JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Components with Health Implications in Wild and Cultivated Mexican Common Bean Seeds (*Phaseolus vulgaris* L.)

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Common bean effects on health have been related to its dietary fiber content and other active compounds. This study assessed the content of flavonoids, coumestrol, phenolic acids, galactooligosaccharides, and phytic acid in wild and cultivated Mexican common bean seeds (raw and cooked) and that of flavonoids, coumestrol, and phenolic acids in germinated bean seeds. The presence of isoflavones in raw bean seeds was not confirmed by the UV spectra. Quercetin, kaempferol, *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, and vanillic acid mean contents were 10.9, 52.3, 10.1, 9.6, 5.4, and 18.2 μg/g, respectively; raffinose, stachyose, verbascose, and phytic acid mean contents were 8.5, 56.3, 5.5, and 11.5 mg/g, respectively, in raw seeds. All compounds were affected by autoclaving, and germination resulted in a de novo synthesis of flavonols, phytoestrogens, and phenolic acids. The impact on health of common bean seed is affected by dietary burden, specific compounds content, and processing. On the other hand, germinated bean seed or beans sprouts may be sources of antioxidants and phytoestrogens.

KEYWORDS: Common bean; *Phaseolus vulgaris*; wild; cultivated; chronic diseases; HPLC; active compounds; antioxidants; isoflavonoids; flavonoids; flavonols; phenolic acids; phytoestrogens; prebiotics; galactooligosaccharides; phytic acid

INTRODUCTION

In 2001, chronic diseases such as obesity, diabetes, cardiovascular disease, and cancer resulted in ∼60% of the 56.5 million total reported deaths in the world. The chronic disease problem is far from being limited to developed regions of the world, contrary to widely held beliefs. Developing countries are increasingly suffering from public health problems related to chronic diseases (*1*). Given the rapidity with which traditional diets and lifestyles are changing in many developing countries, it is not surprising that chronic diseases occur in countries where undernutrition and food insecurity are endemic problems (*1*, *2*). Beyond the appropriate medical treatment for those already affected, the public health approach of primary prevention is considered to be the most cost-effective, affordable, and sustainable course of action to cope with the chronic disease epidemic worldwide (*1*).

Epidemiologic studies have shown the protective effect of plant-based diets on chronic diseases (*3*), and several phytochemicals have been implicated (*4*). These compounds can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of lipid and hormone metabolism, antioxidant, antibacterial, antimutagen, and antiangiogenic effects, reduction of tumor initiation, and promotion and induction of apoptosis (*4*).

Nonsoybean legumes play an important role in the traditional diet of many peoples throughout the world (*2*, *5*). On the other hand, there is evidence that they decrease the risk of cardiovascular disease (*6*), diabetes and obesity (*7*, *8*) and some kinds of cancer (*9*).

The common bean seed (*Phaseolus vulgaris* L.) is an important source of protein, complex carbohydrates, minerals, and dietary fiber, principally in developing countries. Like other legume seeds, the common bean seed contains a number of bioactive substances including enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds that play metabolic roles in humans or animals that frequently consume these foods. These effects may be regarded as positive, negative,

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10.1021/jf051706l CCC: $33.50
Published on Web 02/23/2006

Table 1. Description of Common Bean Seed Samples

sample	status	wt of 100 seeds (g)	seed coat color
FMM38	cultivated	30.3	cream-red
FJAna	cultivated	32.7	cream-red
FJMar	cultivated	32.4	cream-red
N8025	cultivated	22.3	black
Notom	cultivated	32.1	black
Apa 95	cultivated	32.3	grey
FJVic	cultivated	32.2	cream-red
FJAcu	cultivated	23.2	cream-red
FM94050	cultivated	26.0	cream-red
NQro	cultivated	22.7	black
G-12892	wild	5.3	cream
G-12893	wild	9.3	brown
G-12906	wild	11.6	black-brown
G-11025B	wild	10.3	cream

or both (*10*). Some of these substances have been considered as antinutritional factors due to their effect on diet quality. Enzyme inhibitors can diminish protein digestibility, and lectins can reduce nutrient absorption, but both have little effect after cooking (*11*). Phytic acid can diminish mineral bioavailability (*12*). Some phenolic compounds can reduce protein digestibility (*13*) and mineral bioavailability (*12*), and galactooligosaccharides may result in flatulence (*14*). On the other hand, these same compounds may have protective effects against cancer (*9*−*11*). Phytic acid has antioxidant and protective DNA damage effects (*15*, *16*), phenolic compounds such as flavonoids and phenolic acids have antioxidant and other specific properties (*17*−*19*), and galactooligosaccharides may result in prebiotic activity (*20*, *21*).

The epidemiological relationship between the consumption of common bean and the risk or incidence of chronic disease must consider the compositional differences between varieties and/or phenotypes to extend any relationship beyond the influence of dietary fiber as a generality and to understand the role of other compounds, particularly on cancer risk. The aim of this study was to quantify the concentration of flavonoids (daidzein, genistein, kaempferol, and quercetin) coumestan (coumestrol), phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid), galactooligosaccharides (raffinose, stachyose, and verbascose), and phytic acid in wild and cultivated Mexican common bean seeds (raw and cooked) and that of daidzein, genistein, kaempferol, quercetin, coumestrol, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid in germinated seeds.

