Journal of Food and Nutrition Research, 2016, Vol. 4, No. 4, 230-236
Available online at http://pubs.sciepub.com/jfnr/4/4/6

DOI:10.12691/jfnr-4-4-6

Screening Different Zambian Market Classes of Common Beans (*Phaseolus vulgaris)* for Antioxidant Properties and Total Phenolic Profiles

Nyau V.[1,*], Prakash S.[2], Rodrigues J.[3], Farrant J.[3]

[1]Department of Food Science and Nutrition, University of Zambia, Lusaka, Zambia
[2]Department of Chemistry, University of Zambia, Lusaka, Zambia
[3]Department of Molecular and Cell Biology, University of Cape Town, 7701 Rondebosch, South Africa
*Corresponding author: vincentnyau@yahoo.co.uk

Abstract Nutraceutical foods are thought to play an important role in the prevention and management of cardiovascular diseases, diabetes, obesity and some cancers. This study was undertaken to screen the commonly grown Zambian market classes of common beans for nutraceutical value based on the antioxidant activities (DPPH and FRAP) and total polyphenolic profiles. Phenolic phytochemical profiles were determined using Folin Ciocalteu assay and aluminium chloride colorimetric method. The total polyphenol content ranged from 37.3 to 123.7 mg GAE / 100 g DW. Red beans consistently displayed the highest total polyphenol contents in both the methanolic and aqueous extracts, followed by grey mottled, brown and white. Tannin concentration ranged from 10.2 to 55.4 mg GAE / 100 g DW for the aqueous and from 3.1 to 53 mg GAE / 100 g DW for the 70% methanol extract. Flavonoid concentration ranged from 42.1 to 62.6 mg quercetin equivalents / 100 g DW (aqueous extraction) and 95.2 to 123.5 mg quercetin equivalents / 100 g DW (70% methanol extraction). The various classes of common beans displayed varying antioxidant activities. The bean extracts exhibited DPPH free radical scavenging activities with pseudo first-order rate constants (K) ranging between 0.006 min^{-1} and 0.053 min^{-1} and FRAP derived antioxidant power between 1.69 and 6.88 Fe^{2+} / 100 g DW. The red market class displayed the highest antioxidant activity in the aqueous extract, but showed little difference with the grey mottled beans in the methanolic extract. Ranking the market classes based on the free radical scavenging capacities and the FRAP-derived total antioxidant power, the following order was observed: red beans > grey mottled beans > brown beans > white beans. On a comparative basis, white beans displayed far lower antioxidant activities compared to the others.

Keywords: *antioxidant, polyphenols, nutraceuticals, common beans*

Cite This Article: Nyau V., Prakash S., Rodrigues J., and Farrant J., "Screening Different Zambian Market Classes of Common Beans (*Phaseolus vulgaris)* for Antioxidant Properties and Total Phenolic Profiles." *Journal of Food and Nutrition Research*, vol. 4, no. 4 (2016): 230-236. doi: 10.12691/jfnr-4-4-6.

1. Introduction

Plant-derived phenolic compounds are important as nutraceutical constituents in our diet. They have antioxidant properties and may protect against major clinical conditions such as heart disease and cancer in which reactive oxygen species (i.e., superoxide anion, hydroxyl radicals and peroxy radicals) are involved [1,2]. Plant phenolics represent one of the major groups of compounds acting as primary antioxidants or free radical terminators [3,4]. Reactive oxygen species are generated through normal metabolism, environmental factors such as pollution, radiation, pesticides and cigarette smoke in which oxygen participates in the reactions. These species attack cellular components such as DNA, lipids and proteins and are thought to be an initiating factor for several chronic diseases [5]. Dietary antioxidants may prevent these cellular components from oxidative damage and do this by undergoing oxidation themselves [6]. Antioxidant properties that have been described for plant-derived phenolic compounds include: stabilisation of unpaired electrons [7]; scavenging of free radicals from lipid peroxidation [8]; and the ability to chelate transition metal ions, which results in the inhibition of the reactive oxygen species production [2]. In view of the possibility that the antioxidant potential of plant-derived phenolic compounds may reduce the risk of developing chronic diseases, it is important to have a clear idea of the phenolic antioxidant compounds that plant foods contain. Common beans is one of the most important plant food whose contribution to the world nutrition is significant especially in developing countries where they are the main source of protein to the poor majority. They are raw materials of food crops for many regions of the world where they are eaten in large quantities on a daily basis as a rich source of protein, resistant starch, and dietary fibre [9]. They are also an excellent source of nutraceutical constituents such as fiber, protease inhibitors, phytic acid, and polyphenols such as tannins [10]. Further, legumes play important roles in the prevention of chronic diseases

such as diabetes, cancer, hypertension and obesity [11]. The physiological effects of common beans may be due to the presence of abundant phytochemicals including polyphenolics, which possess both anticarcinogenic and antioxidant properties [12,13,14]. In this study, the Zambian market classes of common beans were investigated for their antioxidant activities and phenolic profiles in order to establish their nutraceutical value.

