Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 521–527
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*)

Rocha-Guzmán Nuria Elizabeth [a,*], Herzog Annete [b], González-Laredo Rubén Francisco [a], Ibarra-Pérez Francisco Javier [c], Zambrano-Galván Graciela [a], Gallegos-Infante José Alberto [a,*]

[a] *Instituto Tecnológico de Durango, Departamento de Ingenierías Química y Bioquímica, Boulevard Felipe Pescador 1830 Ote., 34080 Durango, Dgo., Mexico*
[b] *Rheinische Friedrich Wilhelms Universitat Bonn, Department of Nutrition and Food Science, Romerstrase 164, D-53117, Bonn, Germany*
[c] *INIFAP-CEVAG, Carretera al Mezquital Km. 4.5, 34000 Durango, Dgo., Mexico*

Received 24 December 2005; received in revised form 31 July 2006; accepted 24 August 2006

Abstract

Total phenolic content of seven improved common bean cultivars (*Phaseolus vulgaris* L.) namely, Negro Altiplano, Negro Durango, Negro Sahuatoba, Flor de mayo sol, Flor de Mayo Bajío, Flor de Mayo 94044MX and Bayo Madero were analyzed. Acetone and methanol extracts from bean cotyledons were obtained by successive extractions. Total phenolic content was evaluated following Folin–Ciocalteu method; antioxidant activity by the DPPH technique; antimutagenic potential by the Ames method; and preliminary identification was realized by 2D-TLC. Results indicated high correlation between total phenol content and antioxidant activity for acetone extracts, and also high correlation between antioxidant and antimutagenic activities. In contrast, low correlation coefficients were obtained for methanol extracts. Three cultivars (two Negro cultivars and a Flor de Mayo type) showed lower antimutagenic activity than catechin.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Antimutagenic activity; Antioxidant; Common bean; Polyphenol

1. Introduction

Flavonoids constitute the most common group of secondary plant metabolites. They represent a large family of low-molecular-weight phenolics (Koes, Quattrocchio, & Mol, 1994) and there can be divided in different structural classes according to the level of oxidation and the pattern of substitution of the central C-ring (Harborne & Williams, 2000). Due to their contribution to colour and flavour of fruits and vegetables, nuts and seeds, flavonoids form an integral part of human diet. Rich dietary sources of flavonoids are soybean (isoflavones), citrus (flavanones), tea, apple and cocoa (flavanols), celery (flavones), onions (flavonols) and berries (anthocyanins) (Rice-Evans, Miller, Blowell, Bramley, & Pridham, 1995; Ross & Kasum, 2002).

Oxidative stress leads to oxidative damage leading to extracellular macromolecules modification of lipids in cell membranes, proteins in tissues or enzymes and DNA. Reactive oxygen species (ROS) induced damage includes lipid peroxidation, protein disruption and DNA crosslinking/scission (Pietta, 2000). DNA cross-linking results in spontaneous mutagenesis that is implicated in chronic degenerative diseases, particularly cancer (Ames, Shigenaga, & Hagen, 1993). Therefore, supplementation of dietary antioxidants is necessary to diminish the harmful effects of oxidative processes in living organisms (Nordberg & Arner, 2001). Phenolic compounds are able to serve as antioxidants (Decker, 1995), since they are able to suppress formation of

* Corresponding author.
E-mail address: jinfante@itdposgrado-bioquimica.com.mx (J.A. Gallegos-Infante).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.08.021

initiating radical species by inhibition of enzymes or chelating metal ions (iron, copper) involved in initiation of free radical production process. Phenolics also can act as hydrogen donating radical scavengers in aqueous and lipophilic phases (Halliwell, 1998; Jovanovic, Steenken, Tosic, Marjanovic, & Simic, 1994; Kanner, Frankel, Grant, German, & Kinsella, 1994; Shahidi & Wanasundara, 1992). They inhibit xanthine oxidase and protein kinase C which are responsible for generation of superoxide anion. The key enzymes involved in the production of ROS such as cyclooxygenase, lipoxygenase, glutathione *S*-transferase, mitochondrial succinoxidase and NADH oxidase, are also inhibited by flavonoids (Cos et al., 1998).

