

# Proximate Composition, Phenolic Profiles and Antioxidant Capacity of Three Common Bean Varieties (*Phaseolus vulgaris* L.)

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E-mail: [Trust.Beta@umanitoba.ca](mailto:Trust.Beta@umanitoba.ca)**Received:** June 17, 2016**Accepted:** August 08, 2016**Published:** August 10, 2016**Citation:** Fan G, Beta T. 2016. Proximate Composition, Phenolic Profiles and Antioxidant Capacity of Three Common Bean Varieties (*Phaseolus vulgaris* L.). *J Food Chem Nanotechnol* 2(3): 147-152.**Copyright:** © 2016 Fan and Beta. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (<http://creativecommons.org/licenses/by/4.0/>) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

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## Abstract

Proximate composition, phenolic profiles and antioxidant capacity of three bean varieties (Dimeta, Napirira and Nanyati) were investigated. There were significant ( $p < 0.05$ ) differences among varieties in all proximate components. Dimeta bean had the highest content of crude fat, and free and bound phenolic compounds, while Nanyati bean had the highest content of ash and carbohydrate. Napirira bean had the highest content of crude protein and all phytochemicals, as well as the oxygen radical absorbance capacity (ORAC). The phenolic compounds existed mainly in the free form as detected by high performance liquid chromatography (HPLC). A total of five phenolic compounds (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic and sinapinic) were found in these bean varieties while catechin was only detected in Napirira bean. The anthocyanins in these three bean varieties were detected by HPLC-MS. Delphinidin 3-O-glucoside, cyanidin 3-O-glucoside and pelargonidin 3-O-glucoside were the main anthocyanins in Dimeta bean, while cyanidin 3-O-glucoside, pelargonidin 3-O-glucoside and pelargonidin derivatives were the major anthocyanins in both Napirira and Nanyati beans. It could be concluded that significant differences in proximate composition, phenolic profiles and antioxidant capacity occurred among the three varieties with Napirira bean showing superiority as a functional food ingredient.

## Keywords

Common beans, Proximate composition, Phenolic profiles, Antioxidant capacity, *Phaseolus vulgaris* L.

## Introduction

Common beans (*Phaseolus vulgaris* L.) are grown and consumed throughout the world. They play an important role in the nutrition of low-income people especially in developing countries, where they are often the most important dietary source of protein, carbohydrate, dietary fiber, and minerals [1, 2]. Besides the nutritional role, common beans are also rich in antioxidants, which may include a variety of flavonoids such as anthocyanins, flavonols, proanthocyanidins, tannins, glycosides as well as a wide range of phenolic acids. A high consumption of beans is believed to reduce the incidence of cardiac disease, colon cancer, diabetes and hypertension [3, 4].

Phytochemical and nutrient constituents depend both quantitatively and qualitatively on plant genotype [5] and on environmental factors including water and mineral nutrition [6]. Genetic research concerning bean culture is mainly

aimed at improving productivity and resistance to field pests and environmental stress. Although there is evidence that it may also change physicochemical characteristics of seeds and affect their nutritional value, studies concerning these changes in beans are still rare and limited to few cultivars.

Dimeta bean, Napirira bean and Nanyati bean are the main cultivated common bean varieties in central Malawi. Napirira bean is an improved variety bred for disease and pest resistance while Dimeta is an improvement of the local landrace Nanyati. The objectives of the present study was to compare the phytochemical constituents, nutritional and antioxidant properties of three common bean varieties recommended for planting in central Malawi. Hence, this work determines nutritional values of common beans and as a potential source of bioactive components.

## Materials and Methods

### Materials

Three common bean varieties (Dimeta, Napirira and Nanyati) were collected from central Malawi after they were dried in the sun. The dried beans were subsequently milled into powder with an electric mill (Bel-Art Products, Pequannock, NJ, USA), sieved (40 mesh) and stored at 4 °C.

HPLC grade methanol, ethyl acetate, and hexanes were purchased from Fisher Scientific Co. (Ottawa, ON), and used in the extraction and purification. Phenolic compounds were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, purity 99%). MS grade water, methanol and acetic acid were used for HPLC and mass spectrophotometric analysis.

### Physicochemical composition

Approved methods of analysis of the American Association of Cereal Chemists International were used to determine the percentage of moisture, crude protein, crude fat, and ash in the bean samples (AACC, 2000). Total carbohydrate content was calculated by difference, applying the formula:  $100\% - (\% \text{Moisture} + \% \text{Ash} + \% \text{Crude fat} + \% \text{Crude protein})$ .