MATERIALS AND METHODS

Common Bean Seeds. Ten cultivated and four wild varieties of Mexican common bean seeds (**Table 1**) were provided by the germplasm collection of the Instituto de Investigación Forestal, Agrícola y Pecuaria (INIFAP, Bajío, Mexico). Two hundred grams of mature common bean seeds was divided into two batches; one was ground to pass through a 4 mm screen, and the other was autoclaved for 20 min at a 1:3 beans/deionized water ratio (w/v), homogenized, and freeze-dried with cooking broth. Additionally, five cultivated and three wild varieties were germinated in the dark at 27 °C for 72 h, freeze-dried, and ground without the seed coat.

Chemicals. The flavonoids (quercetin and kaempferol), the phenolic acids (*p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, and vanillic acid_, and phytic acid ($InsP_6$) standards were purchased from Sigma Chemical Co. (St. Louis, MO). Isoflavonoids (daidzein and genistein) and coumestrol standards were purchased from LC Laboratories (Woburn, MA). Galactooligosaccharides (raffinose, stachyose, and verbascose) were purchased from Megazyme International (Bray, County Wicklow, Ireland). Solvents and water used for HPLC analysis were of HPLC grade.

Flavonoids, Phenolic Acids, and Coumestrol Content. Phenolics were determined according to the method of Lozovaya et al. (*22*) with some modifications. Briefly, phenolics from bean flour were extracted with 80% methanol and then centrifuged, and the residue was resuspended in 3 mL of 2 N HCl and heated at 95 °C for 2 h to hydrolyze the glycosides. The organic components were extracted from the acidic solution with ethyl acetate. The ethyl acetate was removed under vacuum, and the residue was resuspended in 80% methanol, centrifuged, and used for HPLC analysis. High-performance liquid chromatography analyses were carried out using a separation module 2690 with a PDA detector from Waters (Milford, MA) supplied with a platinum EPS C-18 (7 × 57 mm) Rocket column (Deerfield, IL). Solvent A was water adjusted with acetic acid to pH 2.8, and solvent B was acetonitrile. Injection volume was 40 μL, and flow rate was 2.5 mL/min. For flavonoid elution, the gradient was linear to 10% B in 2.5 min, 12% B in 6 min, 23% B in 18 min, and 35% B in 24 min, after the column was washed with 95% B for 3 min and equilibrated for 3 min at 100% A to start the next sample; total running time was 30 min. Ultraviolet absorbance at 260.6 nm was used to detect quercetin, kaempferol, daidzein, and genistein and UV absorbance at 342.4 nm to detect coumestrol. For phenolic acid elution, the gradient was linear to 6% B in 8 min, 12% B in 14 min, 20% B in 18 min, and 35% B in 24 min, after the column was washed with 95% B for 3 min and equilibrated 3 min at 100% A to start the next sample; total running time was 30 min. Ultraviolet absorbance at 295 nm was used to detect vanillic acid, *p*-coumaric acid, and ferulic acid and UV absorbance at 257 nm to detect *p*-hydroxybenzoic acid.

Galactooligosaccharides Content. Oligosaccharides were determined according to the method of Smiricky et al. (*23*). Briefly, 1.0 g of cooked or raw bean seed flour was homogenized with 100 mL of deionized water and placed in a water bath at 80 °C for 60 min. The sample was transferred into a 250 mL volumetric flask, brought up to volume with deionized water, filtered, and subjected to HPLC analysis. The sample was injected into a Dionex DX-300 HPLC (Dionex Corp., Sunnyvale, CA) fitted with a CarboPac PA-1 (4 × 250 mm) analytical column. Galactooligosaccharides were detected using a Dionex pulsed electrochemical detector equipped with a gold working electrode.

Phytic Acid Content. Inositol hexaphosphate phytate ($InsP_6$) was determined according to the method of Talamond et al. (*24*). Briefly, 0.5 g of cooked or raw bean seed flour was placed into a 16 mL centrifuge tube, 10 mL of 0.5 N HCl was added, and the tube was vortexed. The sample was centrifuged, the supernatant was saved, and 1.5 mL of concentrated HCl was added. The sample was filtered and used for HPLC analysis. The sample was injected into a Dionex DX-300 HPLC (Dionex Corp.) fitted with an Omnipac Pax-100 anion exchange (4 × 250 mm) column. Phytic acid was detected using a conductivity detector.

Statistical Analysis. For each of the quantified compounds, mean values were statistically compared using Tukey's method of multiple comparison (*25*). To descriptively compare materials in their multivariate compound profiles, stars plots of multivariate data were used (*26*). To build dendrograms to identify groups, a divisive hierarchical clustering method was used (*27*). All computations were done using statistical package S-Plus 6 for Windows (*User's Guide*, Insightful Corp., Seattle, WA).

