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Antioxidant Activity in Cotyledon of Black and Yellow Common Beans (*Phaseolus Vulgaris* L.)

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Abstract: Methanol and aqueous acetone cotyledon extracts from two groups of different colored common beans (*Phaseolus vulgaris* L.), black and yellow, were analyzed for total phenolics, lipid antioxidant activity and two-dimensional thin layer chromatography. A higher yield was obtained for methanol extracts in comparison to acetone extracts. However, acetone extracts showed a higher phenol content than methanol extracts independently of the group of beans tested. Furthermore, phenolic content was higher in acetone extracts from black beans than in acetone extracts of yellow beans. A higher antioxidant activity was found in methanol extracts rather than in acetone extracts without differences in the group of beans studied. Most of antioxidant activity was associated to black beans. Thin layer chromatography showed the presence of catechins in black beans but not in yellow beans cotyledons.

Key words: Antioxidant activity, common beans, cotyledon, phenolics, black beans, yellow beans

INTRODUCTION

Common bean seeds are consumed largely by populations in developing countries, as they are an important source of protein, carbohydrate and dietary fiber. Also, legumes provide vitamins, carotenoids (Adsule and Kadan, 1989) and phenolic compounds (Mortinze *et al*., 2002; Mejia *et al*., 1999). This type of compounds has been associated with prevention of several chronic diseases (Shahidi and Naezk, 1995; Anderson *et al*., 1999; Madhuij *et al*., 2004).

Three distinctive parts are recognized in common beans: Seed coat, cotyledon and embryonic axe. The most important part in terms of weight is the cotyledon (Duenas *et al*., 2002).Cotyledon contains proteins and carbohydrates, while seed coat contains the highest concentration of phenolic compounds (Shahdi *et al*., 2001). According to Dueñas *et al*. (2006) distribution of phenolic compounds differs in the cotyledon and seed coat. Non-flavonoid phenolic compounds were located mainly in cotyledon while flavonoids, in the seed coat of lentils (Duenas *et al*., 2002) and in dark peas (Troszynska *et al*., 2002).

Proanthocyanidins (condensed tannins) are predominantly phenolic compounds found in legume seeds. They are distributed in the seed coat, in the border of seed coat and pericarp, in the aleurone layer and accumulated on the embryo (Havsteen, 1983). Proanthocyanidins are compounds responsible for several biologic effects in human health, such as antiallergenic, anti-inflammatory, antiviral, antifungal, antibacterial, antiproliferative and anticarcinogenic (Evans and Packer, 1996). These effects are associated to their antioxidants properties and related to their chemical structure (Park and Levine, 2000; Tsuda *et al*., 1996). In the case of flavonoids (considered primary antioxidants) (Decker, 1997) the position and degree of hydroxylation in the B ring are the most important factors associated with antioxidant activity (Evans and Packer, 1996). Other phenols also present antioxidant activity, which depends on the number and position of hydroxyl groups in the molecule (Dziedzic *et al*., Hudsom, 1983). Proanthocyanidins show free Radical-Scavenging Activity (RSA), influenced by the structure of monomers and degree of polymerization (Hatano, *et al*., 2002).

Studies about phenolic compounds of legumes and antioxidant activity of extracts have been reported for common bean *Phaseolus vulgaris* L., however most studies have focused on seed coat, using Pinto common bean and improved cultivars (Mejia *et al*., 1999; Kahkone, *et al*., 1999). Mexican Bean Program pursues the developing of improved common beans cultivars and high yield driven technologies. In this research antioxidant capacity of polyphenols present in cotyledon is evaluated from two types of improved cultivars of black and yellow common beans (*Phaseolus vulgaris* L.).

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MATERIALS AND METHODS

Improved cultivars of common bean (*Phaseolus vulgaris* L.) were donated by Experimental Field Valle del Guadiana from INIFAP-Durango, Mexico. Samples tested were: Black Otomy, Black 8025, Black Sahuatoba, Black Altiplano, Black Vizcaya, Black Durango, Yellow Pimono 78, Yellow Peruano 87, Yellow Namiquipa, Yellow Regional 87. Seed coat was manually separated from cotyledon and kept refrigerated in hermetic pans until use.