2. Materials And Methods

2.1. Sample Collection

Four market classes of common beans (red, grey mottled, white and brown) commonly grown in Zambia were obtained directly from the farmers immediately after harvest. These four classes of beans are shown in Figure 1. In order to make the samples representative, an attempt was made to collect the seeds of each market class from 15 farmers in each growing area with not less than 0.5 kg per farmer and later mixed homogenously per market class.

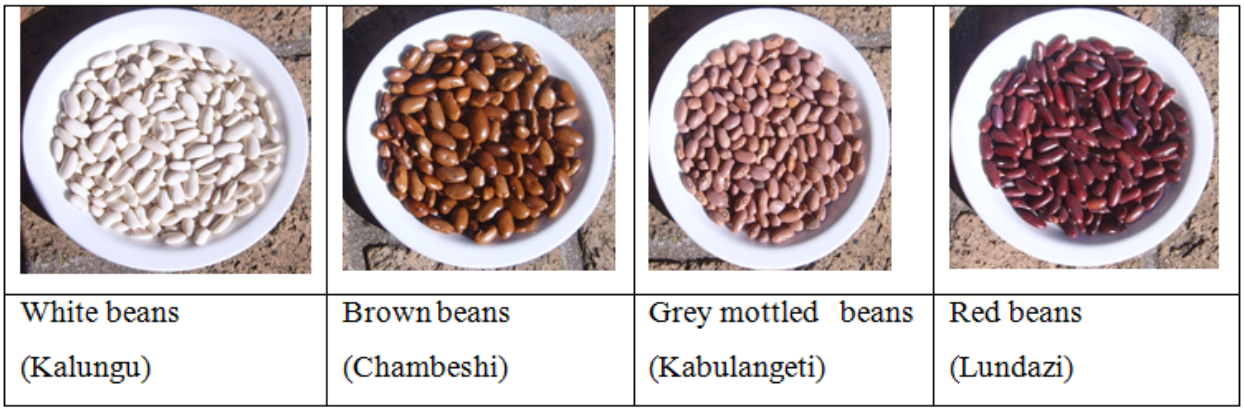

Figure 1. Market classes of common beans investigated

2.2. Preparation of the Extracts

Raw dry common beans were ground into powder of the same consistency using a coffee grinder (Braun, Mexico). The 70% methanol extracts were obtained using Ultrasound-Assisted Extraction (UAE) from the seed flour [15]. Approximately 15 g of seed powder in 150 ml of 70% methanol was sonicated for 30 minutes at 25°C using the Eumax UD500SH 40 kHz ultrasonic bath. After extraction, the mixture was centrifuged at a speed of 10,000 rpm for 15 minutes in Beckman Coulter JE centrifuge. The resulting supernatant was first concentrated to 30 mL by evaporation under reduced pressure in a rotary evaporator (Buchi R-210 model, Switzerland) to remove methanol. The extract was then frozen at -80°C and freeze dried to obtain a powdered methanolic extract using the Telstar LyoQuest -85 freeze dryer. The freeze dried extracts were stored at -4°C until further analysis

2.3. Determination of Total Polyphenols

Powdered samples of common beans (5 g) were placed in 50 mLl of 70% methanol or water and sonicated for 30 minutes at 25°C followed by centrifugation at 10,000 rpm for 15 minute at 4°C to obtain a clear supernatant. Total polyphenols were then determined by the Folin Ciocalteu assay according to the method of [16]. To 100 µL of sample extract, 400 µL of distilled water was added followed by the addition of 250 µl Folin Ciocalteu reagent. 20% Sodium carbonate (1.25 mL) was then added and the mixture was incubated for 40 min. Absorbancies were read at 725 nm after 40 minutes using a spectrophotometer (Ultrospec 1000 model, England) against the blank (70% methanol or water) depending on whether it was the water or 70% methanol extract. The amount of total polyphenols was calculated as gallic acid equilvalents from the calibration curve of gallic acid standard solution and expressed as mg gallic acid equivalents/ 100 g DW. The experiment was conducted three times and all measurements were performed in triplicate.

2.4. Determination of Tannins

The determination of tannins was performed using the Polyvinylpolypyrrolidone (PVPP) tannin binding assay as described by Makkar *et al.* [16]. Approximately 100 mg PVPP was placed in 100 x12 mm test tubes. One mL of distilled water was added to the test tubes containing PVPP followed by the addition of 1 mL of sample extract. The tube was vortexed, and kept at 4°C for 15 minutes. After 15 minutes at 4°C, the tube was vortexed again and then centrifuged for 10 minutes at 10,000 rpm. The supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). Total polyphenols of the supernatant was measured as described in section 2.3 and results of non tannin phenols were expressed as mg gallic acid equivalents / 100 g DW. The amount of polyphenols bound to PVPP as tannin was calculated by subtracting the total polyphenols in the supernatant after the PVPP assay from the amount of total polyphenols determined without PVPP. The experiment was conducted three times and all measurements were performed in triplicate.