Antimutagenic effects are often specific to certain classes of mutagens on certain test systems. There are specific relationships between flavonoid structure and their antimutagenic activities for each class of mutagens. The antimutagenic effect depends on the number and position of phenolic hydroxyl groups as can be seen in flavones. Blocking of hydroxyl groups by alkylation or acetylation decreases antimutagenic activities (Edenharder & Tang, 1997).

In *Phaseolus vulgaris*, phenolic compounds are mainly located in the seed coat with lower amounts in cotyledons (Desphande, Shatze, Salunkhe, & Cornforth, 1982; González de Mejía, Castaño-Tostado, & Loarca-Piña, 1999). Environmental factors such as growing location as well as genetics factors (cultivar) influence the level of total phenolics. The amount and composition of flavonol glycosides, condensed tannins (procyanidins) and anthocyanidins determine the seed coat colour (Feenstra, 1960). Dark pigmented coats such as black beans possess higher contents of phenolics than those with light coloured seed coats (Barampama & Simard, 1993).

Beninger and Hosfield (2003) isolated and identified the flavonoid compounds that contribute to colour in dark red kidney bean seed coats. Three yellow flavonol glycosides were characterized, but the major contribution to their red colour is due to the presence of procyanidins B2, C1 and C2. Other literature reports the presence of catechin and related compounds, such as delphinidin, cyanidin, and phenolic acids such as vanillic, caffeic, coumaric and ferulic in seed coats from common bean cultivars (Madhujith, Amarowicz, & Shahidi, 2004).

Methanol hull extracts of mung bean exhibited antioxidant activity by inhibiting lipid peroxidation in a liposome model system (Duh, Du, & Yen, 1999). Previously isolated condensed tannins as well as pure flavonoid compounds play a crucial role in the overall antioxidant activity of the extracts. Flavonoids such as delphinidin, petunidin and quercetin glucosides are the most active compounds found and showed significant antioxidant activity in comparison to butylated hydroxytoluene (BHT) (Von Gadow, Joubert, & Hansmann, 1997), confirming the structure–activity relationships proposed by Beninger and Hosfield (2003).

Cardador-Martínez, Loarca-Piña, and Oomah (2002) evaluated total phenolics and antioxidant properties of methanolic extracts of seed coats and other bean fractions (*P. vulgaris*, cv Flor de Mayo FM 38). According to this study, the highest concentration of phenolics was obtained in manually separated seed coats. Their phenolics showed high antioxidant activity towards β-carotene-linoleate and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in in vitro model systems. Also seed coats exhibited higher antioxidant activity than other bean extracts. Consequently, many biological functions such as antimutagenicity, are mediated by the antioxidant property (Velioglu, Mazza, Gao, & Oomah, 1998).

Traditional common bean cultivars have been improved in Mexico to enhance their agronomical and physicochemical properties. However, there is still a lack of knowledge on their biological potential and possible utilization as functional foods. Therefore, the objective of this work was to investigate the antioxidant and antimutagenic activities of different colour group improved common beans grown in Mexico.

2. Material and methods

Seven improved common bean (*P. vulgaris* L.) cultivars Negro Altiplano (NA) (Acosta-Gallegos et al., 2001), Negro Durango (ND), Negro Sahuatoba (NS) (Acosta-Gallegos, Ibarra-Perez, Rosales-Serna, Castillo-Rosales, & Kelly, 2001), Flor de mayo Sol (FMS), Flor de Mayo Bajio (FMB), Flor de mayo 94044MX (FM) and Bayo Madero (BM), were harvested in 2004 at the Experimental Field Valle del Guadiana (CEVAG) in Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The histidine-requiring strains *Salmonella typhimurium* TA 98 was kindly supplied by Dr. Javier Espinoza, from Biomedical School at Universidad Nacional Autonoma de México (UNAM).

Seed coats were separated manually and cotyledons ground to a fine powder (mesh size 100). All chemicals used in this work were reagent grade except where indicated (Caledon Lab., Georgetown, ON, Canada). Bean flour samples (20 g) were defatted by extraction with 200 mL of chloroform/methanol (2:1, v/v) and agitated for 1 h at room temperature (RT). Then, successive extractions were performed with 70% aqueous acetone (200 mL), followed by 50% aqueous methanol (200 mL) for 24 h each at RT. Crude extracts were concentrated under vacuum at 40 °C using a rotary evaporator. The resulting aqueous solutions were lyophilized to a powder, which was stored in the dark at 4 °C until analysed. Extractions were performed in duplicate. Experimental samples were permanently protected from light (Waterman & Mole, 1994).

