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Polyphenols and Antioxidant Capacity of Seed Coat and Cotyledon from Brazilian and Peruvian Bean Cultivars (*Phaseolus vulgaris* L.)

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Seed coats and cotyledons from 25 Brazilian and 3 Peruvian bean cultivars were investigated in relation to their phenolic profiles and antioxidant capacity. Condensed tannins, anthocyanins, and flavonols such as kaempferol and quercetin glycosides were mostly found in seed coats. Cotyledons were rich in phenolic acids, such as ferulic, sinapic, chlorogenic, and other hydroxycinnamic acids. In general, the seed coat color pattern and the type of cultivar showed an important influence on the variability of phenolic profiles and levels, respectively. Total phenolics and antioxidant capacity assessed by the DPPH method were higher in seed coats than in cotyledons. The antioxidant capacity had a significant correlation with condensed tannins for all samples and with total anthocyanins in black and red seed coats, whereas in cotyledons, it was more related to the total phenolic content.

KEYWORDS: Brazilian and Peruvian bean cultivars; *Phaseolus vulgaris* L.; polyphenols; antioxidant capacity; seed coat; cotyledon

INTRODUCTION

Polyphenols are common constituents of foods of plant origin and major antioxidants of our diet. The main dietary sources of polyphenols are fruits and beverages. Cereals, chocolate, and dry legumes also contribute to the polyphenol intake (*1*). Legumes, including beans, are a good source of starch, dietary fiber, protein, and minerals and they also serve as a rich source of bioactive constituents (*2*). Dry beans (*Phaseolus vulgaris* L.) are one of the most important and common food legumes consumed by African and Latin American people, and among these countries, Brazil is not only one of the major world bean producers (*3*) but also one of the most important consumers of such pulses (15.7 kg/capita per year) (*4*). On the other hand, in Andean countries like Peru there is a regular bean intake (2.7 kg/capita per year) (*4*). Beans have recently gained increasing attention as a functional food item because like other legume seeds, the common bean seed contains a number of bioactive compounds such as enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic substances that may play metabolic roles in humans or animals that frequently consume this food (*5*). The antioxidant capacity (*6*) and antimutagenic (*7*, *8*) and recently studied antiproliferative effects (*9*) of *Phaseolus vulgaris* L. have been associated with the presence of phenolic compounds, and this fact would explain the numerous scientific studies that suggest that consumption of dry beans is related to several health benefits like reduction of coronary heart diseases (*10*), protective effects against cancer (*11*), and decrease of diabetes and obesity risk (*2*).

Little or no information exists about polyphenol contents in Brazilian and Peruvian bean cultivars, and there is a current concern about changes in Brazilian food habits, that according to some studies, show a fall in common beans intake, a replacement of cereal and legume carbohydrates by lipids, and vegetable protein by animal protein (*12*). This situation could result in an increase in obesity and different degenerative chronic diseases as in developed countries.

The objective of this study was to determine the distribution and contents of phenolic compounds (flavonoids, phenolic acids, total phenolics, and tannins) in seed coat and cotyledon fractions from Brazilian and Peruvian bean cultivars with different colors, and their relation with the antioxidant capacity in vitro, in order to determine their potential as a functional food and to promote their consumption in those countries.

MATERIALS AND METHODS

Materials. Twenty-five bean cultivars of *Phaseolus vulgaris* L. were obtained from the Brazilian company of agricultural research (EMBRAPA) (Brazil) and three (only INIA-6 cultivar was *Phaseolus lunatus* L.) from the Legume and Cereal Program of Agraria University (Lima-Peru). Seeds were sampled from different plants, and after their harvest the mature dry seeds were stored at 4 °C and relative humidity of 85% and analyzed in a period of not more than 1 year. The cultivars were classified visually depending on their seed coat pattern and color (**Table 1**). Seed coat was manually removed, and the fractions obtained were milled under refrigeration into flour (60 mesh). Hypocotyls were removed from the cotyledons and were not considered in this study

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Table 1. Characteristics of Common Bean Samples

no.	cultivar name	color group	seed coat color	origin
1	FT Nobre	A	black	Brazil
2	BRS Triunfo (7762)	A	black	Brazil
3	BRS Campeiro	A	black	Brazil
4	Diamante negro	A	black	Brazil
5	BRS Grafite	A	black	Brazil
6	BRS Valente	A	black	Brazil
7	Uirapuru	A	black	Brazil
8	BRS Timbó	B	red	Brazil
9	CNFRX (7866)	B	red	Brazil
10	BRS Radiante	C	light brown with red stripes	Brazil
11	Iraí	C	light brown with red stripes	Brazil
12	BRS Tropical (8202)	D	light brown with brown stripes	Brazil
13	Pérola	D	light brown with brown stripes	Brazil
14	BRS Requinte	D	light brown with brown stripes	Brazil
15	Talismã	D	light brown with brown stripes	Brazil
16	Magnífico	D	light brown with brown stripes	Brazil
17	Carioca	D	light brown with brown stripes	Brazil
18	Iapar 81	D	light brown with brown stripes	Brazil
19	BRS Pontal	D	light brown with brown stripes	Brazil
20	Jalo precoce	E	yellow brown	Brazil
21	Jalo EEP 558	E	yellow brown	Brazil
22	BRS Vereda	F	pink brown	Brazil
23	IPA-6	G	light brown	Brazil
24	Marfim	G	light brown	Brazil
25	Ouro branco	H	white	Brazil
26	Canario centenario	I	yellow	Peru
27	Pavita molinera	J	white-mottled black	Peru
28	INIA-6	H	white	Peru

because of their insignificant contents of phenolics compounds (data not shown). The average percent of cotyledon and seed coat referred to the total seed weight was of 88.2 ± 1.5 and 9.1 ± 0.6, respectively; whereas 1 g of seed coat and cotyledon flour was equivalent to 44 ± 7 and 4.9 ± 0.8 individual beans seeds, respectively. The final seed coat and cotyledon flours were stored at −18 °C until analysis.

Chemicals. The HPLC standards quercetin, kaempferol, myricetin, catechin, chlorogenic, sinapic, and ferulic acids and the 2,2-diphenyl-1-picrylhydrazyl (DPPH), vanillin, and Folin-Ciocalteu reagents were purchased from Sigma Co (St. Louis, MO). The cyanidin chloride standard was from Extrasynthese (Genay Cedex, France) and the hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Aldrich (Milwaukee, WI). All chemicals and solvents used for extraction and quantification of phenolics were HPLC grade.

