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Phytic Acid, Phytase, Minerals, and Antioxidant Activity in Canadian Dry Bean (*Phaseolus vulgaris* L.) Cultivars

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Ten bean cultivars grown in southern Manitoba in 2006 were evaluated for variability in phytate, phenolic, and mineral contents, phytase activity, and antioxidant properties to elucidate the relationship of these components. Phytic acid content and phytase activity varied significantly among cultivars and market classes, ranging from 16.7 to 25.1 g/kg and from 224 to 361 phytase activity unit/kg of sample, respectively. The bean cultivars with total phenolic content ranging from 2.2 to 5.6 g of catechin equiv/kg of sample exhibited significant variation in antioxidant capacity [1.6−11.2 μM Trolox equiv (TE)/g of dry matter] and peroxyl radical scavenging activity (72−158 μM TE/g) using photochemiluminescence and fluorescence assays, respectively. Multivariate data analysis performed on 22 components analyzed in this study using principal component analysis and cluster methods demonstrate that differences in phytase, antioxidant activity, mineral contents, and bioavailability are much larger within market class than among bean cultivars.

KEYWORDS: Phytic acid; phytase; antioxidant activity; minerals; phenolics; multivariate analysis; beans; cultivar; market class; *Phaseolus vulgaris* L.; IP_6

INTRODUCTION

Phytic acid (*myo*-inositol hexakisphosphate or IP_6), a major phosphorus storage form in plants, and its salts, known as phytates, regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear mRNA export, and, potentially, hormone signaling important for plant and seed development (*1*), as well as animal and human nutrition (*2*). It is often regarded as an antinutrient because of strong mineral, protein, and starch binding properties, which decrease their bioavailability (*3*). Enzymatic degradation of phytic acid by exogenous phytase is already used in feed, particularly to improve mineral and protein utilization (*4, 5*), simultaneously reducing excessive phosphorus accumulation in the environment (*6*). However, several studies have shown potential beneficial health effects of phytates based on antioxidant and anticancer properties due to strong iron chelation, as well as mitigation of cardiovascular disease and renal stone formation (*7*−*11*).

Phytate (50 mg) added to test meal inhibits zinc absorption and calcium retention dose dependently in humans after 7 days (*12*). However, zinc requirements can be met and zinc balance maintained with inclusion of whole grains and legumes rich in phytic acid in the diet, although the risk of zinc deficiency is greater in persons consuming lactoovovegetarian compared with omnivorous diets (*13*). Phytate given as a supplement was quickly absorbed, reaching a maximum concentration in plasma 4 h after ingestion with low absorption rate and urinary excretion (*14, 15*).

Dry beans represent an important crop in Canada, one of the five main producing (0.32 Mt in 2005−2006) countries and the third largest exporter of dry beans, accounting for 11% of world exports (*16*). Consumption of dry beans has been linked to reduced risk of diabetes and obesity (*17, 18*), coronary heart disease (*19, 20*), colon cancer (*21, 22*), and gastrointestinal disorders (*23*). Epidemiological studies confirm the highly significant inverse correlation between bean intake and age-adjusted mortality for colon, breast, and prostate cancers (*24, 25*). Legume consumption (excluding soy food) may protect against prostate cancer in humans according to a recent multiethnic case control study (*25*).

Phytic acid in dry beans has been investigated in relation to mineral content and bioavailability (*26*), and phenolic acids have similarly been evaluated in concert with antioxidant activity. Phytate and polyphenolics play important roles in plant metabolism, stress, and pathogen resistance in addition to their beneficial effects in human diets by acting as anticarcinogens

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or by promoting health in other ways such as in decreasing the risk of heart disease or diabetes (see ref *27* and references cited therein). Phytic acid, phenolic acids, and oligosaccharides recently investigated together in beans differentiated cultivars with high and low metabolic densities (*28*). Similarly, the phytase−phytate−pectin hypothesis (*29*) proposed earlier has only recently been applied to common bean (*30*). However, the complex interaction and association of phytate, phytase, phenolic, mineral content, and antioxidant activity in dry beans has not been investigated.

This investigation extends our previous studies and describes variability in phytic acid and its relationships with antioxidant and phytase activities and mineral, phenolics, and protein contents of Canadian-grown dry bean cultivars. Such information provides a blueprint for developing practical strategies to improve bean quality and enhance market opportunities for bean products in the functional food and nutraceutical industry.

MATERIALS AND METHODS

Dry beans (*Phaseolus vulgaris*) grown and harvested in 2006 from the same field in the southern Manitoba dry bean production area were kindly provided by Agriculture and Agri-Food Canada (Morden, MB). Six market classes of beans, black (cvs. CDC Jet and Onyx), dark red kidney (cv. ROG 802), great northern (cv. Resolute), navy (cvs. AC Cruiser, AC Mast, Envoy, and Galley), pinto (cv. AC Ole), and small red (cv. AC Earlired), were used in this study.

All seed samples were ground in a Hammer mill at 5750 rpm to pass a 1 mm screen. Moisture was determined according to the AOAC vacuum oven method (*31*). Protein (N × 6.25) was determined by a nitrogen combustion method (FP-528, LECO Instruments Ltd., Mississauga, ON, Canada).

