academicJournals

Vol. 8(25), pp. 3330-3333, 4 July, 2013
DOI: 10.5897/AJAR12.225
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Anthocyanin content and antioxidant activities of common bean species (*Phaseolus vulgaris* L.) grown in Mashonaland Central, Zimbabwe

Dzomba P.*, Togarepi E. and Mupa M.

Department of Chemistry, Bindura University of Science Education, P. Bag 1020 Bindura, Zimbabwe.

Accepted 22 April, 2013

Recently, an inverse correlation between bean consumption and age related diseases such as breast and prostate cancer has been established. Furthermore, beans can easily be grown with minimum cost across the globe. In the present study, we investigated anthocyanin, proanthocyanidin and antioxidant activity of five (black, brown, white, brown and black spotted) common bean species (*Phaseolus vulgaris* L.) grown in Mashonaland Central, Zimbabwe. The five common bean species exhibited variations in anthocyanins, proanthocyanidin and antioxidant activity. Anthocyanin content estimated using a UV spectrophotometer by a pH differential method varied significantly in the order: brown (0.59 ± 0.01) > black (0.43 ± 0.01) > white (0.28 ± 0.01). Brown seeds also exhibited superior proanthocyanidin contents (0.18 mg g^{-1} ± 0.01). Bean seeds consisting of greater amounts of anthocyanins exhibited superior antioxidant activity. All species showed antioxidant activity which was concentration-dependent. The present results suggest that screening programs should be done to come up with superior bean species for targeted animal feeds and human food.

Key words: Anthocyanin, proanthocyanidin, antioxidant activities, bean species, *Phaseolus vulgaris* L.

INTRODUCTION

Beans are important, cheap and widely available source of nutrients in a diet. Recently, an increased interest in bean species has been observed because consumption of beans is linked to reduced risk of cardiovascular diseases (Bazzano et al., 2001), diabetes and obesity (Venkateswaran et al., 2002). Several studies confirm a significant inverse correlation between beans intake and age related diseases such as colon, prostate and breast cancer (Hangen and Bennink, 2002; Kolonel et al., 2000). A significant reduction in cancers and coronary diseases implies the presence of phytoactive substances synonymous with polyphenolic compounds. Beans are rich in tannins, anthocyanins and flavonols (Aparicio-Fernandez et al., 2005). Romani et al. (2004) and Aparicio-Fernandez et al. (2005) reported the presence of anthocyanins in black and blue-violet coloured beans, respectively. Akond et al. (2011) investigated the presence of anthocyanins, total polyphenols and antioxidant activity from 29 common beans from USA and International Center for Tropical Agriculture (CIAT) and found out that there was a significant variation in anthocyanins and antioxidant activity in different bean species. Black beans consisted of the highest anthocyanin content and polyphenols. Polyphenolic compounds are famous because they are known to reduce diseases caused by reactive oxygen and nitrogen species (Maestri et al., 2006; Miliauskas, 2006). The naturally electron deficient chemical structure of

*Corresponding author. E-mail: pdzomba@gmail.com or pdzomba@buse.ac.zw. Tel: +263773474525. Fax: +263 7536.

Table 1. Common bean species in Mashonaland Central.

Physical appearance	Average mass (g) n = 100	Percentage weight
White	3.184	10.010
Black	3.268	10.022
Brown	3.434	9.998
Black spotted	3.097	10.008
Brown spotted	3.352	10.021

Mass values reported exclude moisture content of the bean species, n = number of seeds.

anthocyanins makes them highly reactive toward free radicals and, consequently, makes them powerful natural antioxidants. In Zimbabwe, few researches have focused on presence of anthocyanins in beans and no researches have focused on presence of anthocyanins and antioxidant activity of beans grown in Mashonaland Central area. This information is important to budding smallholder farmers, manufacturing industries and consumers since recently consumers' interest has shifted to foods and formulations with natural antioxidants rather than foods with synthetic antioxidants. The reasons for shunning such foods are the growing evidence linking synthetic antioxidants with development of cancers (Bergfeld et al., 2005; Maestri et al., 2006; Wangensteen et al., 2004). In this study, therefore, we investigated naturally occurring anthocyanins and antioxidant activities of five common dried bean species grown in Mashonaland Central Province in an effort to avail critical information in developing practical strategies to enhance bean quality and market prizes.