2.5. Extraction and Analysis of Total Flavonoids

Flavonoids were extracted from common beans samples by the ultrasound-assisted system as described by [17]. Powdered sample (5 g) was placed in 50 mL of 70% methanol or water and was sonicated for 30 minutes at

25°C followed by centrifugation at 10,000 rpm for 15 minute at 4°C to obtain a clear supernatant. Analysis of total flavonoids was done using the aluminium chloride colorimetric method according to [18]. About 500 µL of 70% methanolic extract was mixed separately with 1.5 mL of methanol, 100 µL of 10% aluminium chloride, 100 µL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance of the solutions was measured after 30 minutes at 414 nm using a spectrophotometer (Ultrospec 1000 model, England). The amount of total flavonoids was calculated as quercetin equivalents (mg / 100g DW) using a calibration curve of quercetin standard solution. The experiment was conducted three times and all measurements were performed in triplicate.

2.6. Determination of Free Radical Scavenging Activity of Common Beans

DPPH stable free radicals are reduced to DPPH-H leading to discoloration from purple to yellow and consequently a decrease in absorbance. The degree of discoloration indicates the scavenging potential of the antioxidant compounds [19]. This assay therefore involves the measurement of hydrogen atom transfer or electron donation from a potential antioxidant to free radical molecules [20]. First, it was important to study the kinetic behaviour of the extracts towards DPPH free radicals when the freeze dried extracts from each market class were added at the same concentration. The knowledge of the kinetics of atom transfer is important because free radicals in the organism are short-lived species, implying that the impact of a substance as an antioxidant depends on its fast reactivity towards free radicals [21]. The free radical scavenging kinetic determinations were adapted from [21]. Under the experimental conditions used, the DPPH concentration was in large excess with respect to that of the extracts in order to follow pseudo first-order kinetics. This was done to exhaust the hydrogen donating capacity of the extracts. The excess concentration of DPPH (200 mM) was determined to be the optimum concentration after performing a number of runs with the extracts. This was the only way the excess DPPH concentration could be determined since it was not posible to work it out based on the DPPH: antioxidant molar ratios as the antioxidants in the extracts were not pure compounds. In the assessement of the kinetic behaviour, 2 mL of the extracts were added at the same concentration (400 µg / mL) to 2 mL of DPPH radical solution (200 mM) prepared in 95% methanol. The reaction was run at room temperature within a time period of 80 minutes. The absorbances of the mixture were automatically measured every 10 seconds using the spectrophotometer at 517 nm connected to a computer and the output was displayed using SWIFT 1000 software (Ultraspec 1000 model, England). From the reaction between an antioxidant and DPPH;

(DPPH) + (Y-H) → DPPH-H + (Y), it can be deduced that:

$$-\frac{d[DPPH]}{dt} = k[DPPH][Y-H] \quad (1)$$

Considering that DPPH was in excess and therefore the experiment was under pseudo first-order conditions, one can say:

$$InA = InA_0 - kt \quad (2)$$

Where A_0 is the absorbance of the reaction mixture (DPPH and the extract) at t = 0; A is the absorbance of the reaction mixture (DPPH and extract) at time t.

The pseudo first order rate constant 'k' for the reaction of the antioxidants in the extracts and DPPH in the first seconds of the reaction was calculated from the slopes of *InA* versus time plots.

The percentage of DPPH remaining at any time t can be determined as:

$$\%DPPH_{remaining} = \frac{A_t}{A_0} X100 \quad (3)[21]$$

Where A_0 is the initial absorbance and A_t is the absorbance at time = t, both measured at 517 nm respectively. Plots of percentage DPPH versus time were constructed to show the disappearance pattern of the DPPH with time in the presence of each extract.

2.7. Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was used to determine the ferric reducing antioxidant power of the common beans [22]. The method measures the ferric reducing ability of the antioxidants compounds in the extracts. At low pH, ferric-2,4,6-tri-2-pyridyl-*s*-triazine (TPTZ) complex (Fe^{3+} TPTZ) is reduced to the ferrous form Fe^{2+} in the presence of the antioxidant producing an intense blue colour with an absorption maximum at 593 nm. Powdered sample of common beans (5 g) in 50 mL of 70 % methanol or water was sonicated for 30 minutes at 25°C followed by centrifugation at 10,000 rpm for 15 minutes at 4°C to obtain a clear supernatant. Working FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6); 2.5 ml ferric chloride solution (prepared by dissolving 54 mg ferric chloride in 10 ml distilled water) and 2.5 mL TPTZ solution (prepared by dissolving 31 mg TPTZ in 40 mM HCl at 50°C). The mixture was placed in a water bath at 37°C for 10 minutes. The assay was performed as follows: 1 mL of water and 80 µL of the test sample were pipetted into a cuvette. About 600 µL of the incubated FRAP reagent was added to the cuvette and mixed by inversion. A reagent blank was prepared as above with 80 µL water added instead of the test sample. The change in absorbance was recorded at 593 nm using a spectrophotometer after exactly 4 minutes (Ultrospec 1000 model, England). The amount of Fe^{2+} produced from the reduction of Fe^{3+} by the extract was calculated from the standard curve prepared from ferrous sulphate solution and results were expressed as mg Fe^{2+} / 100 g dry sample. The experiment was conducted three times and all measurements were performed in triplicate

2.8. Statistical Analysis

Statistical analysis was performed using S-PLUS 6 Windows Professional 2001. Experimental results were expressed as mean values ± standard error. Data was analysed using one way analysis of variance (ANOVA) model. Values at $p < 0.05$ were considered statistically significant. Correlation coefficients of variable parameters were analysed by Pearson correlation test.