Assay of Phytic Acid. Phytic acid content was determined on the basis of the modified procedure of Latta and Eskin (*32*), using poly prep prefilled chromatographic columns (Bio-Rad Laboratories, Richmond, CA) containing an AG-1-X8 anion exchange resin (100−200 mesh chloride form, 0.8 × 4 cm) allowing isolation of phytate from bean extract. Briefly, ground bean sample (1 g) was extracted with 2.4% HCl (20 mL) by constant magnetic stirring (RT15 power S1, IKA Werbe GMBH & Co.) for 1 h at room temperature. After centrifugation (9000*g*, 20 min; Sorvall RC5B, Dupont Co., Wilmington, DE), the supernatant was diluted 25× with distilled water, and 10 mL of the diluted supernatant was added to the column. Interfering compounds and inorganic phosphorus were removed by washing with distilled water (10 mL) followed by 0.1 M NaCl (15 mL). Bound phytate was eluted with 0.7 M NaCl (3 × 10 mL), and an aliquot of the eluate (1.5 mL) was vortexed with 0.5 mL of Wade reagent [0.03% iron(III) chloride, 0.3% sulfosalicylic acid]. Absorbance of the salicylate−Fe(III) complex was monitored at 500 nm using a microplate reader (SPECTRAmax PLUS^{384}, Molecular Devices Corp., Sunnyvale, CA). The concentration of phytic acid and IP_6 equivalent was calculated from a similarly prepared standard curve obtained with sodium phytate (0−50 μg/mL, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and IP_6 standard solution (0−40 μg/mL, D-*myo*-inositol, 1312450-1, Calbiochem), respectively.

Phytase Assay. The assay for phytase activity described previously (*33*) was modified for both sample preparation and phytic acid digestion. Briefly, ground bean sample (1 g) was stirred for 10 min in 10 mL of 0.2 M citrate buffer (pH 5.5) and centrifuged (9000*g*, 20 min, 4 °C), and a portion of the supernatant (0.2 mL) was incubated in a water bath (37 °C) for 5 min. Sodium phytate solution (0.2 mL of 9 mM, Sigma-Aldrich Canada Ltd.) in 0.2 M citrate buffer was added to the supernatant to start enzymatic phytate hydrolysis. Incubation was continued for 15 min, and the reaction was stopped by adding 0.4 mL of 15% trichloroacetic acid. The mixture was microfuged (12000 rpm, 10 min, Mini spin plus, Eppendorf, Hamburg, Germany) prior to detection of free inorganic phosphate at 640 nm using a colorimetric detection kit (SensoLyte Malachite Green Phosphate Assay kit, AnaSpec, San Jose, CA). A calibration curve was prepared from known phytase activity standard solutions (0.01−0.25 FTU/2 mL, from *Aspergillus niger*, BASF, Ludwisgshafen, Germany). Data expressed in phosphate were converted to phytase activity (FTU/kg of sample). One phytase activity unit (FTU) is defined as the quantity of enzyme required to liberate 1 μmol inorganic phosphate per minute from sodium phytate at 37 °C.

Assay of Phenolics. Ground bean samples (40 mg) were extracted with aqueous ethanol 80% (v/v) (2 mL) using a vortex mixer at speed 6 (Mini vortex, VWR, model VM-3000, Thorofare, NJ) for 30 min and microfuged at 14000 rpm for 5 min (Mini Spin plus, Eppendorf, Brinkman Instruments). The recovered supernatant was stored at −20 °C in the dark until analysis. Phenolic content of ethanolic extracts was determined according to the procedure described previously (*34*). Briefly, the method consisted of adding 100 μL of extract with 150 μL of a solution of 2% HCl in 80% ethanol in a 96-well ultraviolet flat-bottom plate (Greiner Bio-One Inc., Longwood, FL). The absorbance of the solution was monitored at 280 nm after mixing for 2 min with a spectrophotometer (SPECTRAmax PLUS^{384}, Molecular Devices Corp.), using (+)-catechin (10−100 μg/mL) as standard for total phenolics. Results were expressed in grams of (+)-catechin equivalents per kilogram of sample.

Assay of Antioxidant Activities. Antioxidant activity was evaluated on the basis of photochemiluminescence (PCL) and fluorescence ($ORAC_{FL}$) assays. The PCL assay, based on the method of Popov and Lewin (*35*), was used to measure the antioxidant activity of extracts with a Photochem instrument (Analytik Jena, USA Inc., Delaware, OH) against superoxide anion radicals generated from luminol, a photosensitizer when exposed to UV light. The antioxidant activity of bean extracts was measured using the ACW kit (integral antioxidative capacity of water-soluble substances) (*34*) provided by the manufacturer. Chemiluminescence evolution was monitored by PCLsoft control and analysis software. Lag time (seconds) was used as the radical-scavenging activity. Antioxidant capacity estimated by comparison with a Trolox standard (0.05−6.0 μg/ mL) was expressed as grams of TEAC per kilogram of sample. Antioxidant index was obtained by dividing the antioxidant capacity by lag time multiplied by 1000 (antioxidant activity/lag time × 1000) (*34*). Phenolic extracts were microfuged (5 min at 14000 rpm) prior to analysis.

Antioxidant activity was also measured using the oxygen radical absorbance capacity ($ORAC_{FL}$) according to the procedure described by Prior et al. (*36*). A SpectraMax GeminiEM microplate fluorescence reader (Molecular Devices Co.) was used with excitation and emission wavelengths at 485 and 530 nm, respectively. Sample extracts and Trolox standards were diluted with 75 mM phosphate buffer (pH 7.4) prior to transfer to a 96-well microplate (Fluotrac 200, Greiner Bio-One Inc.). A peroxyl radical was generated by 2,2′-azobis(2-methyl-propionamide) dichloride (AAPH; Sigma Aldrich, USA) during measurement, and fluoresceine was used as the substrate. Measurements were taken every 2 min for 150 min upon addition of AAPH. Final ORAC values were calculated by using a regression equation between the Trolox concentration (0−15 μg/mL) and the net area under the curve [AUC in micromolar Trolox equivalents (TE) per 100 g of sample] and converted to grams per kilogram of sample. The ORAC method was performed on both phenolic extracts and phytic acid eluates.