RESULTS AND DISCUSSION

Flavonols. The flavonols quercetin and kaempferol are the most abundant flavonoids in foods, and their consumption has been related to an inverse association between lung cancer and cardiovascular disease risk. The plausible mechanisms involved in this relationship are modulation of detoxification enzymes and inhibition of some enzymes related to cell proliferation (*28*), but the most recognized effect is their antioxidant capacity [Trolox equivalent antioxidant capacities (TEAC) are 4.7 mM for quercetin and 1.4 mM for kaempferol] (*17*). In the present work, quercetin and kaempferol contents in raw and cooked bean seeds are shown in **Table 2**. Quercetin dry weight content in raw bean seeds ranged from 6.9 to 23.5 μg/g and from 4.3

Table 2. Quercetin, Kaempferol, and Phenolic Acid Contents[a] (Micrograms per Gram, Dry Basis) in Raw and Cooked Mexican Common Bean Seeds

	quercetin		kaempferol		HBA		VA		CMA		FA	
sample	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
FMM38	6.9 de	4.8 d	16.1 d	11.4 ef	13.1 ab	7.6 a	7.2 cd	4.5 cd	6.2 ab	2.1 c	20.8 cd	13.2 c
FJAna	7.0 de	4.3 d	13.8 d	7.1 f	9.7 bc	7.9 a	5.2 d	3.5 d	4.8 b	nd[b]	20.9 cd	17.6 bc
FJMar	8.2 de	6.7 c	209.4 a	123.2a	11.8 ab	7.9 a	7.4 cd	5.3 c	7.1 a	nd	24.3 bc	17.8 bc
N8025	9.7 cde	8.5 bc	19.2 d	15.7 de	9.6 bc	7.5 a	13.0 b	9.8 ab	6.8 ab	4.7 a	36.0 a	27.9 a
Notom	13.4 c	7.8 bc	20.3 d	19.1 d	5.7 cd	4.5 b	8.9 bc	6.7 c	5.4 b	3.8 a	24.5 bc	18.3 bc
Apa 95	7.9 de	4.7 d	20.8 d	13.5 de	7.1 cd	5.4 b	6.0 cd	4.3 cd	5.2 b	3.5 ab	18.2 cd	11.9 c
FJVic	9.4 cde	5.8 cd	204.6 a	65.3 b	8.8 bc	6.9 ab	8.6 c	5.4 c	4.9 b	nd	21.5 cd	15.9 bc
FJAcu	7.7 de	4.8 d	48.1 c	27.3 c	13.8 a	7.6 a	6.1 cd	4.1 d	5.3 b	3.2 b	20.4 cd	17.0 bc
FM94050	8.5 de	4.8 cd	19.1 d	9.7 ef	10.5 b	8.3 a	11.9 b	8.7 b	6.1 ab	3.6 ab	27.6 bc	20.8 b
NQro	23.5 a	12.0 a	19.2 d	12.8 ef	11.1 ab	7.2 a	14.1 ab	11.5ab	5.7 ab	3.8 a	26.8 bc	20.2 b
G-12892	11.3 cd	7.6 bc	23.1 d	14.3 de	9.6 bc	7.3 a	8.6 c	nd	5.6 ab	4.0 a	24.6 bc	19.8 b
G-12893	10.4 cde	7.0 bc	16.1 d	10.1 ef	8.3 bcd	6.5 ab	7.4 cd	6.5 c	3.2 c	2.2 c	25.2 bc	17.8 bc
G-12906	17.9 b	6.1 c	37.0 c	13.2 e	11.3 ab	8.6 a	16.6 a	12.1a	5.3 b	3.9 a	28.4 b	23.3 ab
G-11025B	11.5 cd	6.7 c	64.9 b	18.9 d	10.7 b	7.8 a	13.4 b	9.6 ab	3.9 bc	1.7 c	17.0 cd	13.0 c
mean	10.9	6.5	52.3	27.2	10.1	7.2	9.6	7.1	5.4	3.3	24.0	18.2

[a] Values are means of three determinations. HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CMA, *p*-coumaric acid; FA, ferulic acid. Means in the same column with different letters are different ($P < 0.05$). [b] Not determined.

Table 3. Quercetin, Kaempferol, Daidzein, Genistein, Coumestrol, and Phenolic Acid Contents[a] (Micrograms per Gram, Dry Basis) in 72-h-Germinated Mexican Common Bean Seeds