Flavonoid and Phenolic Acids Extraction. The extraction was performed according to the method of Arabbi et al. (*13*) with some modifications, as follows. Seed coat and cotyledon flours (1 g) were placed in a 50 mL flask, mixed with 20 mL of methanol/water (70:30) or methanol/water/acetic acid (70:30:5) (samples with anthocyanins), and shaken for 2 h at 4 °C. The extracts were filtered under reduced pressure through filter paper (Whatman No. 1), evaporated under vacuum at 40 °C to ∼6 mL in a rotatory evaporator, and made up to 10 mL with water for seed coat samples. An aliquot of 1−6 mL (depending on the flavonoid and phenolic acid concentrations) was added to a polyamide (1 g) SC6 column (Macherey-Nagel Gmbh and Co., Düren, Germany) preconditioned with methanol (20 mL) followed by water (60 mL). For cotyledon samples, the evaporated extracts were resuspended with ∼10 mL water and added to a polyamide column as above. The column was washed with water (20 mL) and further eluted with methanol (50 mL), to elute the neutral flavonols, and with methanol/ammonia (99.5:0.5) (50 mL) to elute the acidic flavonols.

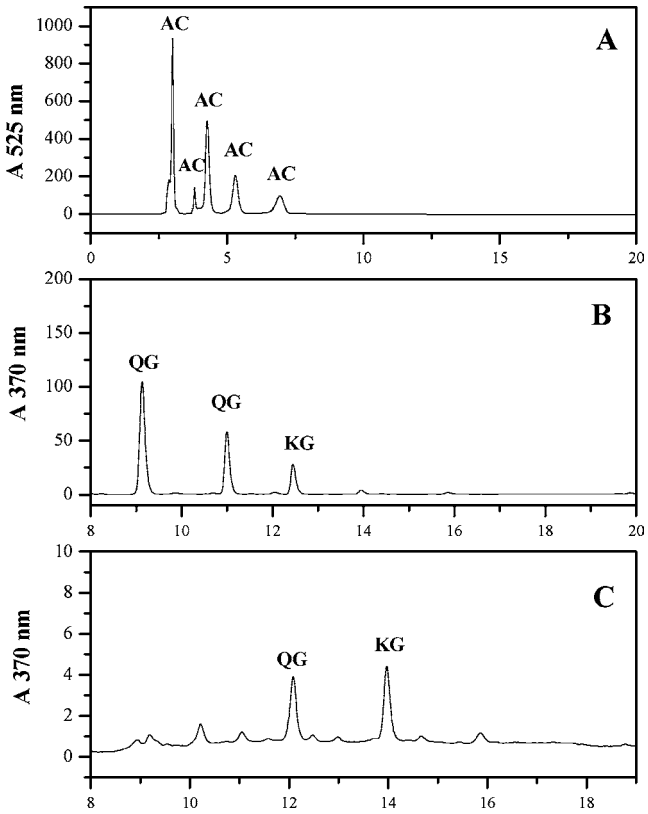

Figure 1. HPLC chromatograms of neutral (**A** and **B**) and acidic (**C**) flavonoid glycosides extracted from FT Nobre (color group A) seed coat. AC, anthocyanins; QG, quercetin glycosides; KG, kaempferol glycosides.

These fractions were evaporated to dryness under reduced pressure at 40 °C, redissolved in HPLC grade methanol (1 mL), filtered through 0.22 μm PTFE (polytetrafluoroethylene) filters (Millipore Ltd., Bedford, MA), and analyzed by HPLC. All extractions were done in duplicate.

HPLC Quantification. Identification and quantification of flavonoids and phenolics acids was achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector controlled by Chemstation software. The column used was 250 × 4.6 mm, i.d., 5μ, Prodigy ODS3 reversed phase silica (Phenomenex Ltd., Torrance, CA), and elution solvents were as follows: A, water:tetrahydrofuran: trifluoroacetic acid (98:2:0.1); and B, acetonitrile. Solvent gradient was the same as that used by Arabbi et al. (*13*). Eluates were monitored at 270, 328, 370, and 525 nm, and samples were injected in duplicate. Calibration was performed by injecting the standards three times at five different concentrations ($R^2 \geq 0.999$). Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. Cochromatography was used when necessary. In the case of quercetin, kaempferol, and myricetin derivatives, results were expressed as milligrams of aglycone, anthocyanin results were expressed as milligrams of cyanidin, phenolic acids (chlorogenic, synapic and ferulic acids), and catechin results were expressed as milligrams of the respective standard. Quantification of hydroxycinnamic acid derivatives was effected on the basis of chlorogenic acid as a reference standard for peaks detected at 328 nm with spectral characteristics similar to chlorogenic acid, but with different retention times (the areas under the curves were combined to obtain a single value). All flavonoid and phenolic acid analyses were done in duplicate and results were expressed by 100 g of sample in fresh weight (FW).

Sample Extraction for Total Phenolics, Tannins, and Antioxidant Capacity Assays. Samples (1 g) were placed in a 50 mL flask and mixed with 20 mL of methanol/water (70:30) or methanol/water/acetic acid (70:30:5) (samples with anthocyanins) for 2 h at 4 °C and centrifuged at 10000*g* for 10 min. The supernatant recovered was stored

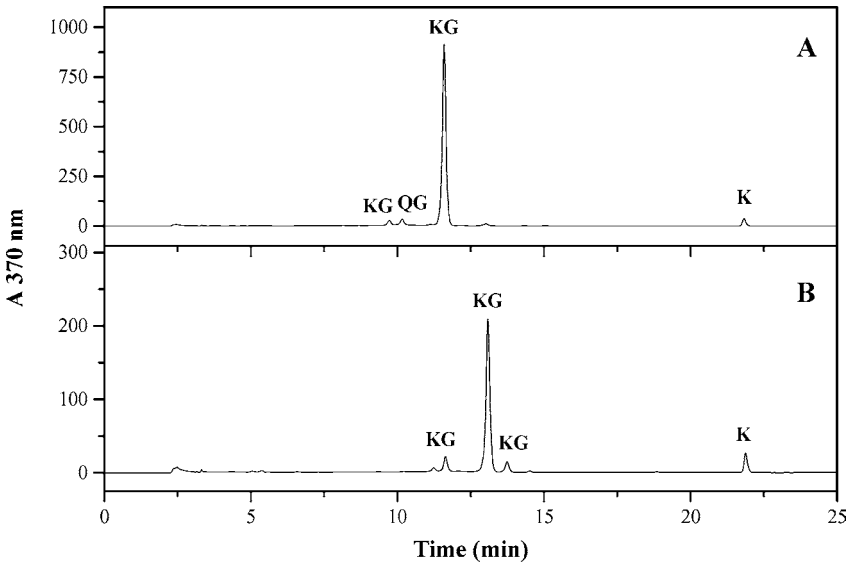

Figure 2. HPLC chromatograms of neutral (**A**) and acidic (**B**) flavonoid glycosides extracted from BRS Tropical (8202) (color group D) seed coat. QG, quercetin glycosides; KG, kaempferol glycosides; K, kaempferol aglycone.