3. Results And Discussions

3.1. Total Polyphenol and Tannins

Table 1 presents the results of the total polyphenol and tannins concentrations of the four market classes of common beans. Total polyphenol concentrations for the four market classes in both extracts were significantly different ($p < 0.05$). The red market class had the highest total polyphenol contents in the aqueous and 70% methanol extracts, followed by the grey mottled, brown and last, the white. The red market class showed stronger absorption than the rest at 280 nm when the aqueous was scanned spectrophotometrically between 200 and 400 nm (see Figure 2). The results of the present study are close to the range (64 – 95 mg GAE / 100 g DW) reported by [23] and less than the range (117 to 427 mg GAE / 100 g DW) reported by [24] for the twelve different market classes of common beans from Italy. Values from 223 to 1247 mg GAE / 100 g DW of total polyphenols for various common beans cultivars, which is very different from what has been observed in this research were reported by [25]. In polyphenol research, several authors have reported different findings on the total polyphenol concentration of common beans. This may be attributed, in part, to the fact that the samples are from diverse growing areas with different environmental conditions, and furthermore, the methods and the solvents used in the extraction of polyphenol compounds differ. The varietal effect may also play a significant role. Previous authors have reported that due to the lack of standardization of the analytical methods, concentrations for polyphenols in a given food are often not easily comparable [26,27].

The results for tannin concentration ranged from 10.2 to 55.4 mg GAE / 100 g DW for the aqueous and from 3.1 to 53 mg GAE / 100 g DW for the 70% methanol extract. The grey mottled and white beans consistently demonstrated the highest and the lowest tannin concentration respectively.

Table 1. Total polyphenol contents and tannin concentrations of common beans

Market classes of common beans		Total polyphenols (mg GAE / 100 g DW)	Tannins (mg GAE / 100 g DW)
Aqueous	Brown	61.6 ± 1.1^{c}	24.7 ± 0.7^{c}
	Red	105.4 ± 0.5^{a}	51.3 ± 2.0^{b}
	Grey mottled	90.3 ± 3.8^{b}	55.4 ± 1.3^{a}
	White	45.2 ± 1.4^{d}	10.2 ± 0.2^{d}
70% methanol	Brown	60.2 ± 3.8^{c}	19.6 ± 1.7^{c}
	Red	123.7 ± 4.3^{a}	49.4 ± 2.7^{b}
	Grey mottled	85.4 ± 2.9^{b}	53.1 ± 1.6^{a}
	White	37.3 ± 2.6^{d}	3.1 ± 0.2^{d}

For each extract (aqueous or 70% methanol), means in the same column with different superscripts were significantly ($p < 0.05$) different.

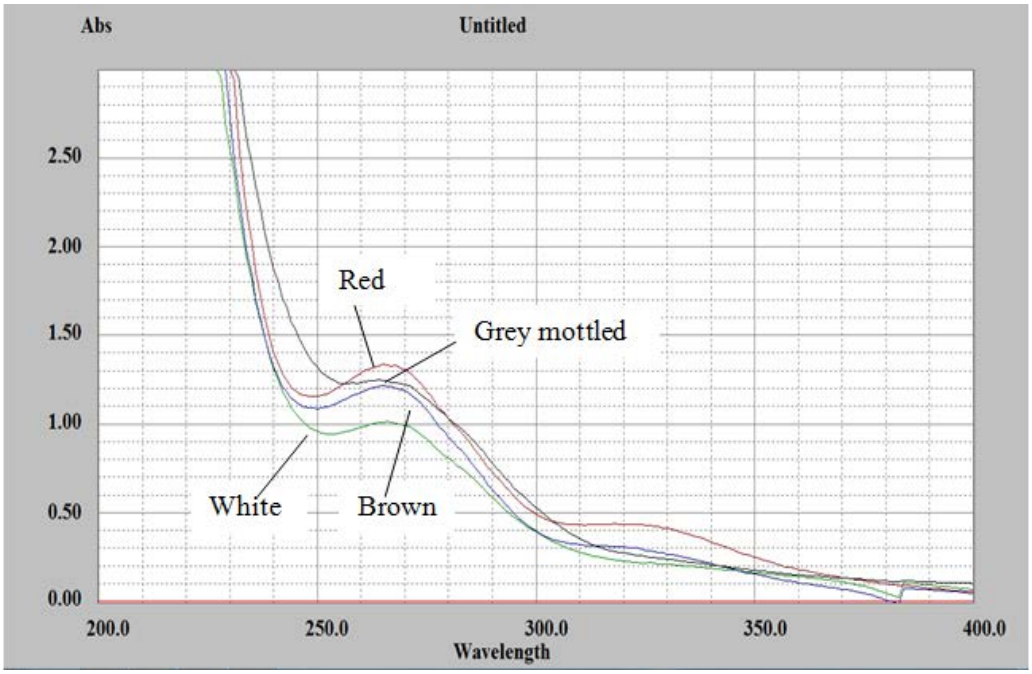

Figure 2. Results of the spectrophotometric scanning at 200 nm to 400 nm for the aqueous extract of common beans

