; H_1, hull 1; MH, manually obtained hull.

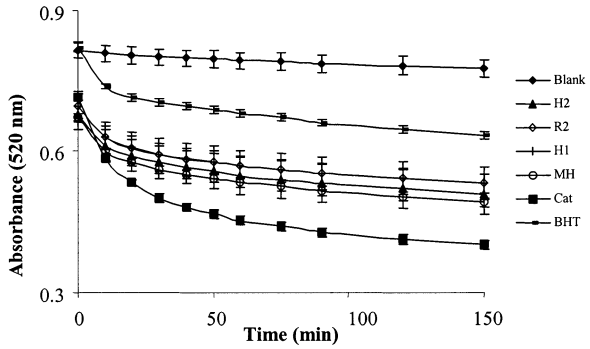

Figure 3. Antiradical activity of milled sample extracts of beans (*Phaseolus vulgaris* cv. FM-38) (BHT and catechin at 1000 μM concentration were used as reference). H_2, hull 2; R_2, residue 2; H_1, hull 1; MH, manually obtained hull. $n = 3$.

antiradical activity (ARA) of extracts from MH, H_2, R_2, and H_1 was significantly more effective than those of catechin but equivalent to about twice the quenching capacity of BHT at 55.1 mg/L (ARA = 34.2%; data extrapolated from BHT curve) (**Table 4**, **Figure 3**). Calculation of the scavenging activity in terms of the TEAC similarly showed that the values for the bean samples MH, H_2, R_2, and H_1 surpassed those of BHT but were lower than those of catechin (**Table 4**). Samples WBF, MDB, R_1, and P that showed no response in the β-carotene test presented weak antiradical activity (15.3, 7.2, 3.2, and 1.2%, respectively). The radical scavenging activity of catechin at 47.9% was similar to that reported previously, at 46.6% (*36*), validating the reproducibility of the assay.

Extracts from MH, H_2, R_2, and H_1 showed dose-dependent DPPH quenching capacity (**Figure 4**); MH and H_2 were more active than R_2 and H_1, especially at 100−250 mg/L. At 125 mg/L, the antiradical activities of MH and H_2 were comparable to those of catechin and BHT, while at 250 mg/L, all bean samples exhibited antiradical activity superior to that of BHT.

Only four (EA/AT, AT, AT/ME, and ME) of the eight fractions obtained by chromatographic separation exhibited antioxidant activity (**Table 5**). Fractions EA/AT and ME had the highest and lowest antioxidant activities of the four fractions, respectively (**Figure 5**). The antioxidant activities (AA and ORR) of fraction EA/AT were equivalent to those of catechin at 197 mg/mL. The AAC values of fractions EA/AT and AT were significantly not different from those of H_2 and R_2 (**Table 4**), respectively. The EA/AT and AT extractable fractions had antioxidant activities (AOX, AA, and ORR values) similar to

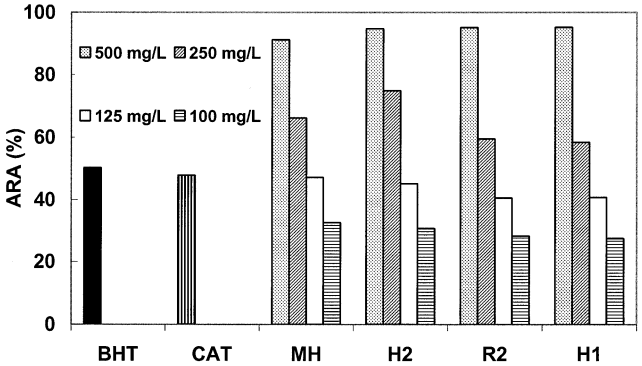

Figure 4. Antiradical activity of milled samples of cv. FM-38 at different concentrations evaluated by the DPPH method (BHT at 500 μM and catechin at 100 μM concentration were used as reference). H_2, hull 2; R_2, residue 2; H_1, hull 1; MH, manually obtained hull; CAT, catechin.

Table 5. Antioxidant and Antiradical Activities of Chromatographic Fractions Obtained from Manually Removed Hull Fraction (MH) of Beans (*Phaseolus vulgaris* cv. FM-38)

	antioxidant activity, β-carotene method[a]				antiradical activity, DPPH method[a]	
sample[b]	AOX[c]	AA[d]	ORR[e]	AAC[f]	ARA[g]	TEAC[h]
EA/AT	5.68	54.34 a	0.47 c	279.03 a	19.59 a	1.54 a
AT	6.60	47.43 a	0.53 c	157.89 b	3.72 d	0.41 c
AT/ME	7.38	38.00 b	0.62 b	132.89 bc	18.71 b	1.46 a
ME	7.43	28.26 c	0.72 a	72.37 c	16.59 c	1.16 b

[a] Means in a column followed by the same letter are not significantly different by Duncan's multiple range test at the 5% level. $n = 3$. [b] EA/AT, AT, AT/ME, and ME represent ethyl acetate/acetone (1:1, v/v), acetone, acetone/methanol (1:1, v/v), and methanol, respectively. [c] Antioxidant value. [d] Antioxidant activity. [e] Oxidation rate ratio. [f] Antioxidant activity coefficient. [g] Antiradical activity. [h] Trolox equivalent antioxidant capacity.