	FMM38	FJAna	FJMar	N8025	Notom	G-12892	G-12906	mean
quercetin	16.5 d	14.2 de	27.9 b	50.4 a	20.0 c	15.1 de	13.3 e	22.5
kaempferol	19.7 b	nd[b]	39.6 a	20.7 b	12.4 c	11.8 c	10.5 c	19.16
daidzein	9.4 e	8.2 e	43.6 b	129.1 a	25.8 d	32.3 c	23.8 d	38.9
genistein	2.6 d	3.5 cd	4.2 cd	9.7 a	6.4 b	5.0 bc	5.6 bc	5.3
coumestrol	2.4 d	3.8 cd	11.7 b	35.6 a	7.2 c	11.4 b	3.1 d	10.7
HBA	2.2 bcd	1.5 cd	4.1 a	3.1 b	1.8 cd	2.8 bc	2.0 cd	2.5
VA	43.2 a	7.7 c	12.2 c	40.8 a	28.4 b	25.7 b	46.3 a	29.2
CMA	nd	nd	nd	15.5 a	10.5 a	nd	nd	13.0
FA	25.5 b	16.7 c	22.6 bc	41.9 a	26.4 b	26.3 b	22.7 bc	26.0

[a] Values are means of three determinations. HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; CMA, p-coumaric acid; FA, ferulic acid. Means in the same row with different letters are different ($P < 0.05$). [b] Not determined.

to 12.0 μg/g in cooked bean seed. The reduction after processing ranged from 12 to 65%. Samples G-12906 and NQro had the higher quercetin contents as raw samples with 17.9 and 23.5 μg/g, respectively and they experienced the greatest reduction after processing (65 and 49%, respectively). Kaempferol dry weight content in raw bean seeds ranged from 13.8 to 209.4 μg/g and from 7.1 to 123.2 μg/g in cooked bean seeds. The reduction after processing ranged from 5 to 71%. Samples FJMar, FJVic, and G-11025 had the higher kaempferol contents as raw samples with 209.4, 204.6, and 64.9 μg/g, respectively. FJVic and G-11025 experienced the greatest reduction after processing (68 and 71%, respectively). The highest quercetin content was in the black samples, whereas the highest kaempferol content was in the cream samples, but there was not an absolute association. The quercetin content was lower than the values reported previously on a fresh weight basis for beans, onions, kale, apple, and broccoli (with 30, 347, 110, 36, and 30 μg/g, respectively), and the kaempferol content was higher than the values previously reported on a fresh weight basis for beans and onions (both with 2 μg/g), whereas kale kaempferol content was highest with 211 μg/g followed by broccoli with 72 μg/g (*29*, *30*).

In the seven accessions that were analyzed as germinated bean seeds without seed coat, quercetin and kaempferol were detected and the values are shown in **Table 3**. The values for quercetin ranged from 13.3 to 50.4 μg/g and those for kaempferol from 10.5 to 39.6 μg/g. Quercetin content in germinated bean seeds was higher than the respective values found in bean seeds except for G-12906, and kaempferol content was lower than the respective values found in bean samples except for FMM38 and N8050. There was a loss of flavonols with the seed coat exclusion as is clear in the sample FJMar, and the germinated bean seed flavonol content was the result of de novo synthesis.

Isoflavones and Coumestrol. The isoflavonoids genistein, daidzein, and equol (a product of intestinal bacterial metabolism of daidzein), along with coumestrol, are compounds known as phytoestrogens that have been related to a risk reduction of cardiovascular disease and cancer, particularly breast and prostate cancer (*9*, *31*−*33*). The plausible mechanism involved in this effect is the estrogen-like activity of these compounds, determined by their capacity to bind estrogen receptors and to induce a weak transcriptional activity (*19*, *34*). Thus, these compounds may result in an antiestrogen activity and protect against cancer inhibiting by competition the estradiol effect and, when estrogen production drops, these compounds may develop a weak estrogen activity. The effect of isoflavones is not exclusively hormonal; genistein is a specific inhibitor of protein tyrosine kinases and DNA topoisomerases I and II and arrests cell grow by interfering with the signal transduction pathways (*31*). Additionally, phytoestrogens exhibit antioxidant activity

(*31*). The presence of daidzein, genistein, and coumestrol in raw and cooked bean seed flour was not confirmed by the UV spectra. However, during the germination of the seven common beans seed samples, there was daidzein, genistein, and coumestrol genesis, and the values are shown in **Table 3**. The values for daidzein ranged from 8.2 to 129.1 μg/g, those for genistein from 2.6 to 9.7 μg/g, and those for coumestrol from 2.4 to 35.6 μg/g. Common bean is not recognized as an isoflavone source, and soybean is the only legume seed with high isoflavone content, with values for genistein of 950 μg/g and for daidzein of 600 μg/g (*33*). However both legumes have the nitrogen-fixing capacity, a process mediated by rhizobial bacteria attracted by isoflavones and coumestrol in the root (*35*); hence, the presence of isoflavones and coumestrol in sprouts and roots of this legume is normal, and its high concentration in soybean is an eccentricity. It is not common to consume germinated bean seeds. It is more common to consume common bean sprouts. This makes the germinated common bean seed and (or) the common bean sprout an alternative source of phytoestrogens.