Interference of proteins in determination of total phenols was evaluated as follows: methanol extract of cotyledon flour (0.1 g) and polyvinylpyrrolidone (Baker, Philipsburg, NJ, USA) (PVP) at a final concentration of 50 mg/mL were suspended in 2 mL of 5 mM phosphate buffer (pH 7.4) and agitated for 1 h. Suspension was then

centrifuged at 2038g (4500 rpm, 20 min) and supernatant recovered for posterior protein quantification. A control experiment was carried out without PVP. Protein determination was realized according to Bradford (1976) method.

Total phenolic content was determined following methodology reported by Waterman and Mole (1994). The total phenolic content was expressed as catechin equivalents (CE, mg catechin/g sample) using a catechin calibration curve (20–120 μg/mL, $r^2 = 0.9927$).

Free radical scavenging capacity was estimated according to Pekkarinen, Stöckmann, Schwarz, Heinonen, and Hopia (1999). Each extract in 50% ethanol, was tested at four concentrations (5, 50, 500 and 5000 μg/mL). The radical scavenging capacity (%RSA) was calculated as the percentage of DPPH absorbance reduction.

Crude extracts were subjected to preliminary identification for flavonoids and condensed tannins using bidimensional thin layer chromatography (2D-TLC) (Karchesy, Bae, Chalker-Scott, Helm, & Foo, 1989). Cellulose in plastic plates was used and two mobile phases selected: butanol–acetic acid–water (BAW; 5:1:2, v/v/v) and 6% acetic acid (AcOH). The fluorescent spots were observed with short and long wavelength UV light and their R_f related to flavonoid structures (Markham, 1982). Selective visualization of procyanidins and other compounds with a reactive phloroglucinol ring was obtained by spraying chromatograms with vanillin acidic reagent followed by heating (Karchesy et al., 1989).

The antimutagenic effect of common bean extracts was tested according to the Ames assay modified by Edenharder and Tang (1997). Genetic markers for each strain were confirmed based on methods reported by Maron and Ames (1983), prior to the anti-mutagenesis study. 1-Nitropiren (1-NP) was used at a concentration of 0.2 μg/mL as positive control for mutagenesis. Standard catechin (1 μg/mL) was used to evaluate their capacity for inhibiting mutation induced by 1-NP. Minimal glucose plates with and without histidine and a zero concentration of 1-NP were considered as controls. Cotyledon bean extracts were used at a concentration of 100 μg/mL. Antimutagenic effect was expressed as percentage of the mutagenicity inhibition.

Statistical analysis was performed by one-way ANOVA. Before the antimutagenic test, dose–response curves were built for mutagen 1-NP and the standard catechin. Antimutagenic effect was expressed as percentage of mutagenicity inhibition following the formula:

$$\%\ \text{Inhibition} = 100 - (R_1/R_2 \times 100),$$

where R_1 is the number of his$^+$ revertants per plate exposed to 1-NP and R_2 is the number of his$^+$ revertants per plate in the absence of phenolic sample.

3. Results and discussion

Successive extraction yields for each cultivar are shown in Table 1. Results obtained showed that cotyledon methanolic extracts yields were higher than acetonic extracts. These results are different to those found by Cardador-Martínez, Castaño-Tostado, and Loarca-Piña (2002) for common bean seed coat phenolics, because they reported acetone extracts with higher yields than methanol extracts. An explanation to this difference could be based in the fact that cotyledon is rich in carbohydrates and proteins, which associate with phenolic compounds and methanol is not able to inhibit these interactions. Therefore, proteins and carbohydrates are partly extracted contributing to the higher yield of methanol extract samples (Hagerman & Butler, 1981).

Extract yields obtained with acetone ranged from 6.03 ± 0.33 (FMS) to 6.83 ± 0.60% (BM). Corresponding range for methanolic samples was 7.16 ± 0.40 (FM) to 8.66 ± 0.26% (NS). For both solvents, the relative yield order by bean groups was: Negro > Bayo > Flor de Mayo. Analysis of variance ($p < 0.05$) showed that cultivar did not influence the overall variation of successive extraction yields; however, the solvent did have a significant effect on it.