MATERIALS AND METHODS

Five dried common bean species were purchased locally from farms surrounding Bindura town (Zimbabwe). Soils ranged from loam to red clay soils. The area receives normal to above normal rainfall. Their characteristics are summarized in Table 1.

Determination of total anthocyanins

Total anthocyanin compounds of the samples were estimated using a UV-spectrophotometer by the pH differential method reported by Abu Bakar et al. (2009) with slight modifications. Two buffer systems, potassium chloride buffer, pH 1.0 (0.0025 M) and sodium acetate buffer, pH 4.5 (0.4 M) were used. Briefly, 400 µl of extract (3 mg of ground beans in 10 ml absolute methanol) was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm. Absorbance (A_d) was calculated as:

$$A_d = (A_{510} - A_{700})\ pH1.0 - (A_{510} - A_{700})\ pH4.5$$

Anthocyanin concentration in the extract was calculated and expressed as cyaniding-3 glycoside (mg g^{-1}) equivalent:

$$A_d \times MW \times DF \times 1000 / (M_a \times 1)$$

Where, A_d is difference in absorbance, MW is a molecular weight for cyaniding-3-glucoside (449.2), DF is the dilution factor of the samples and M_a is the molar absorptivity of cyaniding-3-glucoside (26.900).

Results were expressed as mg of cyaniding-3-glucoside equivalents in 100 g of dried sample.

Determination of proanthocyanidins

Proanthocyanidin content was determined by vanillin-H_2SO_4 assay as described by Chang et al. (2007) with minor modifications. A volume, 1.0 ml aliquots of bean extract (3 mg in 10 ml absolute methanol) were mixed with 2.5 ml of 1.0% (w/v) vanillin in absolute methanol and then with 2.5 ml of 25% (v/v) sulfuric acid in absolute methanol to undergo vanillin reaction with polyphenols in bean species. The blank solution was prepared in the same procedure without vanillin. The vanillin reaction was carried out in a 25°C water bath for 15 min. The absorbance at 500 nm was read and the results were expressed as (+)-catechin equivalent by a calibration method.

Antioxidant activity determination

The 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical assay

$DPPH^{\cdot}$ scavenging activity was assessed according to a method reported by Pothitirat et al. (2009) with slight modifications. A volume, 1 ml of 100 µM DPPH solution in methanol was mixed with 1 ml of powered bean extract, ascorbic acid and butylated hydroxytoluene (BHT) of different concentrations. Extraction was performed by shaking the powered bean seeds (10 g) with 100 ml of methanol overnight. The reaction mixture was incubated in the dark for 20 min and there after the absorbance was recorded at 517 nm using a UV-spectrophotometer against a blank. For the control, 1 ml of DPPH solution in methanol was mixed with 1 ml of methanol and absorbance of solution was recorded after 20 min. The decrease in absorbance of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity as percentage of inhibition (%IP) of DPPH radical:

$$\%\ IP = [(A_c - A_s) / (A_c \times 100)]$$

Where A_c is absorbance of control after 20 min and A_s is absorbance of sample after 20 min.

Statistical analysis

Results are presented as mean value ± standard deviation (of three replicate experiments). Statistical analyses to compare differences in anthocyanins and proanthocyanin content were determined at the significance level of p = 0.05 by applying analysis of variance (ANOVA) followed by multiple comparisons using the least significant difference (LSD) test. Student t-test was applied to

Table 2. Anthocyanin and proanthocyanidin content in common beans (*Phaseolus vulgaris* L.).

Bean species	Anthocyanin content / mg g^{-1}	Proanthocyanidin content / mg g^{-1} catechin
White	0.28 ± 0.01[a]	0.02 ± 0.00[b]
Black	0.43 ± 0.01[a]	0.04 ± 0.00[b]
Brown	0.59 ± 0.01[a]	0.18 ± 0.01[a]
Black spotted	0.45 ± 0.01[b]	0.11 ± 0.01[a]
Brown spotted	0.46 ± 0.02[b]	0.13 ± 0.00[a]

Proanthocyanidin determination was based on a calibration curve y = 0.126x + 0, 11, R^2 = 0.98. a = significant different between the means, b no significant difference. ANOVA and multiple comparisons using the LSD test.