Phenolic Acids. Two classes of phenolic acids can be distinguished: derivatives of benzoic acid (*p*-hydroxybenzoic, vanillic, and gallic acid) and derivatives of cinnamic acid (ferulic, *p*-coumaric, and caffeic acid). There are no epidemiologic studies relating their consumption to health effects, but in vitro studies have demonstrated their antioxidant effects and the capacity to modulate detoxification enzymes. The TEAC values are 2.2 for *p*-hydroxybenzoic acid, 1.8 for vanillic acid, 3.2 for ferulic acid, and 2.3 for *p*-coumaric acid (*18*, *36*). In the present work, the *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid retention times were 5.6, 7.6, 11.3, and 14.7 min, respectively, and their contents in raw and cooked bean seeds are shown in **Table 2**. *p*-Hydroxybenzoic acid in raw bean seed ranged from 5.7 to 13.8 μg/g and from 4.5 to 8.6 μg/g in cooked bean seeds. The reduction after processing ranged from 17.9 to 44.5%. Vanillic acid in raw bean seeds ranged from 5.2 to 16.6 μg/g and from 3.5 to 12.1 μg/g in cooked bean seeds. The reduction after processing ranged from 12.5 to 36.9%. *p*-Coumaric acid in raw bean seeds ranged from 3.2 to 6.8 μg/g and from 1.7 to 4.7 μg/g in cooked bean seeds. The reduction after processing ranged from 26.3 to 66.3%. Ferulic acid in raw bean seeds ranged from 17.0 to 36.0 μg/g and from 11.9 to 27.9 μg/g in cooked bean seeds. The reduction after processing ranged from 15.5 to 36.5%. Ferulic acid was present in the highest concentration followed by *p*-hydroxybenzoic acid and vanillic acid, the concentrations of which were very similar. *p*-Coumaric acid was present in the lowest concentration.

p-Hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid contents in germinated bean seeds without the seed coat are shown in **Table 3**. *p*-Hydroxybenzoic acid concentrations ranged from 1.5 to 4.1 μg/g, those for vanillic acid from 7.7 to 46.3 μg/g, those for *p*-coumaric acid from 10.5 to 15.5 μg/g, and those for ferulic acid from 16.7 to 41.9 μg/g. The germinated bean seed samples had lower *p*-hydroxybenzoic acid content, higher vanillic acid and *p*-coumaric acid contents, and similar ferulic acid content compared with the raw bean seeds. There was a loss of phenolic acids with the exclusion of the seed coat, as is clear by examining the *p*-hydroxybenzoic acid values, and there was de novo synthesis of vanillic acid, *p*-coumaric acid, and ferulic acid but not of *p*-hydroxybenzoic acid.

Total benzoic acid derivative concentrations reported here for raw bean seeds were 19.7 and 14.3 μg/g for cooked bean seeds, whereas the mean total cinnamic acid derivative concentrations in raw bean seeds were 29 and 21.5 μg/g for cooked bean seeds. In germinated bean seeds, the values were 32 and 39 μg/g for benzoic acids and cinnamic acids, respectively. Other foods have been described as the best source of phenolic acids; for example, Manach et al. (*36*) reported concentrations of benzoic acid derivatives in blackberries of 80−270 μg/g and concentrations of cinnamic acid derivatives in blueberries and wheat grain of 2000 μg/g.

Oligosaccharides. A prebiotic is a nondigestible food ingredient that in an intact form may reach the large intestine, where it stimulates the growth and activity of beneficial bacteria or probiotics, leading to a marked change in the gut microflora composition (*20*). Galactooligosaccharides consist of galactose residues linked α-1,6 to the glucose moiety of sucrose. Humans and monogastric animals do not possess the α-galactosidase enzyme to degrade these compounds, so they pass undigested through the stomach and small intestine and reach the large intestine, where they are fermented (*37*). Ricroft et al. (*20*) and De Boever et al. (*21*) used in vitro methodology to determine the capacity of galactooligosaccharides from soybean to stimulate the growth of bifidobacteria and lactobacilli. Besides their prebiotic effect, oligosaccharides, as a result of their fermentation, resulted in copious quantities of gas and short-chain fatty acids (SCFA) (*37*, *38*). The latter have been associated with hypocholesterolemic effects and apoptosis induction (*37*, *39*, *40*). Raffinose, stachyose, and verbascose contents of raw and cooked bean seeds are shown in **Table 4**. Without regard to the accession FM94050, raffinose content in raw bean seeds ranged from 4.4 to 11.4 mg/g and that in cooked bean seeds from 3.2 to 11.0 mg/g. The reduction after processing ranged from 1.7 to 36.8%. Stachyose content in raw bean seeds ranged from 50.9 to 63.8 mg/g and that in cooked samples from 37.9 to 56.7 mg/g. The reduction after processing ranged from 3.8 to 25.5%. Verbascose content in raw bean seeds ranged from 2.2 to 5.1 mg/g and that in cooked seeds from 1.6 to 4.4 mg/g. The reduction after processing ranged from 1.7 to 43.3%. Stachyose was the main oligosaccharide followed by raffinose and verbascose; however, for sample FM94050, the main oligosaccharide was verbascose, with 35.8 mg/g, followed by stachyose with 23.7 mg/g and raffinose with 14.1 mg/g in the raw sample. This has been reported for other legumes (*Phaseolus radiatus* and *Lens esculentus*) (*41*).