Table 2. Seed Coat Flavonoids and Phenolics Acids from Brazilian and Peruvian Bean Cultivars[a]

		flavonoids (mg/100 g sample in fresh weight)						phenolic acids (mg/100 g sample in fresh weight)		
cultivar	color	A	Q	K	M	C	total	CA	HC	total
FT Nobre	A	553 ± 7 a	50 ± 1 e	8.1 ± 0.3 i	nd	nd	611 ± 7 c	nd	nd	nd
BRS Triunfo (7762)	A	334 ± 21 c	57 ± 2 d	12 ± 1 i	nd	nd	403 ± 23 e	nd	nd	nd
BRS Campeiro	A	265 ± 2 d	29 ± 2 g	12 ± 1 i	nd	nd	306 ± 24 g	nd	nd	nd
Diamante Negro	A	429 ± 19 b	69 ± 3 b	11.7 ± 0.5 i	nd	nd	509 ± 22 d	nd	nd	nd
BRS Grafite	A	558 ± 7 a	69 ± 1 b	14.3 ± 0.1 i	nd	nd	642 ± 6 b	nd	nd	nd
BRS Valente	A	450 ± 45 b	61 ± 5 c	9.8 ± 0.4 i	nd	nd	521 ± 49 d	nd	nd	nd
Uirapuru	A	321 ± 6 c	45 ± 1 f	5.5 ± 0.2 i	nd	nd	371 ± 7 f	nd	nd	nd
BRS Timbó	B	48 ± 2 e	95 ± 4 a	166 ± 6 e	nd	nd	308 ± 13 g	nd	nd	nd
CNFRX (7866)	B	16 ± 1 f	29 ± 1 g	97 ± 2 fg	nd	nd	142 ± 4 h	nd	nd	nd
BRS Radiante	C	1.79 ± 0.02 f	0.50 ± 0.02 k	1.11 ± 0.01 i	nd	nd	3.40 ± 0.04 k	nd	nd	nd
Iraí	C	nd	0.75 ± 0.02 k	1.2 ± 0.1 i	nd	nd	2.0 ± 0.1 k	nd	nd	nd
BRS Tropical (8202)	D	nd	3.1 ± 0.3 k	62 ± 1 h	nd	nd	65 ± 1 j	nd	nd	nd
Pérola	D	nd	1.2 ± 0.1 k	54.6 ± 0.4 h	nd	nd	55.8 ± 0.4 j	nd	nd	nd
BRS Requinte	D	nd	1.9 ± 0.2 k	100 ± 5 fg	nd	nd	102 ± 5 i	nd	nd	nd
Talismã	D	nd	1.09 ± 0.02 k	50.4 ± 0.5 h	nd	nd	51.5 ± 0.5 j	nd	nd	nd
Magnífi co	D	nd	1.8 ± 0.1 k	74 ± 2 gh	nd	nd	76 ± 3 ij	nd	nd	nd
Carioca	D	nd	1.6 ± 0.1 k	78 ± 1 fgh	nd	nd	79 ± 1 ij	nd	nd	nd
Iapar 81	D	nd	1.25 ± 0.01 k	106 ± 1 f	nd	nd	108 ± 1 i	nd	nd	nd
BRS Pontal	D	nd	1.2 ± 0.1 k	81 ± 1 fgh	nd	nd	82 ± 1 ij	nd	nd	nd
Jalo Precoce	E	nd	14.6 ± 0.5 i	750 ± 49 a	nd	nd	765 ± 49 a	nd	nd	nd
Jalo EEP 558	E	nd	15 ± 1 i	582 ± 12 b	nd	nd	597 ± 12 c	nd	nd	nd
BRS Vereda	F	nd	15 ± 1 i	301 ± 1 d	nd	nd	316 ± 10 g	nd	nd	nd
IPA-6	G	nd	nd	nd	nd	21 ± 1 a	21 ± 1 k	4.47 ± 0.04 b	12.2 ± 0.2 b	16.6 ± 0.2 b
Marfim	G	nd	nd	nd	nd	16 ± 1 b	16 ± 1 k	2.8 ± 0.2 c	2.3 ± 0.2 d	5.1 ± 0.4 d
Ouro Branco	H	nd	nd	nd	nd	nd	nd	5.6 ± 0.3 a	14 ± 1 a	20 ± 1 a
INIA-6	H	nd	nd	0.80 ± 0.04 i	nd	nd	0.80 ± 0.04 k	nd	6.3 ± 0.1 c	6.3 ± 0.1 c
Canario centenario	I	nd	22 ± 1 h	334 ± 15 c	nd	nd	356 ± 16 f	nd	nd	nd
Pavita molinera	J	nd	5.8 ± 0.3 j	6.0 ± 0.3 i	4.4 ± 0.2	nd	16 ± 1 k	nd	0.92 ± 0.04 e	0.92 ± 0.04 e

[a] Values are means ± SD; nd, not detected. A, anthocyanins; Q, quercetin; K, kaempferol; M, myricetin; C, catechin; CA, chlorogenic acid; HC, hydroxycinnamic acid. Color A, black; B, red; C, light brown with red stripes; D, light brown with brown stripes; E, yellow brown; F, pink brown; G, light brown; H, white; I, yellow; J, white-mottled black. Means in the same column with different letters are significantly different ($p < 0.05$).

in the dark at −18 °C until analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

Total Phenolics. The analysis was performed according to Zielinski and Kozlowska (*14*), with some modifications. A 0.25 mL aliquot was mixed with 0.25 mL of the Folin−Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV/visible spectrophotometer (Amersham Biosciences, Cambridge, U.K.). (+)-Catechin was used as the reference standard, and the results were expressed as mg catechin equivalents/g sample in fresh weight (FW).