Table 3. Result of Antioxidant activity of bean extracts, BHT and ascorbic acid.

Bean	% Antioxidant activity ± SD (n = 3) at different concentrations / (µg/ml)				
	10	25	50	100	250
White	23.12 ± 0.01	46.16 ± 0.01	51.08 ± 0.03	51.08 ± 0.03	76.45± 0.03
Black	39.03 ± 0.05	55.34 ± 0.03	68.90 ± 0.05	79.17 ± 0.03	93.17 ± 0.01
Brown	44.67 ± 0.03*	69.00 ± 0.01*	74.46 ± 0.01	83.55 ± 0.03	98.11± 0.03*
Black spotted	43.11± 0.05*	63.02 ± 0.03	70.38 ± 0.05*	81.33± 0.03*	96.06± 0.05*
Brown spotted	41.07 ± 0.01	56.39 ± 0.01	69.11 ± 0.03*	80.77 ± 0.01*	93.46 ± 0.05
Ascorbic acid	47.93 ± 0.01	69.92 ± 0.01*	78.81 ± 0.05	88.97± 0.05	99.85 ± 0.03*
BHT	43.00 ± 0.05*	61.24 ± 0.03	70.17 ± 0.01*	81.11 ± 0.03*	95.23 ± 0.05*

*. No significant difference between the extracts and the standards. Student t-test p = 0.05.

compare antioxidant activity of the beans and that of standard antioxidants (ascorbic acid and BHT).

RESULTS AND DISCUSSION

Anthocyanin, proanthocyanidin content and seed colour

The results for anthocyanin and proanthocyanidin determination are shown in Table 2. Brown bean species exhibited the greatest anthocyanins and proanthocyanidins content, 0.59 mg g^{-1} ± 0.01 and 0.18 mg g^{-1} ± 0.01, respectively, while white bean species showed the least anthocyanins and proanthocyanidins content 0.28 mg g^{-1} ± 0.01 and 0.02 ± 0.00 mg g^{-1}, respectively. Anthocyanins and proanthocyanidins content decreased significantly in the order: brown (0.59 ± 0.01) > black (0.43 ± 0.01) > white (0.28 ± 0.01). Significant differences in anthocyanin and proantho-cyanin content was observed between the bean species ($p < 0.05$). According to Akond et al. (2011), black beans revealed higher anthocyanin content, while in the present study brown seeds exhibited the greatest anthocyanin content. Low concentrations were found in white bean species. Low concentrations of anthocyanins were found in white bean species because anthocyanins are pigments which confer red/blue colour in plant material (Einbond et al., 2004). The total anthocyanin content in crude extracts containing other phenolic materials is usually determined by measuring absorbance of the solution at a single wavelength (Einbond et al., 2004). This is possible because anthocyanins have a typical absorption band in the 490 to 550 nm region of the visible spectra (Naczk and Shahidi, 2004). The band is far from the absorption bands of other phenolics, which have spectral maxima in the UV range (Giusti et al., 1999). The use of differential or subtractive methods to quantify anthocyanins is highly recommended because of interference from anthocyanin degradation products or melanoidins from browning reactions (Giusti and Wrolstad, 2001).

Antioxidant activity

All the bean species showed antioxidant efficacy assessed by DPPH assay, Table 3 with brown beans recording the greatest antioxidant activity for the selected concentration range of 10 to 250 µg ml^{-1}. At 250 µg ml^{-1}, antioxidant activity of brown beans was 98.11%. White bean species showed the least antiradical activity of 76.45%. The antioxidant activity varied significantly in the order: brown > black > white. (ANOVA and LSD analysis $p = 0.05$). Antioxidant activity increased with concentration of the bean extracts. Similar studies found out that white bean coat consisted of no antioxidant activity (Tsuda et al., 1994) while red and black seed coats consisted of significant antioxidant activity (Wu et al., 2004; Oomah et al., 2005). This implies that dark bean species consist of good antioxidant activity as compared to white bean species. Antioxidant activities of

the extracts were comparable to antioxidant activity of ascorbic acid and BHT (Student t-test p = 0.05).