With the exception of sample FM94050, values reported agree with those of Sathe et al. (*42*) but were higher than the values reported by Reddy and Pierson (*43*) and Muzquiz et al. (*44*). All authors have found stachyose to be the major oligosaccharide.

Phytic Acid. Phytic acid ($InsP_6$) is found in cereals, legumes, nuts, and oilseeds in quantities between 1 and 5% of the weight of the seeds and serves as a storage form for phosphorus. $InsP_6$ has been recognized as an antinutrient due to its indigestibility and its ability to decrease the bioavailability of divalent and trivalent cationic metals such as iron and zinc and to bind proteins and starch, reducing their digestibility. Thus, diets with a high proportion of energy from grains and legumes (high $InsP_6$ content) can lead to nutritional deficiencies (*15*, *45*). However, its unique molecular interactions also are related to beneficial health outcomes. $InsP_6$ interaction with starch and divalent metals results in a low glycemic index and reduces the participation of iron in oxidation metal mediated effects related with a low diabetes, cardiovascular disease, and colon cancer risk (*45*). The phytic acid content of raw and cooked bean seeds is shown in **Table 4**. In raw bean seeds, phytic acid ranged from 7.8 to 17.6 mg/g and from 5.7 to 15.3 mg/g in cooked

Table 4. Galactooligosaccharide[a] and Phytic Acid[b] Contents (Milligrams per Gram, Dry Basis) in Raw and Cooked Mexican Common Bean Seeds

	raffinose		stachyose		verbascose		phytic acid	
sample	raw	cooked	raw	cooked	raw	cooked	raw	cooked
FMM38	8.5 d	6.5 g	57.4 d	50.4 d	3.8 d	3.2 c	7.8 f	7.0 d
FJAna	6.1 h	4.6 j	50.9 e	37.9 g	2.8 gh	1.6 d	8.4 ef	5.7 e
FJMar	7.5 f	7.4 f	56.5 d	54.3 b	2.3 j	2.2 d	13.1 c	8.8 c
N8025	10.7 c	10.6 b	63.8 a	56.3 ab	4.7 c	3.8 bc	12.7 c	8.4 c
Notom	11.2 b	11.0 a	59.6 c	56.1 ab	5.1 b	4.4 b	10.9 d	7.1 d
Apa 95	8.1 e	5.3 i	61.2 b	47.2 e	2.9 g	1.7 d	8.5 ef	5.9 e
FJVic	8.0 e	7.7 e	56.2 d	50.9 cd	2.3 j	2.3 cd	8.2 ef	8.0 c
FJAcu	11.4 b	10.7 b	59.5 c	54.9 b	3.3 e	2.9 c	11.9 cd	8.5 c
FM94050	14.1 a	10.3 c	23.7 f	16.8 h	35.8 a	24.3 a	11.2 d	7.2 d
NQro	10.4 c	10.1 d	59.5 c	52.8 c	3.7 d	3.4 bc	14.5 bc	11.0 b
G-12892	4.4 j	3.5 k	59.1 c	56.7 a	2.8 h	2.7 cd	14.8 b	11.6 b
G-12893	5.0 i	3.2 l	58.4 c	45.5 f	2.2 j	1.8 cd	12.2 cd	8.5 c
G-12906	6.2 h	5.8 h	63.0 a	55.1 b	2.6 i	2.3 cd	9.3 e	8.6 c
G-11025B	7.0 g	4.8 j	59.4 c	52.0 c	3.1 f	1.8 cd	17.6 a	15.3 a
mean	8.5	7.2	56.3	49.0	5.5	4.2	11.54	8.7

[a] Values for galactooligosaccharides are means of two determinations. [b] Values for phytic acid are means of three replicates. Means in the same column with different letters are different ($P < 0.05$).

bean seeds. The reduction after processing ranged from 2.3 to 35.2%. Both lower and similar values were reported previously (*44*).

Whether phytic acid is considered to be an antinutrient or a bioactive compound is not dependent on the phytic acid per se or its source; rather, it is dependent on diet diversity. Thus, in diets based on grains and legumes and low in animal protein, phytic acid compromises mineral balance and health, principally of susceptible persons such as children. Humans who consume a diverse diet in which micronutrient intake and bioavailability are high may be positively affected by phytic acid consumption.

For purposes of clarifying the metabolic differences between bean seed samples, a descriptive scheme that represents the 10 compounds in each raw bean seed sample was developed and is shown in **Figure 1**. Sample FM94050 was omitted due to its unusual oligosaccharide profile. In the scheme, each ratio-like line represents the relative content of one compound. (For example, the line "1" represents the *p*-hydroxybenzoic acid content, that is, the larger one for the FMAcu scheme and but not present for the Notom scheme. It means that FMAcu has the highest *p*-hydroxybenzoic acid content and Notom the lowest; the remainder of the samples have a line 1 of magnitude proportional to its *p*-hydroxybenzoic acid content.)