Crude extracts preparation: Crude extracts were prepared by successive extractions with aqueous acetone (70%) first and aqueous methanol (50%) later. Cotyledons from each common bean cultivar were soaked twice with each solvent by 18 h under continuous stirring. Crude extracts were concentrated using a rotavapor (Büchi R-200/250) and aqueous solutions lyophilized and stored at –20°C.

Total phenols: Determination of phenols was made by Folin Ciocalteu method according to Madhujith *et al.* (2004) Tannic acid was selected as a standard. Results were expressed as tannic acid equivalents (mg tannic acid g^{-1} extract).

Lipid antioxidant activity: Ferric thiocyanate in linoleic acid method was used (Moreno *et al.*, 1999). Results were reported as percentage of Antioxidant Activity (%AA).

Preliminary identification of polyphenolic compounds: A qualitative thin layer chromatography method was carried out in cellulose plates, using *Ter*-Buthanol-Acetic acid (TBA) (3:1:1) and acetic acid 6% as mobile phases (Kar Chesy *et al.*, 1989; Markham, 1982). Chromatograms were visualized with UV light at 254 and 365 nm (lamp Spectronics Co) and revealed with an acidic solution of vanillin in ethanol. Spot color and position (R_f values) were reported as a preliminary guide to polyphenol compounds identification.

Statistical analysis: A factorial design was used. Results were analyzed using ANOVA and LSD test (p = 0.05) by STATISTICA for Windows ver. 4.3 (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Extraction yields of samples are shown in Table 1. No difference was found in function of bean cultivar.

Table 1: Extraction yields from common bean cotyledons

Cultivar	Yield (%) Acetone 70%	Methanol 50%
Black otomy	5.81±1.26	10.05±0.65
Black 8025	4.98±0.02	10.08±1.02
Black sahuatoba	4.12±0.02	10.03±0.04
Black altiplano	4.12±0.65	9.00±0.63
Black vizcaya	4.31±0.32	9.50±1.34
Black durango	4.50±0.66	10.44±1.63
Yellow pimono 78	4.51±1.25	6.95±3.70
Yellow peruano 87	4.62±0.12	7.71±1.81
Yellow namiquipa	4.98±0.02	7.86±0.03
Yellow regional 87	4.59±0.73	6.67±1.04

Each record indicates the mean value of two replicates, Value after±indicates one standard deviation

Table 2: Total phenolics in common bean cotyledons

	Acetone (70%)	Methanol (50%)
Phenolics	Tannic acid equivalents	Tannic acid equivalents
Black otomy	11.37±2.47	9.23±0.10
Black 8025	15.64±2.47	4.96±0.06
Black sahuatoba	14.36±0.99	12.65±0.18
Black altiplano	23.12±2.14	4.97±0.05
Black vizcaya	9.23±0.28	7.10±0.49
Black durango	13.72±0.43	9.24±0.15
Yellow pimono	78 9.24±0.29	9.22±0.12
Yellow peruano 87	10.94±0.34	5.38±0.49
Yellow namiquipa	13.93±0.49	4.95±0.05
Yellow regional 87	13.29±0.43	9.24±0.10

Each record indicates the mean value of two replicates. Value after±indicates one standard deviation

Yields of acetone 70% extracts were lower than those in methanol, independently of cultivar or common bean group analyzed.

Phenol content in crude extracts shows that separation of phenols is more selective in acetone extracts than in methanol extracts (Table 2). Several reports indicated that acetone is more efficient for extraction of proanthocyanidins because this solvent inhibits the tannin-carbohydrate and tannin–protein interactions (Karchesy *et al.*, 1989; Smith *et al.*, 2000; Waterman and Mole, 1994). On the other hand, methanol extracts low molecular weight phenolic compounds, proteins and carbohydrates, The latter two are present in high concentrations in bean cotyledon, thus proteins and carbohydrates may complex with flavonoid or anthocyanins, producing interference at the evaluation (Shirley, 1998).