Mineral Analysis. Mineral content was determined after ground bean sample (1 g) was dry ashed at 475 °C (3−4 h) and dissolved in 1.2 N HCl (25 mL) according to an AOAC method (*31*). Zinc, iron, and manganese contents in the extract were measured as described previously (*37*) after dilution with distilled water on a Varian (model 40P) atomic absorption spectrophotometer. Boron, calcium, copper, magnesium, phosphorus, and potassium contents were measured by inductively coupled argon plasma spectrophotometry (ICP, SpectroFlame, SCS Sciences Canada, Spectro Analytical Instruments, Kleve, Germany).

Statistical Analysis. 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 (*38*). Relationships among the 10 dry bean cultivars based on their content of 22 components were examined by principal component and cluster analyses with the matrix of distances calculated using the average

Table 1. Phytic Acid, IP_6, and Protein Contents, Phytase Activity, and Total Phenolic Content of Dry Bean Cultivars[a]

cultivar	market class	phytic acid	IP_6	phytase (FTU/kg)	phenolics (g of CE/kg)	protein (g/kg)
AC Cruiser	navy (NVY)	24.8 a	18.7 a	341.3 b	2.62 g	218.0 h
AC Earlired	small red (SR)	16.7 c	12.4 c	213.2 e	5.55 a	227.6 f
AC Mast	navy (NVY)	24.9 a	18.8 a	243.5 d	2.19 i	254.1 c
AC Ole	pinto (PT)	23.8 a	17.8 a	257.1 d	4.45 b	252.5 d
CDC Jet	black (BK)	19.9 b	14.8 b	159.5 f	3.11 e	256.2 b
Envoy	navy (NVY)	25.1 a	18.9 a	224.1 e	2.52 gh	244.6 e
Galley	navy (NVY)	23.5 a	17.8 a	330.9 bc	2.47 h	258.6 a
Onyx	black (BK)	18.6 b	14.0 b	169.4 f	3.80 c	244.1 e
Resolute	great northern (GNB)	23.7 a	17.8 a	322.2 c	2.87 f	212.4 i
ROG 802	dark red kidney (DRK)	19.9 b	14.7 b	360.7 a	3.49 d	220.5 g
means	black	19.3 y	14.4 y	164.4 y	3.46 x	250.1 x
	navy	24.6 x	18.6 x	287.7 x	2.45 y	243.8 y
overall means		22.1	16.6	265.5	3.16	238.8

[a] Means in a column with different letters are significantly different ($p < 0.05$).

Euclidean distance, and the phenogram and scattergram were constructed with SAS (*38*).

RESULTS AND DISCUSSION

The method used for phytic acid determination was sensitive, with good repeatability (4.8% relative standard deviation) and high phytate recovery (99%). The phytic acid contents of dry beans differed significantly ($p < 0.0001$) among cultivars and market classes (**Table 1**). Phytic acid level ranged from 16.7 to 25.1 g/kg for AC Earlired and the navy bean cultivar Envoy, respectively, representing a 33% difference in phytate content between these two extreme cultivars. Colored beans, particularly from the black and red market classes, had significantly lower phytic acid content than those of white bean. These values are comparable to the phytic acid content of Canadian dry bean cultivars (17−27 g/kg for AC Mast and CDC Jet, respectively) reported recently (*39*), but higher than those of older Canadian navy bean cultivars (12.5−17 g/kg) grown in Manitoba (*40*) and those found in wild and cultivated Mexican common beans (*28*). The values of phytic acid expressed as IP_6 generally represented about 75% (12.4−18.9 g/kg of IP_6 for AC Earlired and Envoy, respectively) of seed phytate, confirming that phosphate was present mainly as inositol hexaphosphate in dry bean samples. The 75% IP_6 is within the range (70−90%) of phytate reported previously (*26, 41*).

Phytase activity of dry beans differed significantly among cultivars and market classes, with ROG 802 having twice the activity of CDC Jet (**Table 1**). The black bean cultivars, CDC Jet and Onyx, with low phytate contents also expressed the lowest phytase activity, 42% less than those in navy beans. The combination of low phytate and high endogenous phytase in ROG 802 (phytate/phytase ratio of 5.5:1) may enable high phosphorus bioavailability by phytase depletion (dephytinisation), especially when fed to livestock. Differences in phytic acid and phytase of cultivars AC Cruiser, Galley, and Resolute were not significant, with a common phytate to phytase ratio of 7:1. The phytase activity of ROG 802 in relation to Onyx (2.1) was similar to those observed for a red kidney and black turtle beans (2.2) at pH 8 (*42*), whereas those of navy beans were comparable to small white beans reported previously (*43*). On the basis of phytate and phytase levels, dry bean cultivars may be segregated into those with high phytic acid and phytase (AC Cruiser, Galley, Resolute), low phytate and high phytase (ROG 802), low phytate and phytase (Onyx, CDC Jet), low phytate with moderate phytase (AC Earlired), and high phytate with moderate phytase levels (AC Ole). Six bean cultivars with high phytic acid levels (23.5−25.1 g/kg) had moderate to high phytase levels (224−341 FTU/kg).

Variation in phenolic content ranging from 2.2 (AC Mast) to 5.6 (AC Earlired) g of catechin equiv/kg of sample was significant ($P < 0.0001$) among cultivars and market classes. The phenolic content decreased in the following order based on bean market classes: small red > pinto > dark red kidney > black > great northern > navy, the same order observed previously in a study of 39 Canadian dry bean cultivars grown at four locations in Manitoba in 2003 (*44*). White beans, navy and great northern, contained significantly less phenolics than colored beans in accordance with previous studies (*26*). Dry beans contained 218 g/kg (Resolute) to 259 g/kg (Envoy) protein (dm) with significant variation among cultivars and market classes and between white and colored beans. The average protein content of Canadian bean cultivars (239 g/kg) was higher than that of cultivated Mexican varieties (206 g/kg) (*45*).