From these schemes, it is clear that there are samples with high metabolic density (N8025 and NQro) and with low metabolic density (FJAna, Apa95, and G-12893). There are samples with high quercetin content, such as NQro and G-12906, and samples with very high kaempferol content, such as FJMar and FJVic. Also, there are samples with high phenolic acids content, such as N8025, NQro, and G-12906 (and FM94050, which was not included in the schemes), samples with high oligosaccharide content, such as N8025, Notom, FMAcu, and NQro, and samples with high phytic acid content, such as G-11025B, G-12892, and NQro. On the other hand, there are samples with low quercetin content, such as FMM38, FJAna, Apa95, and FMAcu, samples with low kaempferol content, such as FMM38, FJAna, and G-12893, samples with low phenolic acids content, such as FJAna, Apa95, and G-12893, samples with low oligosaccharide content, such as FJAna, FJMar, FJVic, and G-12892, and samples with low phytic acid content, such as FMM38, FJAna, Apa95, and FJVic.

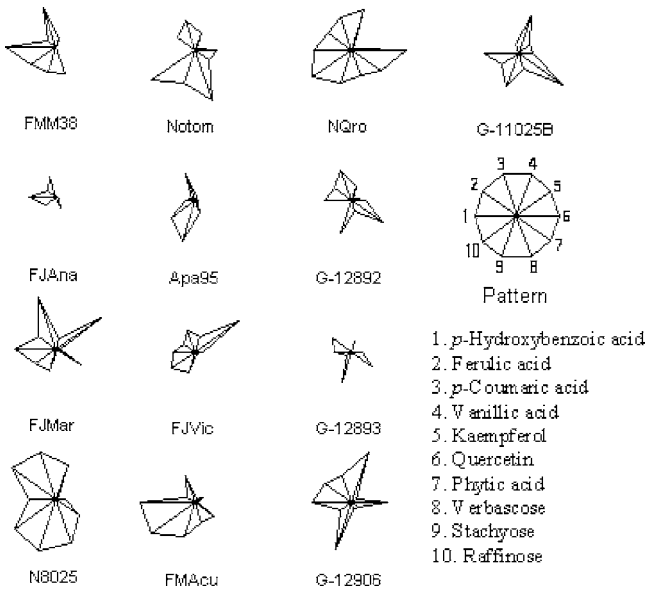

Figure 1. Representative schemes of 10-compound metabolic profiles in each raw bean seed sample. Sample FM94050 was not included. In the figure of each seed sample, the ratio-like lines represent the relative compound content in the same order as in the figure pattern. Each numerated ratio-like line in the figure pattern represents one of the compounds listed above.

The dendrogram obtained from these results (**Figure 2**) shows two main groups. The first group was formed by nine common bean seed samples. In this group, there are three clusters, the first formed by FMM38, FMAcu, Apa95, and FJAna, the second formed by the wild samples G-12892, G-12893, and G-11025B, and the third formed by FJMar and FJVic. The second group was formed by four common bean seeds samples. In this group there are two clusters, the first formed by N8025 and Notom and the second formed by NQro and G-12906. All of the black samples were in this group.

The representative schemes of eight compound concentrations in germinated bean seeds are shown in **Figure 3**. *p*-Coumaric acid was not included because it was detected only in two samples. FJAna exhibited a very low metabolic density, and N8025 exhibited a high metabolic density. FMM38, FJAna,

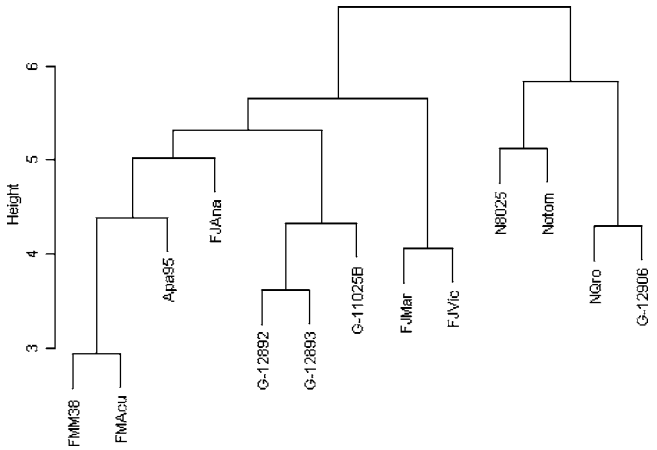

Figure 2. Dendogram representing similarity in the metabolic profiles between raw bean seed samples. Sample FM94050 was not included.