Condensed Taninns. Tannins were assayed with the vanillin−HCl method of Price et al. (*15*). (+)Catechin was used as the reference standard, and tannins were expressed in mg catechin equivalents/g sample in fresh weight (FW). In order to correct for interference from natural pigments in seed coats, a blank sample was prepared by

Table 3. Total Phenolics, Condensed Tannins, and Antioxidant Capacity in Seed Coats from Brazilian and Peruvian Bean Cultivars[a]

cultivar	color	total phenolics[b]	tannins[c]	antioxidant capacity[d]
FT Nobre	A	65 ± 1 ef	288 ± 13 h	296 ± 14 ij
BRS Triunfo (7762)	A	60 ± 1 hij	373 ± 14 d	309 ± 3 ghij
BRS Campeiro	A	49 ± 1 m	263 ± 7 i	249 ± 9 k
Diamante Negro	A	51 ± 2 lm	222 ± 17 k	252 ± 15 k
BRS Grafite	A	73 ± 1 c	369 ± 8 d	327 ± 18 g
BRS Valente	A	62 ± 1 ghi	216 ± 6 k	303 ± 10 hij
Uirapuru	A	63 ± 1 fgh	304 ± 12 g	291 ± 14 j
BRS Timbó	B	64 ± 3 efg	319 ± 11 f	147 ± 5 m
CNFRX (7866)	B	76 ± 2 b	435 ± 15 a	198 ± 12 l
BRS Radiante	C	59 ± 2 ijk	264 ± 15 i	366 ± 13 f
Iraí	C	77 ± 5 b	369 ± 13 d	480 ± 15 b
BRS Tropical (8202)	D	86 ± 1 a	449 ± 7 a	518 ± 22 a
Pérola	D	62 ± 4 ghi	343 ± 8 e	405 ± 18 e
BRS Requinte	D	52 ± 1 lm	262 ± 15 i	315 ± 15 gh
Talismã	D	52 ± 1 lm	228 ± 3 k	315 ± 18 gh
Magnífico	D	66 ± 1 e	322 ± 5 f	366 ± 10 f
Carioca	D	57 ± 4 jk	267 ± 10 i	360 ± 9 f
Iapar 81	D	79 ± 1 b	439 ± 11 a	481 ± 6 b
BRS Pontal	D	53 ± 1 l	241 ± 4 j	369 ± 19 f
Jalo Precoce	E	60 ± 3 hi	282 ± 8 h	369 ± 14 f
Jalo EEP 558	E	69 ± 1 d	372 ± 6 d	418 ± 3 e
BRS Vereda	F	74 ± 1 c	416 ± 13 b	458 ± 18 c
IPA-6	G	69 ± 1 d	394 ± 2 c	438 ± 18 d
Marfim	G	57 ± 1 k	315 ± 6 fg	324 ± 8 g
Ouro Branco	H	0.46 ± 0.02 p	nd	1.05 ± 0.02 o
INIA-6	H	0.87 ± 0.01 p	nd	1.49 ± 0.01 o
Canario centenario	I	4.9 ± 0.1 o	nd	6.1 ± 0.1 o
Pavita molinera	J	8.9 ± 0.2 n	11.5 ± 0.1 l	54 ± 1 n

[a] Values are means ± SD; nd, not detected. Color A, black; B, red; C, light brown with red stripes; D, light brown with brown stripes; E, yellow brown; F, pink brown; G, light brown; H, white; I, yellow; J, white-mottled black. Means in the same column with different letters are significantly different ($p < 0.05$). [b] Milligrams of catechin equivalents per grams of seed coat in fresh weight. [c] Milligrams of catechin equivalents per grams of seed coat in fresh weight. [d] Micromoles of Trolox equivalents per grams of seed coat in fresh weight.

subjecting the original extract to the same conditions of the reaction, but without vanillin reagent.

Antioxidant Capacity. The antioxidant capacity was determined by the DPPH(2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams et al. (*16*) modified by Duarte-Almeida et al. (*17*). A 50 μL aliquot of the extract previously diluted and 250 μL of DPPH (0.5 mM) was shaken, and after 25 min the absorbance was measured at 517 nm using a Microplate spectrophotometer (Benchmark Plus, BioRad, Hercules, CA). The control consisted of a Trolox solution at different concentrations. The antioxidant capacity was expressed as μmol Trolox equivalents/g sample in fresh weight (FW).

Statistical Analysis. Results from HPLC ($n = 4$) and spectrophotometric ($n = 6$) analyses were expressed as means ± standard deviation (SD). Data were subjected to ANOVA analysis, means comparisons to the Newman Keuls test ($p < 0.05$), and Pearson correlations according to the Statistic software package version 5.0 (*18*).

RESULTS AND DISCUSSION

Seed Coat Phenolics. *Flavonoids and Phenolic Acids.* **Figures 1** and **2** show the typical chromatographic profiles of two representative seed coat bean cultivars (group A: black and group D: light brown with brown stripes, **Table 1**).

In agreement with various researchers (*5*, *19*−*21*), flavonol glycosides such as kaempferol and quercetin derivatives were the most widespread flavonoids detected by HPLC analysis in almost all seed coat samples, with the exception of Ouro branco, IPA-6, and Marfim cultivars (chromatograms not shown). However, the distribution of these compounds in the methanolic eluates was quite variable according to the coat color. In the black seed coat group, flavonoid glycosides such as anthocyanins, quercetin, and kaempferol derivatives were concentrated mainly in the methanolic fraction (neutral flavonoids) while in the methanol/ammonia fraction (acidic flavonoids) only two small peaks of quercetin and kaempferol glycosides were detected (**Figure 1**). A different tendency was observed in group D (**Figure 2**), where the main compounds found in either methanol or methanol/ammonia fractions were kaempferol glycosides, with only one small peak of a quercetin glycoside in the methanolic eluate. Interestingly, seed coat samples belonging to the color groups B, D, and E and Vereda cultivar presented the kaempferol aglycone in both fractions (methanol and methanol/ammonia). Similarly, Romani et al. (*19*). also found one small peak of kaempferol aglycone in the hydroalcoholic extract from *P. vulgaris* cv. Zolfino C. Bravo (*22*) stated that flavonoids occasionally occur in plants as aglycones, although they are most commonly found as glycoside derivatives.

Currently, researchers accept the fact that the pigments responsible for seed coat color in beans are flavonoids (*23*). Moreover, the wide variety of seed coat patterns and color in common beans (*P. vulgaris* L.) is controlled by a group of well-defined genes (in seed coat pattern: *T*, *Z*, *L*, *J*, *Bip*, and *Ana*; in color: *P*, *C*, *R*, *J*, *D*, *G*, *B*, *V*, and *Rk*) that appear to regulate the flavonol and anthocyanin biosynthetic pathways (*24*).