The antioxidant activity of dry bean cultivars, measured by the ORAC procedure, showed significant variations in scavenging activity of peroxyl radical among cultivars (**Table 2**). AC Cruiser, AC Ole, and ROG 802 exhibited high [>140 μM Trolox equiv (TE)/g of dry matter], whereas AC Mast and CDC Jet displayed low (<80 μM TE/g) antioxidant capacity. These values, with the exception of navy beans, are within the range reported previously for dry beans (*46*), but generally higher than recent values (*47*). The ORAC values within the black and navy market classes were significantly different, confirming results recently observed in black turtle beans (*47*). Because phytic acid has been shown to have a positive effect due to its antioxidant activity (*10*), its contribution to antioxidant activity was evaluated by the ORAC procedure on the phytate extract before (initial) and after (eluate) elution from the ion exchange column. The ORAC values of the extract prior to elution for two other cultivars (AC Pintoba and Onyx grown in 2004, not included in this study) gave values similar to those of their phenolic extracts and were therefore considered as total antioxidant activity. The contribution of phytic acid to antioxidant activity was highly dependent on cultivar. Generally, cultivars with high ORAC values (AC Cruiser, AC Ole, ROG 802) showed the lowest phytate contribution to antioxidant capacity. Conversely, cultivars with low ORAC values (AC Mast, CDC Jet) displayed high phytate participation in antioxidant capacity. The contribution of phytate to ORAC values was significantly different within the black and navy bean market classes, probably due to differences observed in ORAC values.

The PCL antioxidant assay generated two indicators: lag time (L, in seconds), a parameter of delay in the photochemical generation of superoxide radical anion, and antioxidant capacity (expressed as μM TE/g), a parameter of radical scavenging ability (*34*). Dry bean cultivars exhibited significant differences in antioxidant activity with a 7-fold variation in antioxidant capacity (1.6−11.2 μM TE/g), a 2-fold variation in lag time (71−163 s), and 4-fold variation in antioxidant index (16−70) based on PCL measurements (**Table 2**). The pinto bean cultivar AC Ole exhibited the highest antioxidant capacity and lag time, resulting in the highest antioxidant index. Colored beans displayed higher antioxidant capacity (3.7−11.2 μM TE/g), lower lag time, and higher antioxidant index (>38) than white beans. The low antioxidant capacity of white beans was similar to that reported previously for an acetone (80%) extract (*48*), but generally higher than those reported for narrow-leafed lupins (*34*). The antioxidant potential relative to phenolic content (PAOXI), calculated by dividing the antioxidant capacity (mg of TE/g) by the phenolic content (g/kg), of colored beans (3.4

Table 2. Antioxidant Properties of Dry Bean Cultivars[a]

		$ORAC_{FL}$ method		PCL method			
cultivar	MK[b]	total AA[c] (μM TE/g)	phytate[d] AA (%)	TEAC[e] (μM TE/g)	lag time (s)	antioxidant index[f]	PAOXI[g]
AC Cruiser	NVY	157.9 a	30.4 e	1.79 e	94.8 e	18.9 e	5.85 a
AC Earlired	SR	99.7 e	49.6 c	4.55 c	70.5 f	64.4 b	4.93 b
AC Mast	NVY	76.9 f	55.9 b	1.92 e	120.4 c	15.9 f	4.56 b
AC Ole	PT	150.9 ab	29.7 e	11.24 a	162.6 a	69.5 a	1.58 c
CDC Jet	BK	72.0 f	69.8 a	3.65 d	94.6 e	38.6 c	3.42 c
Envoy	NVY	104.4 e	39.1 d	2.09 e	111.5 cd	18.7 e	4.87 b
Galley	NVY	116.5 d	54.8 b	1.57 e	98.5 de	15.9 f	6.32 a
Onyx	BK	130.2 c	38.0 d	4.71 c	109.9 cd	42.8 c	3.29 c
Resolute	GNB	101.3 e	59.7 b	1.95 e	101.7 de	19.2 e	5.98 a
ROG 802	DRK	143.7 b	30.4 e	5.68 b	144.0 b	39.6 d	2.48 d
means	black	101.1 x	51.6 x	4.22 x	102.9 x	40.9 x	3.35 y
	navy	113.9 x	45.1 x	1.86 y	105.8 x	17.6 y	5.40 x
overall mean		115.3	45.7	3.61	108.8	32.5	4.45

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Market class abbreviation as in **Table 1**. [c] Total antioxidant activity (AA) evaluated by fluorescence ORAC assay. [d] Phytate contribution to the ORAC total antioxidant activity. [e] Trolox equivalent antioxidant activity evaluated by the PCL procedure (μM TE/g). [f] Antioxidant index = TEAC/lag time $\times$ 1000. [g] Antioxidant potential relative to phenolic content.