3.2. Total Flavonoid Concentration

Flavonoids are pigments responsible for seed coat colour in common beans [28]. The flavonoid concentrations of the four market classes of common beans grown in Zambia are shown in Table 2. The concentrations of flavonoids in the aqueous extract were generally lower than in the 70% methanol extract. Flavonoid concentration ranged from 42.1 to 62.6 mg quercetin equivalents / 100 g DW (aqueous extraction) and 95.2 to 123.5 mg quercetin equivalents / 100 g DW (70% methanol extraction). The

concentrations obtained in the present study are in agreement and slightly greater than the range (19 – 84 mg quercetin equivalents / 100 g DW) obtained by [29] for twenty three Italian varieties of common beans. Total quercetin ranging from 5 to 41 mg / 100g DW for twenty landraces of common beans grown in different regions of Uttarakhand, which is lower than in our study has been reported [30]. As mentioned earlier, concentration of phytochemicals may vary depending on a number of factors.

Table 2. Flavonoid content of common beans

Market classes of common beans		Flavonoids content expressed as quercetin equivalents (mg / 100 g dry mass)
Aqueous	Brown	56.3 ± 2.0[b]
	Red	42.1 ± 1.2[c]
	Grey mottled	55.8 ± 0.9[b]
	White	62.6 ± 3.1[a]
70% methanol	Brown	101.4 ± 2.8[c]
	Red	123.5 ± 1.9[b]
	Grey mottled	95.2 ± 1.1[d]
	White	117.7 ± 2.2[a]

For each extract (aqueous or 70% methanol), means in the same column with different superscripts were significantly ($p < 0.05$) different.

3.3. Kinetics of the DPPH Free Radical Reaction with Antioxidants in Common Beans

Figure 3 and Figure 4 present the disappearance pattern of DPPH free radicals with time in the presence of the aqueous and methanol extracts within the time period of 80 minutes. The reaction was performed under pseudo first-order condition that was achieved by making the concentration of DPPH in large excess. The free radical scavenging pattern was characterised by the fast initial decay, followed by the subsequent slower step. The pseudo first-order rate constants (K) of the four market classes of common beans were obtained using equations (1) and (2), and the plot of InA versus time, whose slope was equal to K. Ranking the free radical scavenging capacities of the common bean market classes based on K, the order would be as follows: red beans > grey mottled beans > brown beans > white beans (Table 3). The same order is obtained by ranking the market classes based on the amount of DPPH radicals scavenged after 80 minutes incubation (Table 3). White beans showed far lower antiradical capacity than the other three. Compared to Trolox which was used as a positive reference standard, the free radical scavenging ability of the red, grey mottled and the brown beans can be considered moderate.

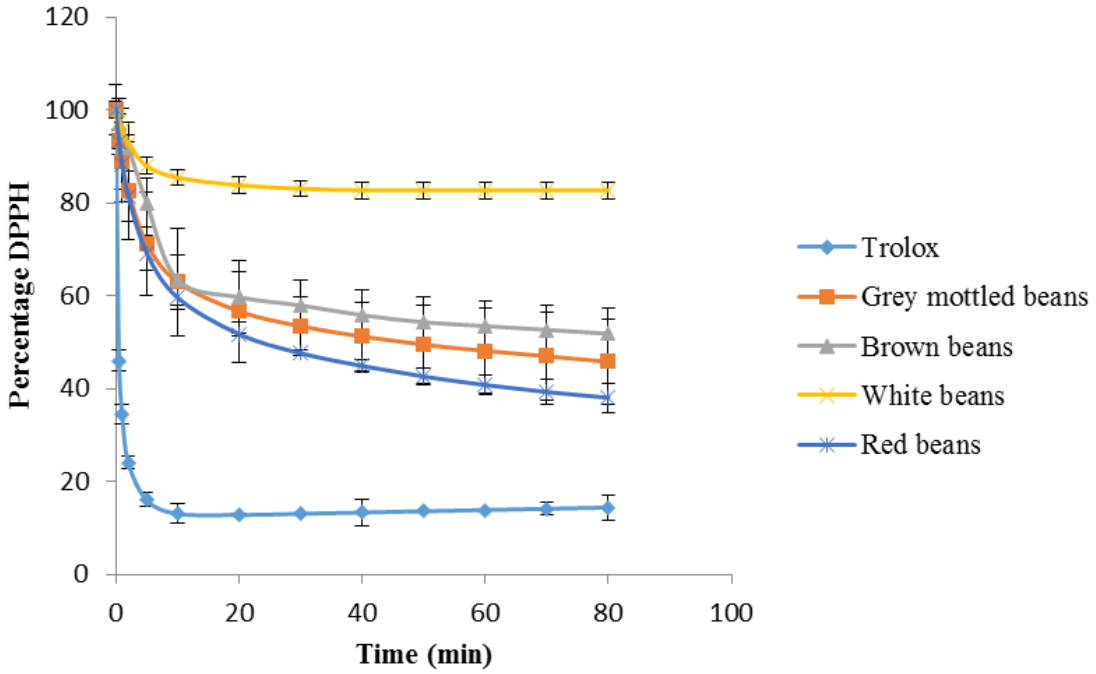

Figure 3. Disappearance pattern of DPPH free radicals with time in the presence of aqueous extracts of common beans