Interesting observations can be made related to the different seed coat phenolic profiles obtained here (**Table 2**). In general, within the same color group all phenolic profiles were similar and they allowed differences among the various seed coat color groups to be distinguished. Anthocyanins were detected in black and red coats, and in small quantity in BRS Radiante cultivar (group C). Quercetin derivatives were more concentrated in black and red bean coats than in groups D and E (brown cultivars). Conversely, these last groups, group B and coats from Vereda and Canario Centenario cultivars were mostly rich in kaempferol glycosides. Phenolic acids were found in white coats such as in Ouro Branco and INIA-6; nevertheless, these cultivars were poor in kaempferol derivatives, and no quercetin glycosides were detected. Pavita Molinera was the only cultivar that contained the flavonol myricetin, besides the typical flavonols quercetin and kaempferol detected, in the majority of the samples. Different patterns were observed for IPA-6 and Marfim coats (group G), which presented catechin and phenolic acids such as chlorogenic and other hydroxycinnamic acids.

A high variability in phenolic contents between the different groups of seed coat colors was observed. Total flavonoids ranged from 0.8 to 765 mg/100 g seed coat or from 0.07 to 69.3 mg/100 g whole bean grain (considering that coat fraction represents 9.1 ± 0.6% of the total bean seed), quantities higher than those reported by other authors (*21*, *25*, *26*), and somewhat similar to the results obtained by Romani et al. (*19*).

Additionally, significant differences in specific flavonoid contents were detected inside each group. Black coats (group A) presented a high quantity of total anthocyanins, ranging from 265 to 558 mg/100 g sample (expressed as cyanidin). Some authors reported high levels of total anthocyanins in black bean seed coat; for instance, Salinas-Moreno et al. (*27*). found a range from 1010 to 1810 mg/100 g in Mexican cultivars, and Takeoka et al. (*28*). a content of 2370 mg/100 g of seed coat from a cultivar developed in an experimental station in the U.S.A. However, these values are not comparable with the results obtained here, because in both works, the total anthocyanins were analyzed by spectrophotometric methods. Present results are more in accordance with those published by Choung et al.

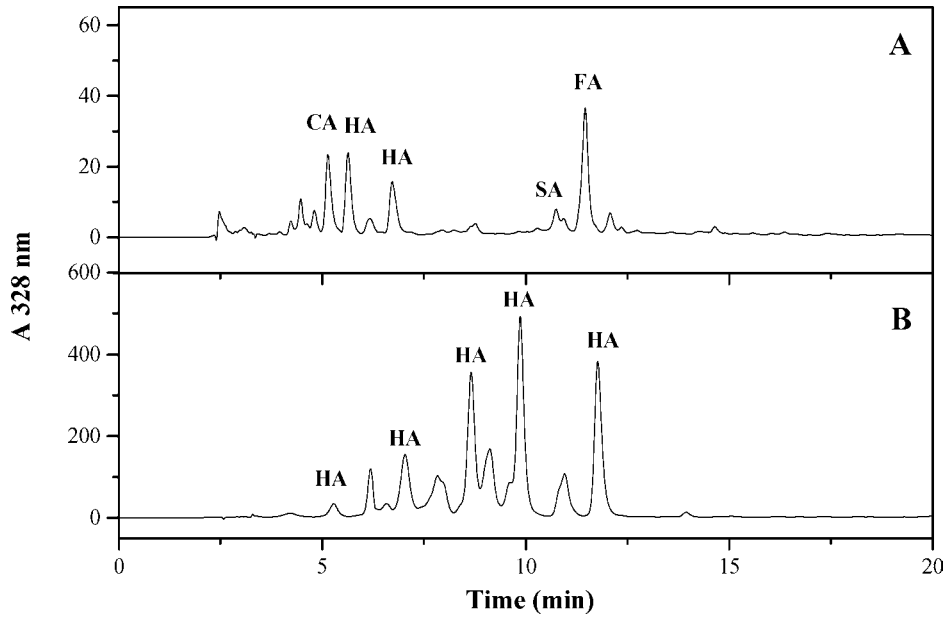

Figure 3. HPLC chromatograms of phenolic acids extracted in the methanol (**A**) and methanol:ammonia (**B**) fractions from FT Nobre (color group A) cotyledon. CA, chlorogenic acid; HA, hydroxycinnamic acid; SA, sinapic acid; FA, ferulic acid.

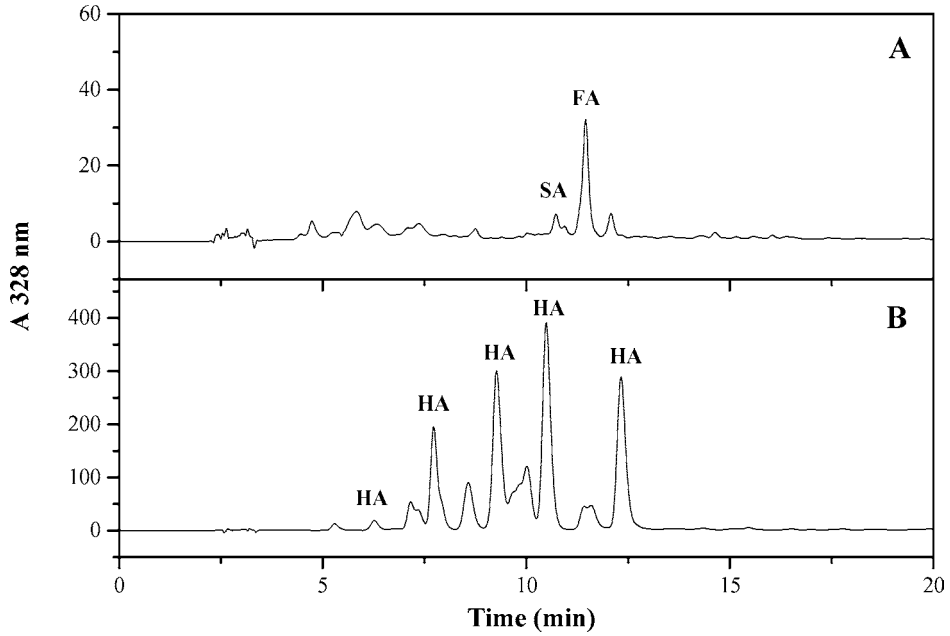

Figure 4. HPLC chromatograms of phenolic acids extracted in the methanol (**A**) and methanol:ammonia (**B**) fractions from BRS Tropical (8202) (color group D) cotyledon. HA, hydroxycinnamic acid; SA, sinapic acid; FA, ferulic acid.

(*29*) and Espinosa-Alonso et al. (*21*). who obtained ranges from 214 to 278 and 212 to 3788 mg/100 g of seed coat, respectively, applying HPLC assays for total anthocyanins quantification. The seed coats with the highest anthocyanin content ($p < 0.05$) were FT Nobre (553 ± 7 mg/100 g) and BRS Grafite (558 ± 7 mg/100 g). Quercetin glycosides levels ranged from 29 to 69 mg/100 g whereas no significant differences were observed between the kaempferol derivatives contents.