Two group cultivars (Negro and Flor de Mayo) were selected randomly for the determination of proteins. The lowest amount of proteins was obtained in extracts from untreated samples (FMB: 0.23 ± 0.01 mg/mL; NS: 0.18 ± 0.05 mg/mL). Proteins remained complexed with phenolic compounds and therefore could not be detected with the Bradford reagent. After PVP-treatment of extracts, the proteins were recovered, resulting in a twofold higher content.

Recent studies have shown the presence of catechin and related flavonoids in beans (Preza y Lerma, 2003); therefore the total phenolic content was calculated as CE.

Table 1
Yield and total phenolic content in common bean cultivar extracts obtained with acetone 70% and methanol 50%

Cultivar	Yield		Total phenolic content (mg catechin equivalents per gram sample)	
	Acetone 70%	Methanol 50%	Acetone 70%	Methanol 50%
Bayo Madero (BM)	6.83 ± 0.60	7.83 ± 0.50	25.35 ± 1.91	15.94 ± 0.58
Flor de Mayo 94044MX (FM)	6.16 ± 0.16	7.16 ± 0.40	24.00 ± 0.67	16.94 ± 0.33
Flor de Mayo Bajío (FMB)	6.16 ± 0.36	7.36 ± 0.33	26.65 ± 0.42	11.23 ± 1.08
Flor de Mayo Sol (FMS)	6.03 ± 0.33	7.50 ± 0.40	20.41 ± 1.75	13.47 ± 0.25
Negro Altiplano (NA)	6.83 ± 0.70	7.36 ± 0.16	18.88 ± 0.92	14.82 ± 1.50
Negro Durango (ND)	6.66 ± 0.36	7.83 ± 0.10	22.76 ± 0.75	16.41 ± 1.25
Negro Sahuatoba (NS)	6.70 ± 0.93	8.66 ± 0.26	19.94 ± 1.25	15.59 ± 1.41

Results obtained from this quantification supported the selectivity of aqueous acetone for polyphenols, which displays 50% higher content than methanol (Table 1). The highest concentration in acetonic extracts was obtained in FMB cultivar (26.65 ± 0.42 CE), followed by the BM cultivar (25.35 ± 1.91 CE). NA cultivar had the lowest total phenolic content (18.88 ± 0.92 CE) of all acetone extracts tested. Phenolics in methanolic extracts ranged from 11.23 ± 1.08 (FMB) to 16.94 ± 0.33 CE (FM). Considering seed coat grouping, the relative order of total phenolic content in acetone extracts was: Bayo (Cream colour) > Flor de Mayo (yellow mottled colour) > Negro (black colour). Whereas no significant differences among cultivar groups could be observed in methanol extracts. It is very difficult to find in the literature data for comparing total phenolic content, particularly when complex extracts are analyzed and different methods are used to quantify it. In the case of Folin–Ciocalteu method different reagent concentrations, timing of addition and incubation, and different standards used to express the results are the main sources of this problem (Prior, Wu, & Schaich, 2005). Total phenolic content in this study, was higher than previously reported (acetone: 5–17, methanol: 4–13 tannic acid equivalents) (Preza y Lerma, 2003).

According to the analysis of variance, significant variability ($p < 0.05$) in the content of total phenolics was due to cultivar, solvent and their statistical interactions. Most of the variance was associated with the *solvent* × *cultivar* statistical interaction.

Brand-Williams, Cuvelier, and Berset (1995) determined three different types of kinetic behaviour: (a) rapid kinetic behaviour refers to antioxidants reaching a steady state in less than 1 min; (b) intermediate reacting antioxidants reach their steady state after 5–30 min; and (c) antioxidants belonging to the third type react slower. According to this study, phenolic compounds present in common bean are slowly acting antioxidants, since their steady state was obtained after 45 min in all concentrations tested.

A wide range of concentration from 5 to 5000 μg/mL was used to assess the capacity of phenolic extracts to scavenge the DPPH radical. Results obtained indicated that acetone extracts were more effective scavengers of DPPH than methanol extracts. Extracts with any of both solvents showed no significant ($p > 0.05$) difference in RSA% at the tested concentrations. Acetone extracts at 5000 μg/mL displayed an average of 2 (BM), 5.7 (FM) and 3.4 (ND) fold higher activity than those at lower concentrations. In contrast, differences were less significant in methanol extracts that is, the scavenge capacity of 5000 μg/mL sample was only 1.2 (BM), 4.9 (FMB) and 1.8 (ND) times greater than the average at lower concentrations.