Table 3. Pseudo-first order rate constant of antiradical (Y-H) in common beans extracts and the amount of DPPH scavenged after 80 minutes of incubation

Market class of common beans	Pseudo-first order rate constant (K) [min^{-1}]		Amount DPPH quenched [%] after 80 minutes incubation	
	Aqueous extract	70% Methanol extract	Aqueous extract	70% Methanol extract
Red	0.043± 0.001[a]	0.053 ± 0.0003[a]	55.3 ± 2.0[a]	61.9 ± 3.1[a]
Grey mottled	0.038 ± 0.0005[b]	0.047 ± 0.001[b]	56.6 ± 0.9[a]	54.2 ± 1.4[b]
Brown	0.019 ± 0.002[c]	0.034 ± 0.003[c]	40.3 ± 1.1[b]	48.1 ± 5.2[c]
White	0.006 ± 0.0006[d]	0.013 ± 0.002[d]	18.5 ± 0.9[c]	17.3 ± 1.0[d]

Trolox K = 1.55, amount quenched in 80 minutes = 85.5%
For each extract (aqueous or 70% methanol), means in the same column with different superscripts were significantly ($p < 0.05$) different.

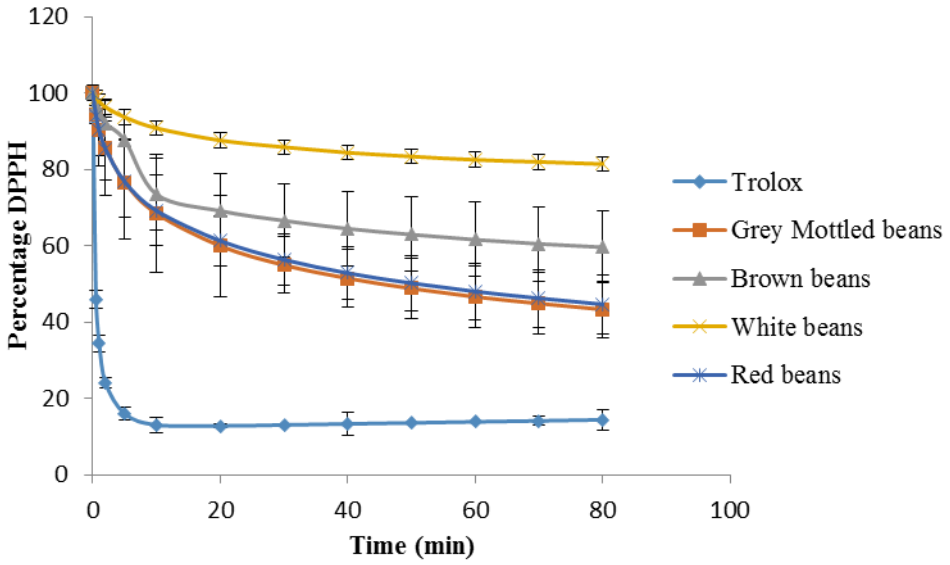

Figure 4. Disappearance pattern of DPPH free radicals with time in the presence of the 70% methanol extracts of common beans

3.4. Ferric Reducing Antioxidant Power of Antioxidants in Common Beans

The FRAP values of the aqueous and methanol extracts of the four market classes of common beans are presented in Table 4. The methanol extracts demonstrated higher FRAP values than the aqueous extracts in all the common bean market classes. FRAP values ranged from 1.69 to 6.88 mmol Fe^{2+} / 100 g DW. The four market classes of common beans can be ranked as follows based on the antioxidant reduction power: red beans > grey mottled beans > brown beans > white beans. Our results are within the range (1.27 to 9.70 mmol Fe^{2+} / 100 g DW) reported previously by [31] for different market classes of common beans grown in North Dakota, Idaho and Washington regions of the United States of America. In comparison to other legumes, all four market classes had higher FRAP values than peas (0.62 to 0.82 mmol Fe2+ / 100 g DW), but lower than lentils (8.75 to 13.92 mmol Fe^{2+} / 100 g DW) reported previously by [31]. Similar comparison can be made with the findings of [32] on the FRAP values of lentils (11.37 to 13.92 mmol Fe^{2+} / 100 g DW), peas (0.43 to 0.86 mmol Fe^{2+} / 100g DW), soyabeans (1.09 to 1.49 mmol Fe^{2+} / 100 g DW) and chickpeas (0.8 mmol Fe^{2+} / 100 g DW).