Conclusions

Bean species showed variations in anthocyanins and antioxidant activities. Generally, bean species consisting of greater anthocyanins exhibited greater antioxidant activity. Darker varieties consisted of greater antioxidant activity. This information may be useful in the choice of bean species for genotype improvement, prizing and human consumption.

ACKNOWLEDGMENTS

We thank Doreen Chiwara for the kind help she gave us in the acquisition of bean species.

REFERENCES

Abu Bakar MF, Mohamed M, Rahmat A, Fry J (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem. 113:479-483.

Akond M, Golam ASM, Khandaker L, Berthold J, Gates L, Peters K, Delong H, Hossain K (2011). Anthocyanin, Total polyphenols and antioxidant activity of common bean. Am. J. Food Technol. 6(5):385-394.

Aparicio-Fernandez X, Manzo-Bonilla L, Loarca-Pina G (2005). Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B1. J. Food Sci. 70:73-78.

Bergfeld WF, Belsito DV, Marks JGJ, Andersen FA (2005). Safety of ingredients used in cosmetics. J. Am. Acad. Dermatol. 52:125-132.

Bazzano LA, He J, Ogden LG, Loria C, Vapputuri S, Myers L, Whelton (2001). Legume consumption and risk of coronary heart diseases in US men and women. Arch. Inten Med.161:2573-2578.

Chang Sub Ku, Jeong Phil Jang, Sung Phil Mun (2007). Exploitation of polyphenol-rich pine barks for potent antioxidant activity. J. Wood Sci. 53:524-528.

Einbond LS, Reynertson KA, Luo,X, Basile MJ, Kennelly EJ (2004). Anthocyanin Antioxidants From Edible Fruits. Food Chem. pp. 23-28.

Giusti MM, Rodriguez-Saona LE, Wrolstad RE (1999). Spectral characteristics, molar absorptivity and color of pelargonidin derivatives. J. Agric. Food Chem. 47:4631-4637.

Giusti MM, Wrolstad RE (2001). Current Protocols in Food Analytical Chemistry John Wiley & Sons, Inc. 2:13.

Hangen L, Bennink MR (2002). Consumption of black and navy beans *Phaseolus vulgaris* reduced azoxymethane-induced colon cancer in rats. Nutr. Cancer 40:60-65.

Kolonel LN, Hankin JH, Whittemore AS, Wu AH, Gallagher RP, Wilkens LR, John EM, Howe GR, Dreon DM, West DW, Paffenbarger RS, (2000). Vegetables, fruits, legumes and prostate cancer. A multiethnic case control study. Cancer Epidemiol. Biomark Prev. 8: 795-804.

Maestri DM, Nepote V, Lamarque AL, Zygadlo JA (2006). Natural products as antioxidants. Res. Signpost pp. 105-135.

Miliauskas G (2006). Screening, isolation and evaluation of antioxidative compounds from *Geranium macrorrhizum, Potentilla fruticosa* and *Rhaponticum carthamoides* Eur. Food Res. Technol. 218:253-261.

Naczk M, Shahidi F (2004). Extraction and analysis of phenolics in food. J. Chromatogr. A. 1054:95-111.

Oomah BD, Cardador-Martinez A, Loaca-Pina P (2005). Phenolics and antioxidative activities in common beans *Phaseolus vulgaris* L. J. Sci Food Agric. 85:935-942

Pothitirat W, Chomnawang MT, Supabphol R, Gritsanapan W (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the *Mangosteen* fruit rind at two stages of maturity. Fitoterapia. 80:442-447.

Romani A, Vignolini P, Galardi N, Mulinacci N, Benedettelli S, Hamle D (2004). Germplasm characterization of zolfino landraces (*Phaseolus vulgaris* L) by flavonoid content. J. Agric. Food Chem. 52: 3838-3842.

Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi S, Kawakishi S, Osawa T (1994). Antioxidative activity of the anthocyanin pigments cyaniding 3-O-ß-D glucose and cyaniding. J. Agric Food Chem. 42: 2407-2410.

Venkateswaran S, Pari L, Saravanan G (2002). Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in rats with streptozotocin induced diabetes. J. Med. Food. 5:97-103.

Wangensteen H, Samuelsen AB, Malterud KE (2004). Antioxidant activity in extracts from coriander. Food Chem. 88:293-297.

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States of America. Agric. Food Chem. 52:4326-4037.