The highest activity for free radical scavenging was obtained after 45 min at 5000 μg/mL (Fig. 1). Acetone extracts from FMB (67.35 ± 0.21%) and BM (62.28 ± 0.20%) cultivars were the most active, while NA had the lowest activity in both solvents, acetone: 32.21 ± 1.03% and methanol: 9.90 ± 0.35%. The highest DPPH radical

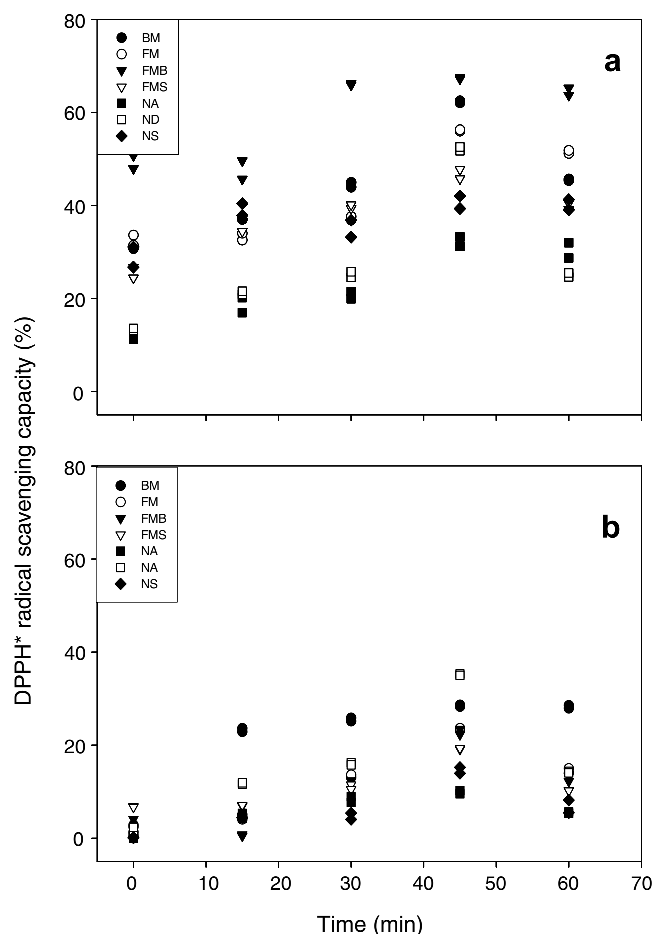

Fig. 1. Free radical scavenging activity at 5000 μg/mL of common bean extracts obtained with: (a) 70% acetone and (b) 50% methanol.

scavenging capacity value in methanol extracts was obtained for the cultivar ND (35.15 ± 0.20%).

At every concentration used, all bean samples tested exhibited lower antiradical capacity than catechin. At lower concentrations the black cultivars showed the highest capacity and Flor de Mayo, the lowest one. However, at the highest concentration, the free radical scavenging capacity of Flor de Mayo types exceeded that of black type cultivars.

Antioxidant activity toward DPPH of acetone extracts was found to correlate strongly with their total phenolic content ($r^2 = 0.9605$ and $p < 0.05$) as indicated in Fig. 2; such correlation indicates that phenolic compounds might be responsible for the antioxidant activity exhibited by samples in this study. Thus total phenolic content can be used to predict the capacity of the phenolic extracts to scavenge the DPPH radical. However, this correlation was not observed for methanol extracts (Fig. 2). This might be due to the presence of carbohydrates in the extracts. If they take up an electron, they are not able to delocalize it and form a stable free radical, so they give it back to the medium shortly afterwards.