Table 4. Ferric Reducing Antioxidant Power (FRAP) values for common beans

Market classes of common beans	FRAP value (mmole Fe^{2+} / 100 g DW)	
	70 % Methanol extract	Aqueous extract
Red	6.88 ± 0.04[a]	4.86 ± 0.05[a]
Grey mottled	6.74 ± 0.08[b]	4.70 ± 0.03[b]
Brown	4.46 ± 0.14[c]	2.25 ± 0.02[c]
White	2.96 ± 0.04[d]	1.69 ± 0.03[d]

For each extract (aqueous or 70% methanol), means in the same column with different superscripts were significantly ($p < 0.05$) different.

3.5. Correlation of Antioxidant Activities and Phenolic Contents of Common Beans

The correlations between the antioxidant activity and total polyphenols, tannins and flavonoids of common beans were established using regression analysis. For the aqueous extract, the correlations between the ferric reducing antioxidant power and phenolic compounds were as follows: total polyphenols (0.9684), tannins (0.9549), and flavonoids (0.0988). The correlations between the DPPH free radical scavenging and phenolic compounds were: total polyphenols (0.8642), tannins (0.9462) and flavonoids (0.0127). For the methanol extract, the following correlations were observed for ferric reducing antioxidant power: total polyphenols (0.8841), tannins (0.9724) and flavonoids (-0.6898), and with the DPPH free radical scavenging: total polyphenols (0.7826), tannins (0.7850) and flavonoids (-0.6887). In both extracts, total polyphenols and tannins showed a positive linear relationship with the antioxidant activities. This suggests that their contribution to the antioxidant activities of the investigated market classes of common beans is considerable. These results are consistent with the findings of previous researchers, who reported such positive correlations for total polyphenols and tannins with antioxidant activity [18,32]. Surprisingly, there was no correlation between antioxidant activity and flavonoids content of the aqueous extracts, whereas negative correlations were observed in the methanol extracts. Low correlations between flavonoids and antoxidant activity have been reported previously in other studies [3,33]. It is known that only flavonoids of certain structure and containing certain groups, particularly hydroxyl groups in certain positions in the molecule determine antioxidant properties [33]; and that in general, these properties depend on the ability to donate hydrogens or electrons to a free radical [3].

4. Conclusion

This study revealed that market classes of common beans grown in Zambia possess antioxidant properties that may be valuable for human health. The red beans have the highest free radical scavenging activity and FRAP – derived total antioxidant power, followed by the grey mottled, brown and white beans. Similarly, red beans is the richest in total polyphenol concentration, followed by grey mottled, brown and white beans, and there is a strong

positive correlation between antioxidant activity and total polyphenol content.

Acknowledgements

This work was supported by the University Science, Humanities, Engineering Partnership in Africa (USHEPiA). We are thankful for the financial assistance.

References

[1] Rhodes, M.J.C., Price, K.R., Identification and analysis of plant phenolic antioxidants, *Eur. J. Cancer prev*., 6, 5188, 1997.

[2] Duthie, G., Crozier, A., Plant-derived phenolic antioxidants, Curr. *Opin Clin Nutr Metab Care*, 3(6), 447-451, 2000.

[3] Miliauskas, G., Venskutonis, P., Van Beek, T., Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chem.*, 85, 231-237, 2004.

[4] Saura-Calixto, F., Serrano, J., Goñi, I., Intake and bioaccessibility of total phenols in a whole diet, *Food Chem.*, 101, 492-501, 2007.

[5] Muller, H. E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M., Antioxidant content of whole grain breakfast cereals, fruits and vegetables, *J. Am. Col. Nutr.*, 19, (3 Suppl), 312S-319S, 2000.

[6] Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M., Free radical scavenging properties of wheat extracts, *J. Agric. Food Chem.*, 50 (6), 1619-1624, 2000.

[7] Duthie, G., Gardner, P.T., Kyle J., Plant polyphenols: are the new magic bullet, *Proc. Nutr. Soc.*, 62, 599-603, 2003.

[8] Nijveldt, R.J., Nood, E., Hoorn, D., et al, Flavonoids: a review of probable mechanisms of action and potential applications, *Am J Clin Nutr*., 74, 418-425, 2001.

[9] Geil, P.B., Anderson, J.W., Nutrition and health implications of dry beans: a review, *Am J Clin Nutr*. 13, 1994.

[10] Guzman□-Maldonado, S. H., Paredes-Lo´pez, O., (1999), Biotechnology for the improvement of nutritional quality of food crop plants, In *Molecular Biotechnology for Plant Food Production*; Paredes- Lo´pez, O., Ed.; Technomic Publishing Company, Inc.: Lancaster, PA, 590-591, 1999.

[11] Kushi, L.H, Meyer, K.A., Jacobs, D.R., Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies, *Am J. Clin. Nutr*., 70(suppl), 451S-458S, 1999.

[12] Gonzalez, M. E., Castano-Tostado, E., Loarca-Pina, G., Antimutagenic effects of natural phenolic compounds in beans, *Mutat. Res. Genet. Toxicol. En*Viron. *Mutagen*. *44*, 1-9, 1999.

[13] Martinez, A.C., Loarca-Pinã, G., Oomah, B. D., Antioxidant activity in common beans (*Phaseolus* V*ulgaris* L.), *J. Agric. Food Chem*., *50*, 6975-6980, 2002.