Table 3. Mineral Content (Milligrams per Kilogram) of Dry Bean Cultivars[a]

cultivar	MK[b]	Ca	Mg	K	P	B	Cu	Zn	Fe	Mn
AC Cruiser	NVY	1381 de	2002 b	17286 f	6207 b	13.7 b	8.8 a	25.0 c	55.0 b	13.3 d
AC Earlired	SR	1344 ef	1677 f	17305 f	5727 d	11.3 c	0.4 h	18.9 e	34.1 e	13.2 d
AC Mast	NVY	1649 b	1952 c	17944 d	6564 a	11.1 c	3.1 e	18.3 e	44.1 de	15.8 c
AC Ole	PT	1328 f	1758 e	18638 b	6194 b	10.2 e	3.6 d	21.5 d	43.6 d	13.3 d
CDC Jet	BK	1229 g	1843 d	16166 h	5044 f	10.5 de	2.4 f	26.6 b	46.1 cd	13.8 d
Envoy	NVY	2025 a	1841 d	16945 g	5695 d	9.4 f	6.1 c	27.1 ab	53.3 bc	17.2 b
Galley	NVY	2035 a	2078 a	19464 a	6447 a	10.9 cd	1.0 g	21.2 d	42.2 d	19.4 a
Onyx	BK	1408 d	1746 e	18428 c	5483 e	14.7 a	0.1 i	18.8 e	49.4 bcd	13.3 d
Resolute	GNB	1516 c	1693 f	17585 e	6034 c	10.2 e	0.9 g	18.5 e	28.0 e	13.5 d
ROG 802	DRK	823 h	1525 g	17090 g	5660 d	11.1 c	7.1 b	28.3 a	66.6 a	10.8 e
means	BK	1300 x	1804 x	17070 y	5220 y	12.2 x	1.5 y	23.5 x	47.4 x	13.6 x
	NVY	1749 x	1958 x	17768 x	6209 x	11.3 x	5.1 x	23.1 x	49.2 x	16.2 x
overall mean		1456	1804	17595	5901	11.2	3.6	22.6	46.3	14.2

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Market class abbreviation as in **Table 1**.

for black beans) was significantly lower than those of white beans (5.4 for navy beans), mainly reflecting differences in antioxidant capacity. Thus, cultivars AC Ole and Galley with the highest and lowest antioxidant capacity produced diametrically opposed, lowest and highest, PAOXI, respectively.

Cultivars varied significantly in mineral content (**Table 3**). The amount of Ca in the seeds varied among cultivars and ranged from 823 to 2035 mg/kg of dry weight. A similar range of Ca content (731−1929 mg/kg) has been reported for dry bean cultivars grown in Ethiopia (*49*). Navy bean contained 35% more Ca on average than black beans and twice that of the dark red kidney cultivar ROG 802, thereby confirming a similar previous result (*50*). The variation (60%) in Ca content observed in this study was higher than those reported for Mexican accessions of cultivated and wild common beans (*45, 51*) and other bean genotypes (*52, 53*). The average concentration of Ca in white bean cultivars was significantly (40%) higher than those of colored beans. Our mean Ca content for navy bean (1749 mg/kg) was similar (1.77 g/kg) to those reported by the USDA (*54*). Cultivars Galley and ROG 802, with the highest and lowest Ca contents, also represented the maximum and minimum (2078 and 1525 mg/kg) Mg contents, respectively. The range of Mg content in Canadian bean cultivars was similar to those reported previously (*50, 51, 53*) but lower than those of 36 common bean cultivars grown under similar conditions in Washington state (*52*) and white bean cultivars from Sudan (*55*). Potassium was the most abundant (17595 mg/kg) mineral with a range (from 19484 to 16166 mg/kg) higher than those reported previously (*50, 53, 55*). Differences in K content of navy bean AC Cruiser and AC Earlired and between Envoy and ROG 802 were not significant. Phosphorus content of Canadian dry bean cultivars (from 5044 to 6447 mg/kg) was higher than previously reported (*53, 55, 56*), although the relative variation (22%) was lower than that reported for colored beans (36%). The high phosphorus content corresponded to those generated during the phytase assay. Three of the navy bean cultivars, AC Mast, Galley, and AC Cruiser, had the highest (>6200 mg/kg), whereas black beans had the lowest phosphorus content, respectively. The common bean is considered to have a high demand for boron according to Stass et al. (*57*). Boron contents of beans ranged from 9.4 to 14.4 mg/kg, values higher than those reported in dry bean (*58*). A similar range (10.0−15.4 mg/kg) has been observed for B concentration in flag leaves of spring wheat cultivars with contrasting field responses to B and cold (*59*). In this regard, Envoy and Onyx represent cultivar differences in B uptake and accumulation and probably suggest a higher sensitivity to cold temperatures and risk of frost damage for Envoy. Two groups of cultivars (AC Earlired, AC Mast, ROG 802 and AC Ole, CDC Jet, Resolute) showed no significant differences in B contents. Copper was the least abundant (3.6 mg/kg), but most variable (0.1−8.8 mg/kg), mineral in Canadian dry bean cultivars and was similar to levels reported previously (*58*). Cu contents of cultivars AC Cruiser, Envoy, and ROG 802 were within the range (from 5 to 10 mg/kg) reported for colored beans (*54*). Galley and Resolute showed no significant differences in Cu content, and together with AC