Compounds present in black bean cotyledon extracts according to thin layer chromatography were: Catechin, di and tri glycosides of flavonoids (Table 3 and 4). For yellow beans, presumable compounds were proanthocyanidin polymers and di and tri glycosides of flavonoids (Table 5 and 6). It is important to note the major difference associated with the absence of catechin in yellow beans. Catechins are an important class of flavonoids. Epidemiological studies have shown that catechins may reduce the risk for several diseases (Arst *et al.*, 2000). Catechins are

Table 3: R_f values and spot colors observed in TLC for black beans acetone (70%) extracts

	Mobile phase											
	TBA (3:1:1)						HOAc 6%					
	Visualization UV light				Vanillin		Visualization UV light				Vanillin	
	254 nm		365 nm		Spot color	R_f	254 nm		365 nm		Spot color	R_f
Cultivar	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f
Black otomy			Fluorescent		Pink	0.05			Fluorescent			
	Blue	0.52	blue	0.52		0.11	Blue	0.82	blue	0.82	Pink	1
		0.58				0.35						
	Purple	0.70	Fluorescent			0.52			Yellow	0.94		
		0.94	yellow	0.94		0.88						
Black 8025	Blue	0.52	Fluorescent		Pink	0.05	Blue	0.82	Fluorescent		Pink	1
			Blue	0.52		0.11			blue	0.82		
		0.58	Fluorescent			0.35						
	Purple	0.70	blue	0.94		0.52			Yellow	0.94		
		0.94				0.88						
Black sahuatoba			Fluorescent		Pink	0.05			Fluorescent			
	Blue	0.52	blue	0.52		0.11	Blue	0.82	blue	0.82	Pink	1
		0.58				0.35						
	Purple	0.70	Fluorescent			0.52			Yellow	0.94		
		0.94	yellow	0.94		0.88						
Black altiplano	Blue	0.52	Fluorescent		Pink	0.05	Blue	0.82	Fluorescent		Pink	1
			Blue	0.52		0.11			blue	0.82		
		0.58	Fluorescent			0.35						
	Purple	0.70	blue	0.94		0.52			Yellow	0.94		
		0.94				0.88						
Black vizcaya			Fluorescent		Pink	0.05			Fluorescent			
	Blue	0.52	blue	0.52		0.11	Blue	0.82	blue	0.82	Pink	1
		0.58				0.35						
	Purple	0.70	Fluorescent			0.52			Yellow	0.94		
		0.94	yellow	0.94		0.88						
Black durango	Blue	0.52	Fluorescent		Pink	0.05	Blue	0.82	Fluorescent		Pink	1
			Blue	0.52		0.11			blue	0.82		
		0.58	Fluorescent			0.35						
	Purple	0.70	blue	0.94		0.52			Yellow	0.94		
		0.94				0.88						

Table 4: R_f values and spot colors observed in TLC for black beans methanol (50%) extracts

	Mobile phase											
	TBA (3:1:1)						HOAc 6%					
	Visualization UV light				Vanillin		Visualization UV light				Vanillin	
	254 nm		365 nm		Spot color	R_f	254 nm		365 nm		Spot color	R_f
Cultivar	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f
Black otomy			Fluorescent						Yellow and			
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								
Black 8025			Fluorescent						Yellow and			
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								
Black sahuatoba			Fluorescent						Yellow and			
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								
Black altiplano			Fluorescent						Yellow and			

Table 4: Contiuned

	Mobile phase											
	TBA (3:1:1)						HOAc 6%					
	Visualization UV light				Vanillin		Visualization UV light				Vanillin	
	254 nm		365 nm		Spot color	R_f	254 nm		365 nm		Spot color	R_f
Cultivar	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								
Black vizcaya			Fluorescent						Yellow and			
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								
Black durango			Fluorescent						Yellow and			
	Pruple	0.52	blue	0.41	Pink	0.05	Pruple	0.94	fluorescent	0.70	Pink	0.88
		0.58				0.35			yellow	to		
		0.94	Fluorescent							0.94		
			yellow	0.94								

Table 5: R_f values and spot color observed in TLC for yellow beans acetone (70%) extracts