The histidine-requiring strain of *S. typhimurium* TA98 was maintained, propagated, and routinely tested for the

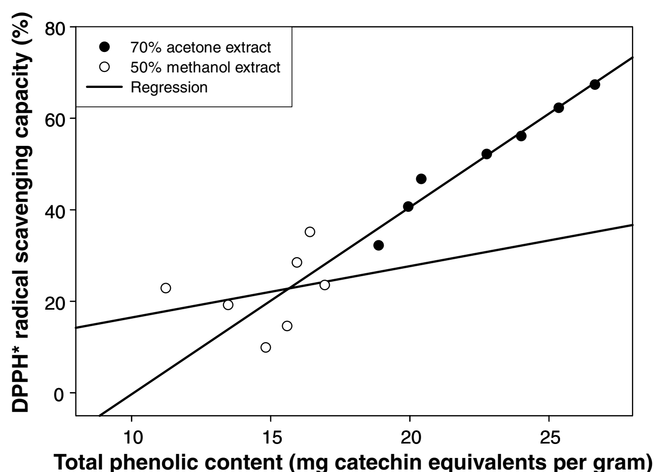

Fig. 2. Total phenolic content vs. DPPH radical scavenging activity for 70% acetone extracts ($r^2 = 0.9605$) and 50% methanol extracts ($r^2 = 0.0694$).

presence of a genetic marker. The dose–response curve of 1-NP mutagenicity in the tester strain TA98 indicated that the highest doses (0.42 and 0.5 μg/mL) were toxic to the bacteria. There was a substantial dose–response relationship up to 0.33 μg/mL, thus 0.2 μg/mL was chosen for all antimutagenicity assays. Catechin was neither mutagenic nor toxic to the bacteria at all concentrations tested. The antimutagenic activity was dose-dependent upto a concentration of 1 μg/mL and decreased at higher concentrations. Since the strongest effect was observed at a concentration of 1 μg/mL, it was chosen as the antimutagenic control. Phenolic compounds present in the acetone extracts from beans are potent anti-mutagens against the direct mutagen 1-NP in *S. typhimurium* TA98 (Fig. 3). Acetone extracts were not mutagenic to the bacteria at 1 μg/mL. All cultivars inhibited more than 50% of the 1-NP mutagenicity. The FMB and BM cultivars exhibited the highest antimutagenic activity: 76.83 ± 2.58 and $73.97 \pm 1.78\%$, respectively. Inhibitory effect of four cultivars (BM, FM, FMB and ND) was superior to the standard catechin. However, González de Mejía et al. (1999) reported that phenolic compounds from common bean coats extracted with 50% methanol did not exhibit any antimutagenic effect against 1-NP in *S. typhimurium* YG1024, a derivative of tested strain TA 98, which contains the gene that overproduces nitroreductase, an enzyme responsible for nitroarene and aromatic metabolism. Edenharder, Leopold, and Kries (1995) showed that solvents were crucial to extract antimutagenic agents from some fruit and vegetables. Thus solvent partition may be the major factor to explain these differences. The antimutagenic potencies were correlated with total phenolics content ($r^2 = 0.784$ and $p < 0.05$) (Fig. 4). Therefore, phenolic compounds are the major components responsible for the antimutagenicity of common bean extracts. Analysis of variance indicated that cultivars had a significant influence on antimutagenic activity.

The yield of successive extraction was higher for methanol extracts than for acetone extracts owing to the lack of selectivity from methanol which partly extracts biopolymers (carbohydrates) associated to phenolic compounds. However, methanol extracts contained a minimum of proteins. Data obtained for total phenolics support the selectivity of acetone which extracted higher amounts of phenolic compounds. Antioxidant activity of acetone extracts was more potent than methanol extracts. Acetone extracts displayed an antioxidant activity that strongly correlated ($r^2 = 0.96$) with the total phenolic content. This correlation was not observed for methanol extracts, which might be due to the carbohydrates present in the extract. In a preliminary identification, spots colour and their relative displacement in the TLC chromatograms showed the presence of catechin. In addition, phenolics belonging to the group of flavones, flavonones lacking a free 5-OH and flavonols lacking a free 5-OH but with the 3-OH substituted were revealed. Phenolic compounds present in the acetone extracts from beans were potent anti-mutagens against the direct mutagen 1-NP in *S. typhimurium* TA98.