Earlired and Onyx represented cultivars with low copper (≤1 mg/kg) content. Cultivar Onyx with the highest B content had the lowest Cu content. Dry beans, particularly pinto beans, area good source of dietary Cu with respect to both concentration and bioavailability (*60*). Dry bean cultivars can be segregated into three groups based on zinc content: low (<19 mg/kg, AC Earlired, AC Mast, Onyx, and Resolute), average (20−25 mg/kg, AC Cruiser, AC Ole, and Galley), and high (>25 mg/kg, CDC Jet, Envoy, and ROG 802). Zinc contents of cultivars were within the range (17−28 mg/kg) reported for colored beans (*53*), but showed only half the variation reported for Mexican accessions of cultivated and wild common beans (10−33 mg/kg) (*45*). Cultivars ROG 802 and Resolute with the highest and lowest Zn contents, respectively, were reversed in their order of Fe contents. Differences in Fe contents (42−49 mg/kg) of five cultivars, AC Mast, AC Ole, CDC Jet, Galley, and Onyx, were not significant. The variation in Fe content observed in this study was similar to that reported for over 1000 accessions of common beans (34−89 mg/kg) (*27*) and higher than that reported (48−74 mg/kg) for Mesoamerican and Andean genotypes (*53*). On the basis of Fe and Zn contents, cultivars AC Earlired and Resolute can be classified as low-Fe and low-Zn genotypes, whereas cultivars ROG 802, AC Cruiser, and Envoy conform to the high-Fe and high-Zn genotypes. Differences in Mn contents (13.2−13.8 mg/kg) were minimal in 6 of the 10 cultivars, whereas those of the navy bean cultivars AC Mast, Envoy, and Galley were highly significant. The average Mn content of colored beans (12.8 mg/kg) was significantly lower than that of white beans (15.6 mg/kg) and corresponded with previously reported values (*53*). The average Ca, B, and Mn contents of the Canadian dry bean cultivars were similar to those of cultivated accessions ($n = 1031$) evaluated in the micronutrients project at the Centro Internacional de Agricultura Tropical (CIAT) (*61*). However, the average P, Cu, Zn, and Fe values were closer to those reported for wild beans ($n = 119$) (*61*).

Dry beans rich in phytate can be potentially antagonistic to the utilization of zinc and iron, although the mechanism of the antagonistic action remains largely unresolved (*62*). The phytate/zinc molar ratio has been suggested as an indicator of zinc bioavailability because phytic acid found in legumes is thought to be a major contributor to reduced zinc availability in foods (*63*). Furthermore, the phytate × calcium/zinc molar ratio has been used to evaluate wild and weedy accessions of common bean as a better predictor of zinc bioavailability (*45*). Similarly, the phytate/iron molar ratio has been used as an indicator of iron bioavailability in beans (*53*). The bean cultivars can be segregated into those with high (≥100) (AC Cruiser, AC Mast, AC Ole, Galley, Onyx, Resolute) and low (<100) (AC Earlired, CDC Jet, Envoy, ROG 802) phytate to zinc ratios (**Table 4**). Similar differences in phytate zinc molar ratio have been reported previously in beans (*45*). However, the bioavailability of zinc from these cultivars, based on their phytate to zinc molar ratio (70−135), were low because ratios above 20 might be expected to reduce zinc utilization as a result of phytate antagonism, especially for nonruminant farm animals (*62*). The phytic acid × (calcium/zinc) [PA × (Ca/Zn)] molar ratio ranged from 1.4 (ROG 802) to 5.6 (AC Mast) for the Canadian bean cultivars, values generally lower than those reported previously (*45*). The colored bean cultivars AC Earlired, CDC Jet, Onyx, and ROG 802 had low PA × (Ca/Zn) molar ratios of 2.97, 2.30, 3.50, and 1.4, respectively. Growth-depressing effects due to zinc bioavailability based on PA × (Ca/Zn) molar ratio are considered to be severe when the ratio exceeds 3.5 (*63*). Thus, cultivars with high phytate to zinc ratio, primarily white beans, would potentially cause significant decline in zinc bioavailability. Similar trends of high phytate × (Ca/Zn) ratio and phytate/iron ratio were observed for white beans, further confirming their potential inhibitory effects on zinc and iron bioavailability. ROG 802 may therefore be considered to be the best cultivar in regard to mineral bioavailability due to its low phytate/mineral ratio and its high phytase content, which can modify the influences of both iron and zinc utilization (*62*). Ratios of 1:1 for calcium/magnesium and 5:1 for zinc/copper are considered to be appropriate for human health (*64*), and cultivars Envoy and, to a lesser extent, AC Mast and AC Ole, met these criteria.

Table 4. Molar Ratio of Phytic Acid to Mineral Content[a]

cultivar	MK[b]	PA/Zn	PA × (Ca/Zn)	PA/Fe	Ca/Mg	Fe/Zn	Zn/Cu
AC Cruiser	NVY	100.2 d	3.45 c	35.7 de	0.69 g	2.40 ab	2.8 b
AC Earlired	SR	87.6 e	2.97 d	41.9 bcd	0.80 e	1.81 cd	43.7 b
AC Mast	NVY	135.0 a	5.57 a	47.9 b	0.85 d	2.41 ab	5.9 b
AC Ole	PT	109.7 c	3.63 c	46.1 bc	0.76 f	2.04 c	6.0 b
CDC Jet	BK	74.3 f	2.30 e	36.6 de	0.67 h	1.73 de	11.1 b
Envoy	NVY	91.7 e	4.63 b	39.8 cde	1.10 a	1.97 cd	4.4 b
Galley	NVY	106.7 cd	5.45 a	45.9 bc	0.98 b	1.99 c	22.1 b
Onyx	BK	100.5 d	3.50 c	32.8 e	0.81 e	2.62 a	207.1 a
Resolute	GNB	126.9 b	4.80 b	72.1 a	0.89 c	1.51 e	19.8 b
ROG 802	DRK	69.6 f	1.40 f	25.3 f	0.54 i	2.35 b	4.0 b
means	W	113.4 x	4.83 x	49.4 x	0.90 x	2.38 x	11.0 y
	C	87.5 y	2.71 y	36.8 y	0.71 y	2.42 x	54.4 x
	BK	84.8 y	2.78 y	35.1 x	0.72 x	2.09 x	99.6 x
	NVY	109.4 x	4.84 x	42.6 x	0.90 x	2.19 x	8.6 y
overall mean		99.97	3.73	42.89	0.80	2.05	31.1

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Market class abbreviation as in **Table 1**. W, white beans; C, colored beans.