	Mobile phase											
	TBA (3:1:1)						HOAc 6%					
	Visualization UV light				Vanillin		Visualization UV light				Vanillin	
	254 nm		365 nm		Spot color	R_f	254 nm		365 nm		Spot color	R_f
Cultivar	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f
Yellow pimono 78	Blue	0.47	Fluorescent		Pink	0.05			Fluorescent			
			Blue	0.47		0.17	Blue	0.47	blue	0.82	Pink	0.94
	Purple	0.70	Fluorescent			0.35	Purple	0.70	Fluorescent			
			Yellow	0.94		0.88		0.94	yellow	0.94		
Yellow Peruano 87	Blue	0.47	Fluorescent		Pink	0.05			Fluorescent			
			Blue	0.47		0.17	Blue	0.47	blue	0.82	Pink	0.94
	Purple	0.70	Fluorescent			0.35	Purple	0.70	Fluorescent			
			Yellow	0.94		0.88		0.94	yellow	0.94		
Yellow Namiquipa	Blue	0.47	Fluorescent		Pink	0.05			Fluorescent			
			Blue	0.47		0.17	Blue	0.47	blue	0.82	Pink	0.94
	Purple	0.70	Fluorescent			0.35	Purple	0.70	Fluorescent			
			Yellow	0.94		0.88		0.94	yellow	0.94		
Yellow regional 87	Blue	0.47	Fluorescent		Pink	0.05			Fluorescent			
			Blue	0.47		0.17	Blue	0.47	blue	0.82	Pink	0.94
	Purple	0.70	Fluorescent			0.35	Purple	0.70	Fluorescent			
			Yellow	0.94		0.88		0.94	yellow	0.94		

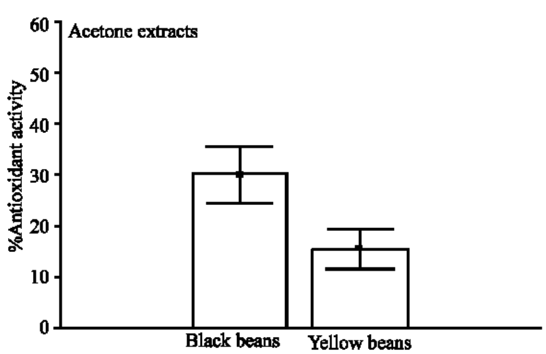

Fig. 1: Antioxidant activity in black and yellow bean cotyledons extracted with acetone (70%)

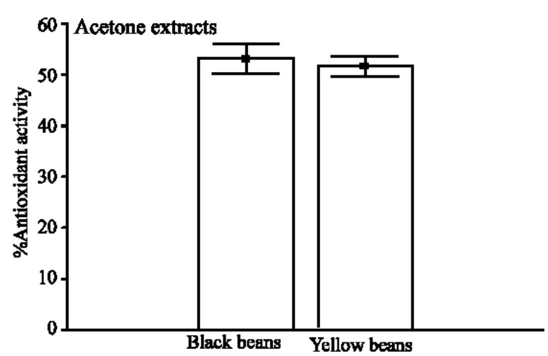

Fig 2: Antioxidant activity in black and yellow bean cotyledons extracted with methanol (50%)

Table 6: R_f values and spot colors observed in TLC for yellow beans methanol (50%) extracts

	Mobile phase											
	TBA (3:1:1)						HOAc 6%					
	Visualization						Visualization					
	UV light				Vanillin		UV light				Vanillin	
	254 nm		365 nm		Spot color	R_f	254 nm		365 nm		Spot color	R_f
Cultivar	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f	Spot color	R_f
Yellow pimono 78	Pruple	0.35	Fluorescent blue	0.35	Pink	0.05	Pruple	0.58	Fluorescent blue	0.70	Pink	0.94
		0.52				0.17		0.82				
		0.58				0.32		0.94				
Yellow peruano	Pruple	0.35	Fluorescent blue	0.35	Pink	0.05	Pruple	0.58	Fluorescent blue	0.70	Pink	0.94
		0.52				0.17		0.82				
		0.58				0.32		0.94				
Yellow namiquipa	Pruple	0.35	Fluorescent blue	0.35	Pink	0.05	Pruple	0.58	Fluorescent blue	0.70	Pink	0.94
		0.52				0.17		0.82				
		0.58				0.32		0.94				
Yellow regional 87	Pruple	0.35	Fluorescent blue	0.35	Pink	0.05	Pruple	0.58	Fluorescent blue	0.70	Pink	0.94
		0.52				0.17		0.82				
		0.58				0.32		0.94				

strong antioxidants, (Cook and Samman, 1996) thus antioxidant activity could be important in black beans as compared to yellow beans

Results of lipid antioxidant activity are shown in Fig. 1 and 2. The methanol extracts inhibited more efficiently lipid oxidation than acetone extracts. Most reports about antioxidant activity research on legumes have used seed coats. Few works, as this one, have reported active polyphenols from bean cotyledons. Dueñas *et al.* (2002) postulated differences between phenols present in seed coats and cotyledons. Results obtained in our experiments support earlier findings, because no differences were found in antioxidant activity in methanol extracts (58% for both bean groups) and acetone black bean extracts showed higher antioxidant activity (34%) than yellow beans (18%).