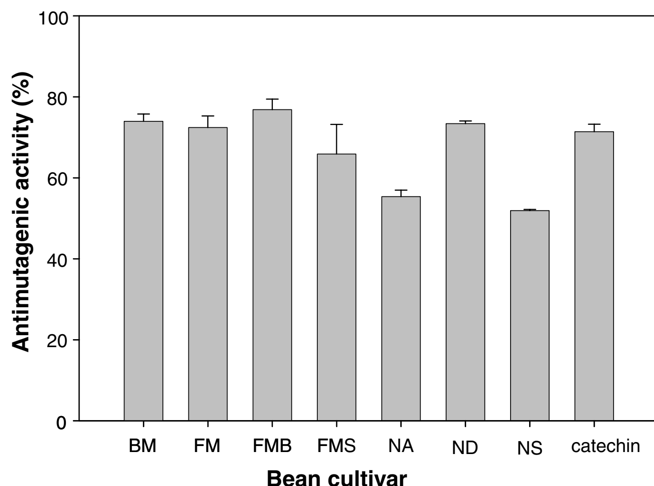

Fig. 3. Antimutagenic effect of common bean cotyledon extracts against 1-NP mutagenicity in *Salmonella typhimurium* TA98.

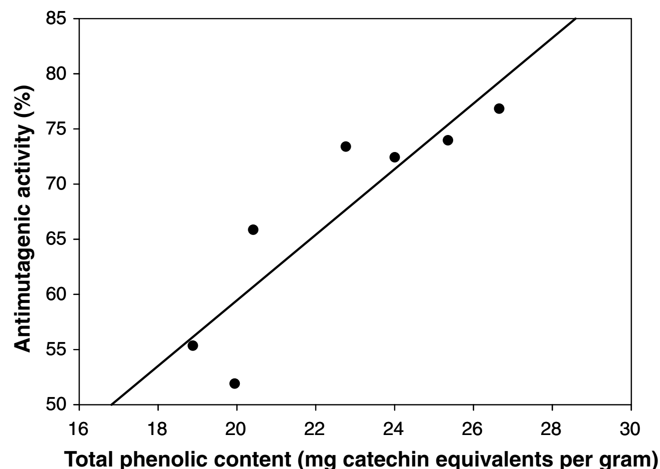

Fig. 4. Antimutagenic activity vs. catechin equivalents in 70% acetone extracts.

All cultivars inhibited more than 50% of 1-NP mutagenicity.

The correlations indicate that phenolic compounds were responsible for the antioxidant and antimutagenic activity exhibited in this study. Thus total phenolic content can be used to predict the ability of the phenolic extracts to scavenge DPPH radical and to decrease the mutagenicity induced by 1-NP.

Common beans can be considered an important source of phenolic compounds, which exhibit antioxidant and antimutagenic activity. Thus they may be helpful in the prevention of degenerative diseases such as cancer. A reported anticarcinogenic effect of common bean phenolic extracts is supporting its potential use as a functional food (Preza y Lerma, 2003). However, further work is required to determine mechanisms involved in the antioxidant and antimutagenic effects. In addition, more in vivo evidence and identification of active phenolics involved is needed.

References

Acosta-Gallegos, J. A., Ibarra-Perez, F. J., Rosales-Serna, R., Castillo-Rosales, A., Cazares-Enriquez, B., Fernandez-Hernandez, P., et al. (2001). Registration of "Negro Altiplano" Common Bean. *Crop Science, 41*, 1650.

Acosta-Gallegos, J. A., Ibarra-Perez, F. J., Rosales-Serna, R., Castillo-Rosales, A., & Kelly, J. D. (2001). Registration of "Negro Sahuatoba" Opaque Black Bean. *Crop Science, 41*, 1646–1647.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7915–7922.

Barampama, Z., & Simard, R. E. (1993). Nutrient composition, protein quality and antinutritional factors of some varieties of dry bean (*Phaseolus vulgaris*) grown in Burundi. *Food Chemistry, 47*, 159–167.

Beninger, C. W., & Hosfield, G. L. (2003). Antioxidant activity of extracts, condensed tannin fractions and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of Agricultural and Food Chemistry, 51*, 7879–7883.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenchaft und Technology, 28*, 25–30.

Cardador-Martínez, A., Castaño-Tostado, E., & Loarca-Piña, G. (2002). Antimutagenic activity of natural phenolic compounds present in the common bean (*Phaseolus vulgaris*) against aflatoxin B_1. *Food Additives and Contaminants, 19*, 62–69.

Cardador-Martínez, A., Loarca-Piña, G., & Oomah, B. D. (2002). Antioxidant activity in common bean (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 50*, 6975–6980.

Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Poel, B., et al. (1998). Structure–activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products, 61*, 71–76.

Decker, E. A. (1995). The role of phenolic, conjugated linoleic acid, carnosine and pyrroloquinoline as nonessential dietary antioxidants. *Nutrition Reviews, 53*, 49–58.

Desphande, S. S., Shatze, S. K., Salunkhe, D. K., & Cornforth, D. P. (1982). Effect of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry bean (*Phaseolus vulgaris* L.). *Journal of Food Science, 47*, 1846–1849.

Duh, P. D., Du, P. C., & Yen, G. C. (1999). Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food Chemical Toxicology, 37*, 1055–1061.

Edenharder, R., Leopold, C., & Kries, M. (1995). Modifying actions of solvent extracts from fruit and vegetable residues on 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) induced mutagenesis in *Salmonella typhimurium* TA98. *Mutation Research, 341*, 303–318.

Edenharder, R., & Tang, X. (1997). Inhibition of mutagenicity of 2-nitrofluorene, 3-nitrofluorene and 1-nitropyrene by flavonoids, coumarins, quinones and other phenolic compounds. *Food and Chemical Toxicology, 35*, 357–372.

Feenstra, W. J. (1960). Biochemical aspects of seed coat color inheritance in *Phaseolus vulgaris* L.. *Medical Landbouwhogeschool Wageningen, 60*, 1–53.

González de Mejía, E., Castaño-Tostado, E., & Loarca-Piña, G. (1999). Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research, 441*, 1–9.

Hagerman, A. E., & Butler, L. G. (1981). The specificity of proanthocyanidin–protein interactions. *Journal of Biological Chemistry, 256*, 4494–4497.

Halliwell, B. (1998). Antioxidants in human health and disease. *Annual Review of Nutrition, 16*, 253–265.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481–504.

Jovanovic, S. V., Steenken, S., Tosic, M., Marjanovic, B., & Simic, M. G. (1994). Flavonoids as antioxidants. *Journal of the American Chemical Society, 116*, 4846–4851.

Kanner, J., Frankel, E., Grant, R., German, B., & Kinsella, E. (1994). Natural antioxidants in grapes and wines. *Journal of Agricultural and Food Chemistry, 42*, 64–94.

Karchesy, J. J., Bae, Y. S., Chalker-Scott, L., Helm, R. F., & Foo, L. Y. (1989). Chromatography of proanthocyanidins. In R. W. Hemingway & J. J. Karchesy (Eds.), *Chemistry and significance of condensed tannins* (pp. 139–151). New York, NY: Plenum Press.

Koes, R. E., Quattrocchio, F., & Mol, J. N. M. (1994). The flavonoids biosynthetic pathway in plants: function and evolution. *BioEssays, 16*, 123–132.

Madhujith, T., Amarowicz, R., & Shahidi, F. (2004). Phenolic antioxidants in beans and their effects on inhibition of radical-induced DNA damage. *Journal of the American Oil Chemists' Society, 81*, 691–696.

Markham, K. R. (1982). *Techniques of flavonoid identification* (pp. 16–24). New York, NY: Academic Press.

Maron, D. M., & Ames, B. N. (1983). Revised methods for *Salmonella* mutagenicity test. *Mutation Research, 113*, 173–215.

Nordberg, J., & Arner, E. S. J. (2001). Reactive oxygen species, antioxidants and the mammalian thioredoxin system. *Free Radical Biological & Medicine, 31*, 1287–1312.

Pekkarinen, S. S., Stöckmann, H., Schwarz, K., Heinonen, I. M., & Hopia, A. I. (1999). Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoelate. *Journal of Agricultural and Food Chemistry, 47*, 3036–3043.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035–1042.

Preza y Lerma, A. M. (2003). Antioxidant capacity of phenolic extracts from common bean cultivars (*Phaseolus vulgaris* L.) (pp. 96–101). M.Sc. Thesis, Instituto Tecnológico de Durango, Dgo., México.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Rice-Evans, C. A., Miller, N. J., Blowell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

Ross, J. A., & Kasum, C. M. (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annual Review of Nutrition, 22*, 19–34.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Von Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalahus linearis*), α-tocopherol, BHT and BHA. *Journal of Agricultural and Food Chemistry, 45*, 632–638.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites* (pp. 66–103). Oxford: Blackwell Scientific Publications.