A statistically significant correlation ($r = 0.66$; $p = 0.0001$) existed between calcium and magnesium, and both were positively correlated ($r = 0.93$ and 0.73; $p < 0.0001$) with manganese content, whereas iron and zinc were positively correlated ($r = 0.73$ and 0.75; $p < 0.0001$) with copper contents (**Table 5**). Zinc and iron had a significantly higher correlation of 0.75 ($p < 0.0001$) than those reported previously (0.52) across different genotypes (*61*). Potassium was positively correlated with phosphorus ($r = 0.68$; $p < 0.0001$) and inversely with zinc ($r = -0.57$; $p = 0.002$). Phosphorus was positively correlated with phytic acid and IP_6 contents ($r = 0.59$; $p < 0.001$) and phytase activity ($r = 0.54$; $p < 0.005$). Calcium, magnesium, and manganese contents were positively correlated with PAOXI ($r = 0.53-0.58$; $p < 0.005$), which in turn was highly correlated with antioxidant activity measured by chemiluminescence ($r = 0.92$; $p < 0.0001$). However, the antioxidant index was inversely correlated with calcium, magnesium, and manganese ($r = -0.50$; $p < 0.01$) contents. Calcium, magnesium, and phosphorus, minerals involved in bone health, were moderately correlated ($r = 0.51-0.59$; $p < 0.01$) with phytic acid content, probably due to phytate binding properties, a high phytate content corresponding to a high mineral concentration.

Comparison of the phytic acid content of bean cultivars with phenolic content and antioxidant and antiradical activities revealed strong correlation (**Table 5**). Phytic acid and IP_6 contents were inversely related to phenolic content ($r = -0.67$, $p < 0.0001$), which correlated significantly with antioxidant index ($r = 0.92$; $p < 0.0001$) as well as antioxidant capacity ($r = 0.63$; $p < 0.0005$), whereas phytate content showed a moderate inverse correlation with AI only ($r = -0.58$, $p < 0.001$). High and low phytate cultivars AC Mast and AC Earlired (24.95 and 16.68 g/kg) corresponded to low and high phenolic contents (2.2 and 5.6 g of catechin equiv/kg), which were in turn related to low and high antioxidant indices (15.9 and 64.4),

Table 5. Correlation Coefficients for Phytic Acid, Phytase, Phenolics, Minerals, and Antioxidant Activities of Beans[a]

	AA[b]	lag	AI[c]	PAOXI[d]	phytate	IP6[e]	Ca	Mg	P	Zn	Fe	Mn
phenolics	0.63 b	−0.05	0.92 a	−0.37	−0.67 a	−0.67 a	−0.42	−0.54	−0.20	−0.30	−0.26	−0.45
AA		0.63 a	0.85 a	−0.83 a	−0.22	−0.23	−0.48	−0.47	−0.11	0.02	0.10	−0.47
AI				−0.66 a	−0.58 b	−0.59 b	−0.51 d	−0.52 c	−0.29	−0.11	−0.12	−0.50 d
PAOXI					0.24	0.26	0.58 c	0.54 c	0.35	−0.26	−0.36	0.53 c
phytate						0.98 a	0.51 d	0.54 c	0.59 b	0.07	0.05	0.46
IP_6							0.54 c	0.55 c	0.60 b	0.05	0.04	0.46
phytase							0.13	−0.01	0.54 c	0.15	0.25	−0.13
Ca								0.66 a	0.39	−0.25	−0.31	0.93 a
Mg									0.46	−0.10	−0.07	0.73 a
K									0.68 a	−0.57 c	−0.22	0.36
Cu										0.73 a	0.75 a	−0.18
Zn											0.75 a	−0.14

[a] Letters indicate significance: a, $p < 0.0001$; b, $p < 0.001$; c, $p < 0.005$; d, $p < 0.01$. [b] Total antioxidant activity (AA) evaluated by fluorescence ORAC assay. [c] Antioxidant index = TEAC/lag time × 1000. [d] Antioxidant potential relative to phenolic content. [e] Phytic acid content expressed as D-myoinositol.

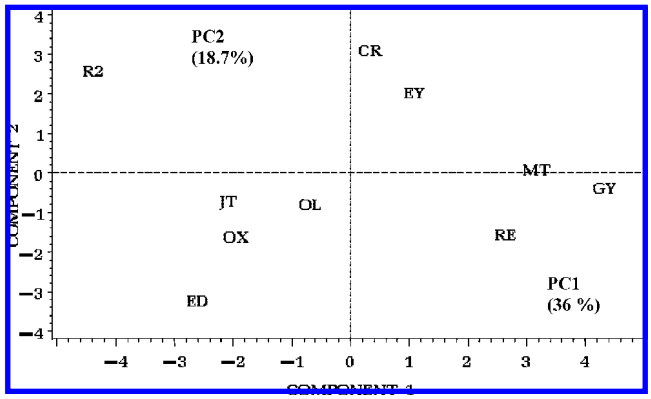

Figure 1. Classification of dry bean cultivars grouped according to principal components 1 and 2. Cultivars AC Cruiser, AC Earlired, AC Mast, AC Ole, CDC Jet, Envoy, Galley, Onyx, Resolute, and ROG 802 are denoted, respectively, CR, ED, MT, OL, JT, EY, GY, OX, RE, and R2.

respectively, suggesting a low antioxidant capacity but stronger antiradical generation properties (*35*). PA eluate, which contained only phytates, exhibited antioxidant activity. Thus, PA acts as one of the beans antioxidant compounds, and its contribution can be either low (AC Cruiser) or high (CDC Jet). The ORAC method used for evaluating antioxidant activity had higher reproducibility (lower coefficient of variation) and therefore showed more significant variations among cultivars than the PCL assay. The antioxidant methods were not correlated, indicating differences in mode of operation and measurements.