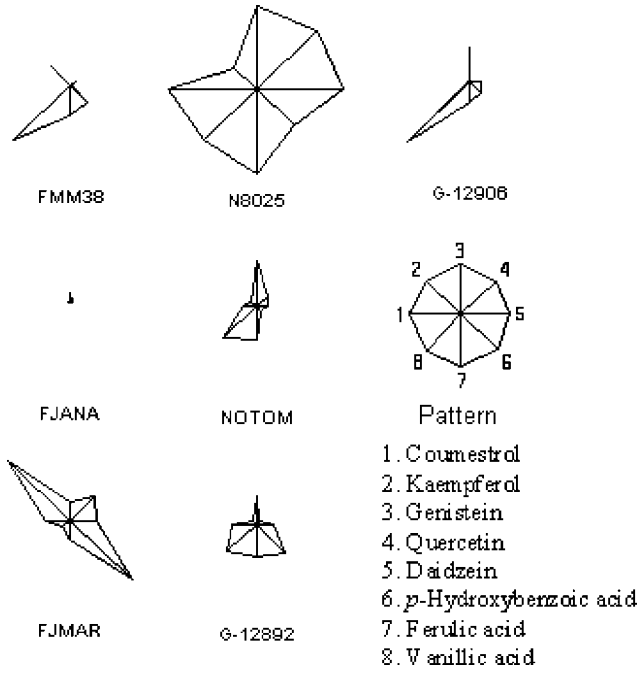

Figure 3. Representative schemes of eight-compound metabolic profiles in each germinated bean seed sample. In the figure of each seed sample, the ratio-like lines represent the relative compound content in the same order as in the figure pattern. Each numerated ratio-like line in the figure pattern represents one of the compounds listed above.

G-12892, and G-12906 were samples with low flavonoid and coumestrol contents, whereas N8025 was a sample with a high concentration of flavonoids, coumestrol, and phenolic acids. The dendrogram obtained from these results (**Figure 4**) shows one group of six samples with the sample N8025 separated from the group. If the plausible effect on health of germinated seeds or beans sprouts is evaluated, the components of common bean must be considered due to the dramatic difference between component concentrations exemplified by sample FJAna, a poor source of phenolics, and the sample N8025, a major source of phenolics (**Figure 3**). This may have relevance not only to human health but to plant health and development as well.

Legumes have been associated with a low risk of cardiovascular disease, diabetes, and cancer. However, soybean and nonsoybean legumes must be considered separately when the health effects are considered, due to the differences in their metabolic profiles. Soybean health effects relate to a lower risk of cardiovascular disease and prostate and breast cancer and

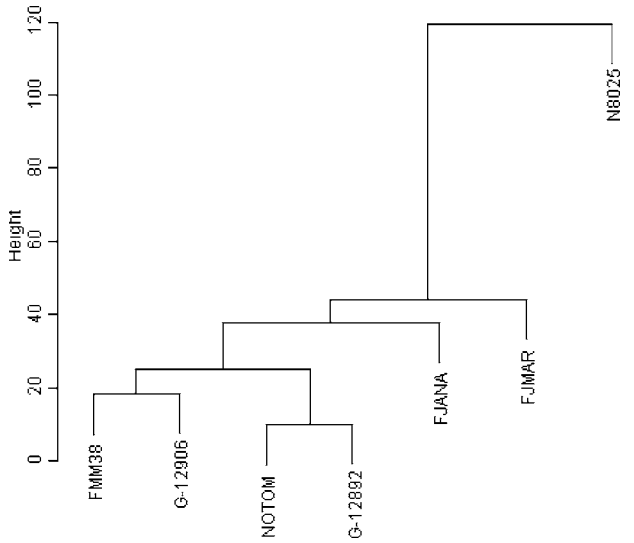

Figure 4. Dendogram representing similarity in the metabolic profiles between germinated been seed samples.

are associated with the isoflavone content. There are no measurable flavonols in soybean (*46*−*48*). In common bean, there are no measurable isoflavones, but they are a good source of flavonols, phenolic acids, and other phenolics, and their health effects are associated with a lower risk of cardiovascular disease and colon cancer. These diseases are associated with the dietary fiber content; however, fiber per se affects cardiovascular disease (*10*, *49*), but to date results are inconclusive regarding cancer of the colon (*50*).

In the present work, wild and cultivated Mexican common bean seeds have been described with regard to their content of compounds not commonly regarded as impacting health. No particular differences were found between wild and cultivated Mexican common bean; however, high variability was found in the concentration of compounds present. In the metabolic relationship among bean seeds samples, there was a divergence between phenotypes (**Figure 2**), black samples in general having a high metabolic density in relation to the remaining samples.

In conclusion, Mexican common bean seeds, besides being a good source of dietary fiber, are an important source of flavonols, phenolic acids, galactooligosaccharides, and phytic acid. Additionally, germinated bean seed and bean sprouts may be a source of an interesting mix of phytoestrogens and antioxidants, two categories of compounds associated with human health.

ACKNOWLEDGMENT

We express our gratitude to H. Guzmán Maldonado and J. Acosta Gallegos, from INIFAP, for the common bean samples provided, to M. E. Valverde from Cinvestav-IPN for technical assistance, and to E. González de Mejía and E. Jeffrey, University of Illinois, for assistance in developing this work.

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Received for review July 15, 2005. Revised manuscript received November 8, 2005. Accepted November 9, 2005. Partial financial support from the USAID-TIES University of Illinois−Universidad de Querétaro program is acknowledged.

JF051706L