[14] Aparicio-Fernández, X., García-Gasca, T., Yousef, G.G., Lila, M. A., González de Mejía, E., Loarca-Piña G., Chemopreventive activity of polyphenolics from black Jamapa Beans (*Phaseolus vulgaris* L.) on HeLa and HaCaT cells, *J. Agric. Food Chem*., 54, 2116-2122, 2006.

[15] Dobiáš, P., Pavlíková, P., Adam M., Eisner A., Beňová B., Ventura K., Comparison of pressurised fluid and ultrasonic extraction methods for analysis of plant antioxidants and their antioxidant capacity, *Cent. Eur. J. Chem*., 8: 87-95, 2010.

[16] Makkar, H.P.S., Hagerman, A., Mueller-Harvey, I., Quantification of tannins in tree foliage – a laboratory manual, FAO/IAEA Working Document, IAEA, Vienna, 23-24, 2000.

[17] Wang, J., Zhao, Y.M., Guo, C.Y., Zhang, S.M, Liu, C.L., Zhang, D.S., Bai, X.M., Ultrasound-Assisted extraction of total flavonoids from Inula Helenium, *Pharmacogn Mag*. April-Jun; 8(30), 166-170, 2012.

[18] Pourmorad, F., Hosseinimehr. S., Shahabimajd N., Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, *Afr. J. Biotechnol*., 5, 1142-1145, 2006.

[19] Pal, D.K., Kumar, S., Chakraborty, P., Kumar, M.A., A study on the antioxidant activity of *Semecarpus anacardium* L. f. Nuts, *J. Nat. Rem*., 8, 160-163, 2008.

[20] Becker, E.M., Nissen, L.R. & Skibsted, L.H., Antioxidant evaluation protocols: Food quality or health effects, *Eur. Food Res. Technol*. 19, 561-571, 2004.

[21] Villanño, D., M.S. Fern´andez-Pach´on, M.S., Moy´a, M.L., Troncoso, A.M. & Garc´ıa-Parrilla, M.C., Radical scavenging ability of polyphenolic compounds towards free radicals', *Talanta* 71, 230-235, 2007.

[22] Benzie, I. F. F., *Clin. Biochem.* 29, 111-116, 1996.

[23] Deshpande, S.S., Cheryan, M., Determination of phenolic compounds of dry beans using vanillin, redox and precipitation assays, *J. Food Scie. 52*, 332-334, 1987.

[24] Heimler, D., Vignolini, P., Dini, M.G., Romani, A., Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L dry beans, *J.Agric. Food Chem.*, 53, 3053-3056, 2005.

[25] Wu, X., Beecher, G.R., Holden J.M., Haytowitz, D.B., Gebhardt S.E., Prior R.L., Concentrations of anthocyanins in common foods in the United States and Estimation of normal consumption, *J. Agric. Food Chem.* 54, 4069-4075, 2006.

[26] Harnly, J.M, Bhagwat, S., Lin L.Z., Profiling methods for the determination of phenolic compounds in foods and dietary supplements, *Anal. Bioanal*. Chem. 389, 47-61, 2010.

[27] Amarowicz, A., Carle, R., Dongowski, G. et al., Influence of postharvest processing and storage on the content of flavonoids and phenolic acids in foods, *Mol. Nutr. Food Res*., 53, S151-S183, 2009.

[28] Beninger, C.W., Hosfield, G.L. & Nair M.G., Glycosides from the seed coat of a new Manteca-type dry beans (phaseolus vulgaris L.), *J. Agric. Food Chem*. 46, 2906-2910, 1998.

[29] Dinelli, G., Bonetti, A., Minelli, M., Marotti I., Catizone P., Mazzanti A., Content of flavonols in Italian beans (*Phaseolus vulgaris*, L.) ecotypes, *Food Chem.* 99, 105-114, 2006.

[30] Mishra, J., Tattan, T.D., Jadon, V.S., Manju P., Gusain, Aradhana., HPTLC Profile of Quercetin Content of Common Beans (Uttarakhand) Landraces growing in Uttarakhand, *Am J. of Food Tech.* 7(2), 96-100, 2012.

[31] Xu, B.J., Yuan, S.H., Chang, S.K.C., Comparative Analyses of Phenolic Composition, Antioxidant Capacity, and Color of Cool Season Legumes and other selected food legumes, *J. Food Scie.* 72, 2, 2007.

[32] Hajimahmoodi, M., Sadeghi, N., Jannat, B., Oveisi, M.R., Madani, S., Kiayi, M., Akrami, M.R. & Ranjbar, A.M., Antioxidant Activity, Reducing Power and total Phenolic Content of Iranian Olive Cultivar, *J. Bio. Scie.* 8(4), 779-783, 2008.

[33] Luís, Â., Domingues F., Gil C., Duarte A.P., Antioxidant activity of extracts of Portuguese shrubs: *Pterospartum tridentatum*, *Cytisus scoparius* and *Erica*, *J. Med. Plants Res.* 3(11), 886-893, 2009.