Principal component analysis was performed on the 22 constituents analyzed in this study as a starting model to evaluate their complex interactions. The PCA generated six factors with eigenvalues exceeding 1.0 (Kaiser's rule) that accounted for 93% of the total variance. The first component (PC1) accounting for 36% of total variance had large positive loadings for the PA × (Ca/Zn) (0.341) molar ratio, an indicator of zinc bioavailability. The second component (PC2, 18.7%) was primarily influenced by positive loadings of Cu (0.462), Zn (0.367), and Fe (0.375). Although smaller variation was assigned to PC3 (13.4%), it was influenced by ORAC value (0.437), phytase (0.330), K (0.356), and P (0.337).

The score plot of the first two principal components, accounting for 55% of the total variance (**Figure 1**), revealed strong differences between market class and abundance of phytase and minerals in bean cultivars. Thus, white beans (AC Cruiser, Envoy, AC Mast, Resolute, Galley) formed a distinct group on the right side (positive) of the plot and were mainly dominated by high PA × (Ca/Zn) molar ratio (>3.4). The PCA plot grouped the colored beans (AC Earlired, Onyx, CDC Jet, AC Ole) (lower left quadrant) primarily on the basis of phytase and phytate contents. Cultivar ROG 802 was differentiated by its high phytase, Zn, and Fe contents. Addition of the third component in the PCA plot did not change the overall grouping of the bean cultivars despite the high cumulative variance (68%). Although smaller variations were assigned to PC4 (11.9%), PC5 (8.3%), and PC6 (5.1%), they were nevertheless important delineating factors for bean cultivars. The dendogram (**Figure**

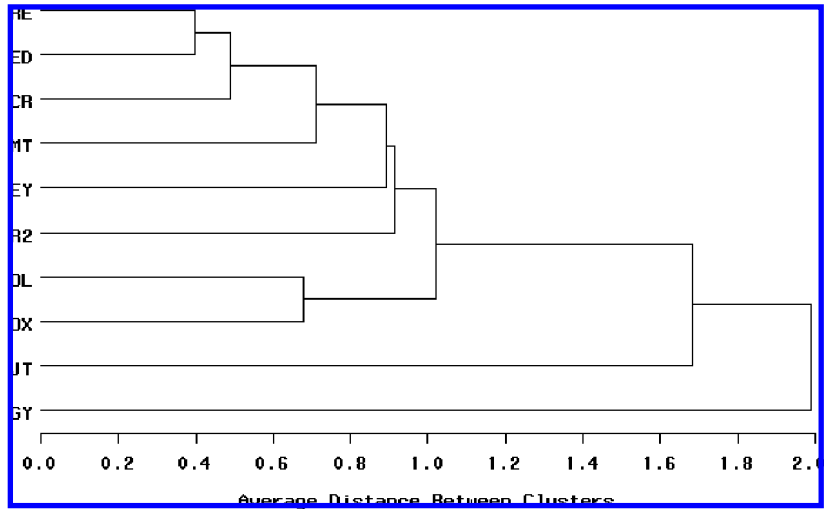

Figure 2. Dendogram of cluster analysis performed on 22 constituents of dry bean cultivars Resolute (RE), AC Earlired (ED), AC Cruiser (CR), AC Mast (MT), Envoy (EY), ROG 802 (R2), AC Ole (OL), Onyx (OX), CDC Jet (JT), and Galley (GY).

2) obtained from cluster analysis based on the same 22 variables displayed three discrete clusters. Galley and CDC Jet separated from the other cultivars with the highest average distance based on phytase, K, and P contents and ORAC and antioxidant values. Resolute and AC Earlired were the two most similar cultivars on the basis of their minimal distance because of their low Zn, Cu, and Mg contents and similar ORAC values. AC Ole and Onyx were also equidistant with similar Mg, K, and Mn contents and PA × (Ca/Zn) molar ratio. The three navy bean cultivars AC Cruiser, AC Mast, and Envoy grouped together with Resolute, AC Earlired, and ROG 802, yielding a distinct profile. Multivariate data analysis using PCA and cluster methods indicated that differences in phytase, antioxidant activity, mineral contents, and bioavailability were much larger within market class than among bean cultivars.

Cultivars with low PA content and high phytase activity as well as low calcium and magnesium contents but high contents of the three essential elements iron, zinc, and copper (e.g., ROG 802) exist in the Canadian germplasm. This cultivar and similar genotypes could potentially be released with superior essential minerals as a value-added trait. The other extreme, high phytate, phytase, iron, and zinc contents and antioxidant activity but low phenolic content, is exemplified by AC Cruiser. This or other cultivars with similar composition may be considered for weight control management due to slow protein bioavailability in the large intestine because of high phytate leading to incomplete protein digestion in the small intestine. AC Earlired represents the cultivar with low phytate, phytase, iron, and zinc contents and antioxidant activity but high phenolic contents. Bean cultivar and market class can therefore be segregated on the basis of their relative variation in phytic acid, phenolic, protein, and mineral contents, phytase activity, and antioxidant properties. Bean cultivars exhibited distinguishing differences in phytate, phenolics, phytase, antioxidant activities, mineral contents, and availability. Therefore, mineral content and bioavailability should not be overlooked in the selection of cultivars for phytochemical improvements. Such information can be used to closely scrutinize the strategy of breeding program for selecting superior dry bean cultivars for targeted food and feed purposes.

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