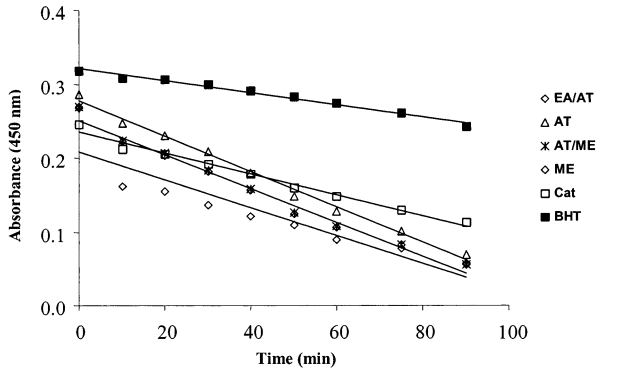

Figure 5. Antioxidant activity of fractions separated on silica gel chromatographic column from manually removed hull (MH) fraction of beans (*Phaseolus vulgaris* cv. FM-38) assayed by the β-carotene bleaching method (BHT and catechin at 1000 μM concentration were used as reference). EA/AT, AT, AT/ME, and ME represent ethyl acetate/acetone (1:1, v/v), acetone, acetone/methanol (1:1, v/v), and methanol, respectively.

those of the MH fraction (**Table 4**), suggesting that the latter may be represented in its entirety by fractions EA/AT and AT. Antiradical activities (ARA and TEAC) of EA/AT and AT/ME fractions were significantly higher than those of ME and AT and surpassed those of BHT, with the exception of AT (**Table 5**, **Figure 6**). When the values for phenolics were compared against the antioxidant and antiradical activities of the chromatographic fractions, a poor relationship existed among the parameters. For example, fraction AT/ME had lower antioxidant

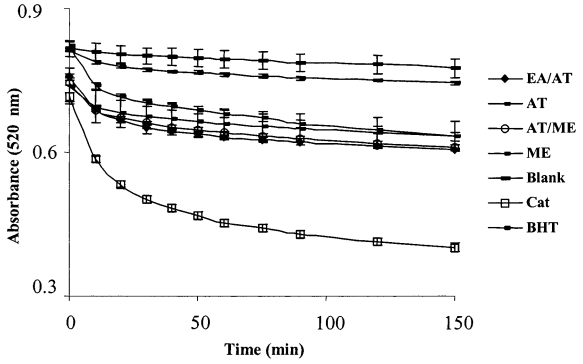

Figure 6. Antiradical activity of chromatographic fractions obtained from manually removed hull fraction (MH) of beans (*Phaseolus vulgaris* cv. FM-38) (BHT and catechin at 100 μM concentration were used as reference). EA/AT, AT, AT/ME, and ME represent ethyl acetate/acetone (1:1, v/v), acetone, acetone/methanol (1:1, v/v), and methanol, respectively.

activity (half of AAC value) than fraction EA/AT, although its phenolic content was 7 times higher than that of the latter. These results suggest that the antioxidant activity of the fractions remains unaccounted for by its phenolics, since the latter most likely is covalently bound or unglycosylated.

In our study, phenolic content in column fractions, expressed as milligrams per gram of sample, showed only moderate correlation with antioxidant activity, AAC ($r = 0.676$, $p < 0.05$). This is in contrast with a previous report (*37*) that the antioxidant activities of extracts fractionated from the hulls of fava beans, broad beans, lentil, and peas differed and were dependent on their contents of phenolic compounds.

Our data showed that bean phenolics exhibited antioxidant activity. On the other hand, when antiradical activity was evaluated, milled samples MH, R_2, H_1, and R_1, including fractions EA/AT, AT, AT/ME, and ME, showed better scavenging effects on the free radical DPPH, suggesting that these phenolics are better as free radical scavengers than as antioxidants. The results of this study provide evidence that bean hulls, and to a lesser extent other bean fractions obtained by dry dehulling, exhibit interesting antioxidant properties, expressed by their capacity to either scavenge free radicals or inhibit lipid peroxidation. The antioxidant effects of the manually separated hulls and their fractionated methanolic extracts may partly explain the reported antimutagenic activities. Further elucidation of the composition of the phenolic extract from the seed coat of beans and variability in antioxidant activity due to cultivars is currently in progress. This study demonstrates the feasibility of producing fractions of bean by dry milling that have elevated levels of phenolic antioxidants and enhanced antioxidant activities. The data suggest that it would be possible to manipulate the dry milling process to produce a fraction or fractions that contain the desired concentration of one or more of these antioxidants. Thus, a diet rich in beans and bean products (obtained by an optimized milling process) may be useful in the treatment of pathologies in which free radical production plays a key role.

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