These results are interesting, because methanol extracts showed antioxidant activity that seemed independent of the presence of catechins. Activity can be attributed

REFERENCES

Adsule, R.N. and S.S. Kadam, 1989. In: Handbook of world food legumes: Nutritional. chemistry, processing technology and utilization, D.K. Salunkhe, S.S. Kadam, (Eds.), CRC Press, Boca Raton, Fl., 2: 75-97.

Anderson, J.W., B.M. Smith and C.S. Washnock, 1999. J. Clin. Nutr., 70: 464-474.

Arts, I.C.W. and P.C.H. Hollman, 1998. J. Agric. Food Chem., 46: 5156-5162.

Arts, I.C.W., B. van de Putte and P.C.H. Hollman, 2000. J. Agric. Food Chem., 48: 1746-1751.

Cardador-Martinez, A., G. Loarca-Piña and B.D. Oomah, 2002. J. Agric. Food Chem., 50: 6975-6980.

Cook, N.C. and S. Samman, 1996. J. Nutr. Biochem., 7: 66-76.

Decker, E.A., 1997. Nutrition Rev., 55: 396-407.

Dueñas, M., T. Hernandez and I. Estrella, 2002. Eur. Food Res. Tech., 215: 478-483.

Dueñas, M., T. Hernandez and I. Estrella, 2006. Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. Food Chem., in Press.

Dziedzic, S.Z. and B.J.F. Hudson, 1983. Food Chem., 12: 205-209.

González de Mejía, E., E. Castaño-Tostado and G. Loarca-Piña, 1999. Mutation Res., 441: 1-9.

Hatano, T., H. Miyatake, M. Natsume, N. Osakabe, T. Takizawa and H. Ito, 2002. Phytochemistry, 59: 749-758.

Havsteen, B., 1983. Biochem. Pharmacol. 32: 1141-1148.

Kähkönen, M.P., A.I. Hopia, H. J. Vuorela, J.P.Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. J. Agric. Food Chem., 47: 3954-3962.

Karchesy, J.J., Y. Bae, L. Chalker-Scott, R.F. Helm and L.Y. Foo, 1989. In Chemistry and Significance of Condensed Tannins, J.J. Karchesy, R.W. Hemingway, (Eds.), Plenum Press, pp: 139-151.

Markham, K.R., 1982. Techniques of flavonoid identification, Academic Press, New York, pp: 16-24.

Madhujith, T., M. Naczk and F. Shahidi, 2004. J. Food Lipids, 11: 220-233.

Park, J.B. and M. Levine, 2000. Am. Soc. Nutr. Sci. 11: 1297-1302.

Rice-Evans, C.A. and L. Packer, 1996. Flavonoids in health and disease. Marcel Dekker, New York, pp: 123-132.

Sánchez-Moreno, C., J.A., Larrauri and F. Saura-Calixto, Food Res. Int., 32: 407-412.

Shahidi, F. and M. Naczk, 1995. in Food phenolics: sources, chemistry, effects and applications. Technomic Publishing Company Inc. Lancaster, PA., pp: 1-5, 13-18, 235-273.

Shahidi, F., U.D. Chavan, M. Naczk and R. Amarowicz, 2001. J. Agric. Food Chem., 49: 926-933.

Shirley, B.W., 1998. Seed Sci. Res., 8: 415-422.

Smith, M.A., K.A. Marley, D. Seigles, K.W. Singletary and B. Meline, 2000. J. Food Sci., 213: 1023-1025.

Troszynska, A., I. Estrella, M. L. Lopez-Amoros and T. Hernandez, 2002. Lebensmittel-Wissenscha und Tech., 35: 158-164.

Tsuda, T., K. Ohshima, S. Kawakishi and T. Osawa, 1996. J. Agric. Food Chem., 42: 248-251.

Waterman, P.G. and S. Mole, 1994. Analysis of phenolic plant metabolites. Blackwell Sci. Publ., London, pp: 66-103.