The red bean group (B) had lower anthocyanin levels (from 16 to 48 mg/100 g sample) when compared to the black one, but presented higher levels of kaempferol (97 to 166 mg/100 g) and quercetin (29 to 95 mg/100 g) glycosides. BRS Timbó cultivar had the highest quercetin glycosides content ($p < 0.05$) among all the analyzed coat samples (95 ± 4 mg/100 g). The other big group with brown seed coat color (D) showed a high variability in kaempferol glycosides contents, ranging from 50.4 to 106 mg/100 g and no significant differences were observed in quercetin derivatives levels.

The two remarkable samples that contained the highest kaempferol glycosides levels were Jalo Precoce and Jalo EEP 558 (group E), with 750 ± 49 and 582 ± 12 mg/100 g respectively. In contrast, samples from group C exhibited low contents of flavonols.

Total phenolic acids were detected in a range of 5.1 ± 0.4 to 16.6 ± 0.2 mg/100 g (expressed as chlorogenic acid) in group G, and no flavonol derivatives were found.

Among the Peruvian cultivars, Canario centenario presented a high quantity of kaempferol glycosides (334 ± 15 mg/100 g). Beninger and Hosfield (*23*) found a high level of two kaempferol glycosides (108 mg/100 g of fresh whole bean weight) in a new manteca type dry bean with a yellow seed

Table 4. Cotyledon Flavonoids and Phenolics Acids from Brazilian and Peruvian Bean Cultivars[a]

		flavonoids (mg/100 g sample in fresh weight)			phenolic acids (mg/100 g sample in fresh weight)				
cultivar	color	Q	K	total	CA	SA	FA	HC	total
FT Nobre	A	nd	nd	nd	0.42 ± 0.05 b	0.11 ± 0.01 e	0.49 ± 0.01 e	39.2 ± 0.4 a	40.2 ± 0.4 a
BRS Triunfo (7762)	A	nd	nd	nd	0.27 ± 0.02 d	nd	0.11 ± 0.01 q	35.6 ± 0.3 c	35.9 ± 0.3 c
BRS Campeiro	A	nd	nd	nd	0.17 ± 0.01 g	nd	0.13 ± 0.01 q	20.7 ± 0.5 hi	20.9 ± 0.5 ij
Diamante Negro	A	nd	nd	nd	0.19 ± 0.01 fg	0.14 ± 0.00 c	0.51 ± 0.01 d	28 ± 1 e	29 ± 1 ef
BRS Grafite	A	nd	nd	nd	0.18 ± 0.01 g	nd	0.11 ± 0.00 q	14 ± 1 m	14 ± 1 m
BRS Valente	A	nd	nd	nd	0.31 ± 0.02 c	nd	0.66 ± 0.00 a	24.3 ± 0.4 f	25.3 ± 0.4 g
Uirapuru	A	nd	nd	nd	0.22 ± 0.00 ef	nd	0.47 ± 0.01 f	23.1 ± 0.1 g	23.8 ± 0.1 h
BRS Timbó	B	nd	nd	nd	0.20 ± 0.01 fg	nd	0.56 ± 0.01 c	19.6 ± 0.9 ijk	20 ± 1 ij
CNFRX (7866)	B	nd	nd	nd	nd	0.16 ± 0.00 b	0.36 ± 0.01 j	11.1 ± 0.1 o	11.6 ± 0.2 o
BRS Radiante	C	nd	nd	nd	nd	nd	0.19 ± 0.01 no	23 ± 2 g	23 ± 2 h
Iraí	C	nd	nd	nd	nd	nd	0.37 ± 0.00 ij	19 ± 1 ijk	20 ± 1 jk
BRS Tropical (8202)	D	nd	nd	nd	nd	0.09 ± 0.00 g	0.42 ± 0.00 g	32.7 ± 0.4 d	33.2 ± 0.4 d
Pérola	D	nd	nd	nd	nd	nd	0.20 ± 0.01 n	22.9 ± 0.3 g	23.1 ± 0.3 h
BRS Requinte	D	nd	nd	nd	nd	nd	0.15 ± 0.01 p	28.4 ± 0.5 e	28.5 ± 0.5 f
Talismã	D	nd	nd	nd	nd	nd	0.16 ± 0.00 p	21 ± 1 h	22 ± 1 i
Magnífic o	D	nd	0.21 ± 0.00 d	0.21 ± 0.00 d	nd	0.19 ± 0.01 a	0.50 ± 0.02 de	29 ± 1 e	30 ± 1 e
Carioca	D	nd	nd	nd	nd	0.10 ± 0.01 f	0.22 ± 0.01 m	19 ± 1 jk	19 ± 1 kl
Iapar 81	D	nd	0.39 ± 0.01 b	0.39 ± 0.01 c	nd	0.15 ± 0.00 c	0.34 ± 0.00 k	38 ± 1 a	39 ± 1 b
BRS Pontal	D	nd	nd	nd	nd	nd	0.18 ± 0.01 o	21 ± 1 hi	21 ± 1 ij
Jalo Precoce	E	0.19 ± 0.01 a	0.75 ± 0.01 a	0.93 ± 0.00 a	nd	nd	0.39 ± 0.01 hi	18 ± 1 kl	19 ± 1 kl
Jalo EEP 558	E	0.17 ± 0.00 a	0.30 ± 0.01 c	0.48 ± 0.01 b	nd	nd	0.11 ± 0.00 q	11.2 ± 0.2 o	11.3 ± 0.2 o
BRS Vereda	F	nd	0.21 ± 0.01 d	0.21 ± 0.01 d	0.24 ± 0.00 e	nd	0.11 ± 0.01 q	29.5 ± 0.3 e	29.9 ± 0.3 ef
IPA-6	G	nd	nd	nd	0.45 ± 0.04 a	0.13 ± 0.00 d	0.47 ± 0.02 f	37.2 ± 0.5 b	38 ± 1 b
Marfim	G	nd	nd	nd	0.30 ± 0.02 c	0.08 ± 0.01 g	0.34 ± 0.02 k	28 ± 1 e	29 ± 1 ef
Ouro Branco	H	nd	nd	nd	0.18 ± 0.01 g	nd	0.34 ± 0.01 k	17 ± 1 l	18 ± 1 l
INIA-6	H	nd	nd	nd	nd	nd	0.32 ± 0.01 l	4.5 ± 0.1 p	4.8 ± 0.1 p
Canario centenário	I	nd	0.19 ± 0.00 e	0.19 ± 0.00 e	nd	0.13 ± 0.01 d	0.64 ± 0.02 b	20 ± 1 ij	21 ± 1 ij
Pavita molinera	J	nd	nd		0.19 ± 0.02 fg	nd	0.39 ± 0.01 h	12.6 ± 0.2 n	13.2 ± 0.3 n

[a] Values are means ± SD; nd, not detected. Q, quercetin; K, kaempferol; CA, chlorogenic acid; SA, sinapic acid; FA, ferulic acid; HC, hydroxycinnamic acid. Color A, black; B, red; C, light brown with red stripes; D, light brown with brown stripes; E, yellow brown; F, pink brown; G, light brown; H, white; I, yellow; J, white-mottled black. Means in the same column with different letters are significantly different ($p < 0.05$).

coat color, and their data indicated that these flavonols are responsible for imparting the yellow color to the seed coat.

Total Phenolics, Condensed Tannins, and Antioxidant Capacity. Important differences were observed in total phenolics, condensed tannins, and antioxidant capacity of the seed coats, as shown in **Table 3**.

Total phenolic contents varied from 0.46 to 86 mg catechin equivalents/g seed coat. BRS Tropical (8202) and Iapar 81 cultivars (from group D) presented the highest values ($p < 0.05$) with 86 ± 1 and 79 ± 1 mg catechin equivalents/g, respectively, and the lowest contents were for white coats Ouro branco (0.46 ± 0.02 mg catechin equivalents/g) and INIA-6 (0.87 ± 0.01 mg catechin equivalents/g).

Condensed tannins ranged from 11.5 to 449 mg catechin equivalents/g seed coat or from 1 to 41 mg catechin equivalents/g whole bean grain (considering that coat fraction represents 9.1 ± 0.6% of the total bean seed). Guzman-Maldonado et al. (*30*) and De Mejia et al. (*31*) also found similar ranges (7−32.4 and 17−38 mg catechin equivalents/g, respectively) in whole grains of Mexican beans. Cultivars from group D like BRS Tropical (8202) and Iapar 81, the same cultivars with the highest total phenolics, also had the highest tannin levels (449 ± 7 and 439 ± 11 mg catechin equivalents/g seed coat, respectively) ($p < 0.05$) while the Peruvian cultivar Pavita molinera presented the lowest one (11.5 ± 0.1 mg catechin equivalents/g seed coat). On the other hand, no tannins were detected in white and yellow coats.

Depending on the different color groups, the variability of total phenolics and tannins was moderate, whereas more accentuated differences were observed among the antioxidant capacity of seed coats. Several statements concerning the relationship between tannin levels and bean coat colors are reported by various authors. Espinosa-Alonso et al. (*21*). showed that total phenol and condensed tannin contents in *P. vulgaris* tended to increase according to clearness of seeds, and conversely, Barampama and Simard (*32*) reported that beans with light colored coats have lower tannin contents than beans with dark pigmented coats such as black beans. Other authors simply did not find a significant correlation between the bean seed coat color and the content of tannins (*30*, *33*). In spite of this controversial situation, the results of the present work suggest that certain groups of seed coat colors such as brown and red groups tended to have higher levels of total phenolics and condensed tannins than the black group, and in contrast, less colored coats such as white and yellow types did not exhibit considerable contents of these compounds. On account of the high variability observed inside each group, it can be inferred that the effect of the type of cultivar also would have to be considered.

As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress, by acting directly on reactive oxygen species or by stimulating endogenous defense systems (*1*). Correlation coefficients (provided in a supplementary table included in the Supporting Information) were calculated so as to know what phenolic compound(s) is (are) more related with antioxidant capacity in seed coats. Similarly to Heimler et al. (*6*), neither total flavonoids nor specific flavonols had correlation with antioxidant capacity. In contrast, total phenolics and tannins showed a significant positive correlation with antioxidant capacity in coats ($r = 0.88$ and $r = 0.86$, respectively). Furthermore, total phenolics and condensed tannins presented a highly significant correlation ($r = 0.97$). These results indicate that the main phenolic com-

Table 5. Total Phenolics and Antioxidant Capacity of Cotyledons from Brazilian and Peruvian Bean Cultivars[a]

cultivar	color	total phenolics[b]	antioxidant capacity[c]
FT Nobre	A	0.77 ± 0.01 d	2.1 ± 0.1 f
BRS Triunfo (7762)	A	0.81 ± 0.01 c	2.2 ± 0.1 f
BRS Campeiro	A	0.62 ± 0.01 j	1.6 ± 0.1 k
Diamante Negro	A	0.70 ± 0.03 g	1.8 ± 0.1 hij
BRS Grafite	A	0.63 ± 0.01 j	1.87 ± 0.04 ghi
BRS Valente	A	0.72 ± 0.02 f	1.9 ± 0.1 ghi
Uirapuru	A	0.65 ± 0.02 i	1.8 ± 0.1 hij
BRS Timbó	B	0.67 ± 0.02 hi	2.3 ± 0.1 e
CNFRX (7866)	B	0.47 ± 0.00 l	1.8 ± 0.1 ij
BRS Radiante	C	0.68 ± 0.01 gh	2.41 ± 0.02 cde
Iraí	C	0.83 ± 0.01 b	2.67 ± 0.04 ab
BRS Tropical (8202)	D	0.81 ± 0.01 c	2.4 ± 0.1 e
Pérola	D	0.74 ± 0.01 e	2.37 ± 0.05 de
BRS Requinte	D	0.72 ± 0.01 f	2.4 ± 0.1 de
Talismã	D	0.82 ± 0.01 c	2.7 ± 0.1 a
Magnífico	D	0.83 ± 0.02 bc	2.60 ± 0.15 b
Carioca	D	0.65 ± 0.02 i	2.5 ± 0.1 c
Iapar 81	D	0.90 ± 0.02 a	2.63 ± 0.02 ab
BRS Pontal	D	0.70 ± 0.00 g	2.49 ± 0.15 cd
Jalo Precoce	E	0.74 ± 0.01 e	2.0 ± 0.1 g
Jalo EEP 558	E	0.69 ± 0.01 g	1.7 ± 0.1 j
BRS Vereda	F	0.66 ± 0.01 i	2.38 ± 0.04 de
IPA-6	G	0.66 ± 0.01 i	1.9 ± 0.1 gh
Marfim	G	0.66 ± 0.01 i	2.08 ± 0.04 f
Ouro Branco	H	0.47 ± 0.01 l	1.03 ± 0.04 m
INIA-6	H	0.42 ± 0.01 m	0.81 ± 0.06 n
Canario centenário	I	0.53 ± 0.00 k	1.13 ± 0.03 l
Pavita molinera	J	0.42 ± 0.01 m	0.88 ± 0.02 n

[a] Values are means ± SD. Color A, black; B, red; C, light brown with red stripes; D, light brown with brown stripes; E, yellow brown; F, pink brown; G, light brown; H, white; I, yellow; J, white-mottled black. Means in the same column with different letters are significantly different ($p < 0.05$). [b] Milligrams of catechin equivalents per grams of cotyledon in fresh weight. [c] Micromoles of Trolox equivalents per grams of cotyledon in fresh weight.

pounds in seed coats may be condensed tannins, and these polymers would be responsible for the antioxidant capacity rather than total flavonoids or flavonols. Hagerman et al. (*34*). found that condensed and hydrolyzable tannins of high molecular weight are effective antioxidants with even greater activity than simple phenolics, e.g., flavonoid monomers. More recent work has shown that tannin extracts from *P. vulgaris* were as active, or slightly more active, than pure flavonoid compounds (*35*).

Coats with anthocyanins (groups A and B) presented another tendency. The antioxidant capacity had a high positive correlation ($r = 0.82$) with anthocyanin levels rather than condensed tannins and total phenolics. In consequence, in black and red groups, anthocyanins are the main compounds that would contribute significantly to their antioxidant capacity. This could be the reason for the fact that coats from group B (red) were the only samples that exhibited a moderate antioxidant capacity in spite of their high levels of total phenolics and tannins.

In addition, a low negative correlation ($r = -0.43$) between anthocyanins and condensed tannins was also observed, similarly to Espinosa-Alonso et al. (*21*).

Cotyledon Phenolics. *Flavonoids and Phenolic Acids.* In contrast to what was observed in seed coats, chromatographic profiles were quite similar for almost all cotyledon samples without showing significant dependency on seed coat color patterns (**Figures 3** and **4**). The only phenolics detected by HPLC were hydroxycinnamic acids that differed in their distribution between the methanolic fractions. Free phenolic acids, like ferulic, synapic, and chlorogenic acids, were eluted in the methanolic fraction, while other hydroxycinnamic acids were released in the methanol:ammonia eluate.

According to **Table 4**, only small amounts of free phenolic acids were detected in cotyledons, whereas other hydroxicinnamic acids (probably conjugated acids) represented the major phenolic compounds. Ferulic acid was distributed in all cotyledon samples in a range of 0.11 to 0.66 mg/100 g of cotyledon. Chlorogenic acid was detected specially in cotyledons from black and light brown beans (0.17 to 0.45 mg/100 g cotyledon) and sinapic acid was found in some cultivars (0.08 to 0.19 mg/100 g cotyledon), independently of the bean color. Cotyledons from cultivars that presented the highest levels of kaempferol derivatives in the seed coats (Vereda, Canario Centenario, and group E) were the only ones that contained quercetin and kaempferol glycosides, but in low levels.

To our knowledge, this is the first time that chlorogenic acid has been reported in *Phaseolus vulgaris* L., instead of other phenolic acids derived from benzoic acid like *p*-hydroxybenzoic, vanillic, and syringic acids (*5*, *21*) that have been found in whole bean. Besides the ferulic, sinapic, and *p*-coumaric acids, Luthria and Pastor-Corrales (*36*) also found caffeic acid in some whole black beans. All the authors mentioned above based the quantification of phenolic acids on acid or basic hydrolysates, which could explain some of the differences found in this work, e.g., the presence of caffeic acid in black coats in contrast to chlorogenic acid detected here.

Although the bean color did not significantly influence the phenolic acid profiles, cotyledons from groups A and D (black and brown cultivars, respectively) tended to have higher phenolic acids contents than cotyledons from white cultivars. Total phenolic acids ranged from 4.8 mg/100 g (INIA-6 white cultivar) to 40.2 mg/100 g (FT Nobre black cultivar), or from 4.2 to 35.4 mg/100 g whole bean grain (considering that cotyledon fraction represents 88.2 ± 1.5% of the total bean seed), values higher than those reported by Espinosa-Alonso et al. (*21*) and similar to those found by Luthria and Pastor-Corrales (*36*).

Total Phenolics and Antioxidant Capacity. In **Table 5** is observed that cotyledons had less total phenolics (from 0.42 to 0.90 mg/100 g cotyledon) and lower antioxidant capacity (from 0.81 to 2.70 μmol Trolox equiv/g cotyledon) than seed coats and no condensed tannins were detected, as also was observed by Guzman-Maldonado et al. (*30*). In general, the variability among the samples was not as high as that observed in seed coats; however, it was more evident in cotyledons from white and yellow beans which showed the lowest levels of total phenolics and antioxidant capacity.

Considering all samples (results not shown), a moderate positive correlation ($r = 0.66$, $n = 112$, $p < 0.05$) was found between the amounts of total phenolic acids and total phenolics, which was higher for cotyledons from black beans ($r = 0.90$, $n = 28$, $p < 0.05$), indicating that in this group, the major phenolic compounds are phenolic acids. In addition, there was a significant positive correlation between total phenolics and the antioxidant capacity ($r = 0.84$, $n = 112$, $p < 0.05$) and a low direct correlation between phenolic acids and antioxidant capacity ($r = 0.49$, $n = 112$, $p < 0.05$).

In conclusion, seed coats exhibited higher concentrations of phenolic compounds than cotyledons. Seed coat phenolics like condensed tannins, anthocyanins, and other flavonoids such as quercetin and kaempferol glycosides were mostly present in seed coats, whereas cotyledons were rich in cinnamic acid derivatives. Although some color groups were not enough represented, the seed coat color appeared to be related to phenolic profiles. From a quantitative aspect, the type of cultivar had a significant

influence on the variability observed in all phenolic levels. However, other external factors such as environmental and agronomic conditions would have to be considered as well. Seed coats from BRS Grafite and FT Nobre black cultivars were the major sources of anthocyanins, BRS Timbó red coat showed the highest level of quercetin glycosides while Jalo Precoce, Jalo EEP 558, Canario Centenario, and BRS Vereda coats are promising for their high levels of kaempferol derivatives. This work provides evidence that phenolic compounds like condensed tannins are strongly correlated with the antioxidant capacity in seed coats. In this way, BRS Tropical (8202) seed coat was the sample with the highest condensed tannin and antioxidant capacity levels. For black and red seed coat groups, anthocyanins were the flavonoids with a major effect on antioxidant capacity. Although in lower levels, cotyledon fractions exhibited scavenging capacity as well, which was related to the total phenolic content and moderately with their total phenolic acid contents.

These results could be helpful for the selection or genetic improvement of potentially functional cultivars and for promoting their use as an important option for healthy nourishment in these countries.

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Supporting Information Available: Correlation coefficients for anthocyanins, flavonoids, flavonols, tannins, total phenolics, and capacity antioxidant of all seed coat bean samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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