### Extraction of free phenolic compounds

Free phenolic compounds were extracted according to the methods of Chen et al. [7] with some modifications. The milled bean seeds (1 g) was defatted twice with 20 mL hexanes to remove lipid by shaking on a wrist action shaker (Burrell Scientific Pittsburgh, PA, USA) for 15 min under dark conditions at room temperature followed by centrifuging at 10,000 rpm (Sorvall RC 6+ Centrifuge, Thermo Fisher Scientific, Asheville, NC, USA) and 4 °C for 10 min. The lipid-free residue was extracted three times with 80% methanol (20 mL) to extract free phenolic compounds. Each time, the mixture was shaken on a wrist action shaker for 1 h and dark conditions at room temperature. The mixture was then centrifuged at 10,000 rpm for 10 min at 4 °C. The combined supernatants were filtered and evaporated to dryness under vacuum at 38 °C. The dried extracts were dissolved in 5 mL 50% methanol. The extracts were stored at 4 °C. Extraction and all analysis were performed in duplicate.

### Extraction of bound phenolic compounds

Bound phenolic compounds were extracted according to the methods described by Chen et al. [7] with modifications. The residue collected after methanol extraction was hydrolyzed with 40 mL of 2 M NaOH at room temperature for 2 h. The mixture was shaken using a wrist action shaker under dark conditions at room temperature. The hydrolyzed mixture was adjusted to a pH of 1.5-2.0 with 6 M HCl. After centrifugation at 10,000 rpm for 10 min at 4 °C, the supernatant was defatted using 50 mL hexanes and extracted three times with 50 mL ethyl acetate. The combined ethyl acetate fractions were evaporated to dryness under vacuum at 35 °C with a rotary vacuum evaporator. The dried extracts were dissolved in 5 mL 50% methanol. The extracts were stored at 4 °C. Extraction and all analysis were performed in duplicate.

### Determination of total phenolic content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu colorimetric method with minor modification [8]. Briefly, 200 µL of appropriately diluted crude free phenolic and bound phenolic extracts or standard solutions were added to 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent, and then neutralized with 1.5 mL saturated sodium carbonate (75 g/L). The absorbance was measured at 725 nm (Ultrospec 1100 pro, Biomicon Ltd., Cambridge, CB4 0FJ, England) after 2 h of reaction at room temperature in the dark. Gallic acid solution was prepared and used as standard at concentrations ranging from 25 to 150 mg/L. TPC was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of bean flour. All tests were done in duplicate.

### Determination of oxygen radical absorbance capacity

The extraction of antioxidant compounds from bean samples were done as described by Limomon et al. [9], with slight modifications. To 0.500 g of sample, 5 mL of acidified methanol (HCl : methanol : water, 1 : 80 : 20, V/V/V) was added in 10 mL centrifuge tubes and shaken at room temperature for 2 h using a horizontal rotary shaker (RKVSD, ART Inc., Laurel, MD, USA). The mixture was then centrifuged at 5 °C for 10 min at 10000 rpm (Sorvall RC-6 Plus Centrifuge, Thermo Fisher Scientific Inc., Asheville, NC, USA) and the supernatant collected and used for the oxygen radical absorbance capacity (ORAC). The assay was conducted using a previously described method [10]. Briefly, the peroxy radical was generated by 2,2'-azobis[2-methylpropionamide] dihydrochloride (AAPH), and fluorescein was used as the substrate. The plate-to-plate transfer of fluorescence working solution, buffer solution (blank), Trolox (standard), appropriately diluted samples, and AAPH were carried out by Precision 2000 automated microplate pipetting system (BIO-TEK Instruments, Inc., Winooski, VT). The fluorescence was monitored by FL\_800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) controlled by software KC4 3.0 (version 29) with an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm every minute. The total reaction time was 50 min. A regression equation was obtained from a plot of Trolox concentrations versus net area under the fluorescence decay curve (AUC). This was used to calculate ORAC values which were expressed as Trolox equivalents.

## HPLC for analysis of phenolic compounds

HPLC assay was conducted as described by Yu et al. [11] with some modifications. The chromatographic separation was carried out on an HPLC (Waters 2695) equipped with a photodiode array detector (Waters 2996). The analytical column was a 4.6×150 mm, Gemini 5 µm C18 110A column (Phenomenex, Torrance, California, USA). The mobile phase consisted of A (0.1% acetic acid in water) and B (0.1% acetic acid in methanol). The conditions were set as follows: 35 °C column temperature, 0.9 mL/min flow rate, 20 µL injection volume. A 70 min gradient was programmed as follows: 0-11 min, 9-14% B; 11-14 min, 14-15% B; 14-17 min, 15% B; 17-24 min, 15-16.5% B; 24-28 min, 16.5-19% B; 28-30 min, 19-25% B; 30-36 min, 25-26% B; 36-38 min, 26-28% B; 38-41 min, 28-35% B; 41-46 min, 35-40% B; 46-48 min, 40-48% B; 48-53 min, 48-53% B; 53-65 min, 53-70% B; 65-66 min, 70-9% B; 66-70 min, 9% B. The phenolic acids were detected at a wavelength of 280 nm. Phenolic compounds were identified by comparing retention times with those of their respective standards. The contents of phenolic compounds were quantified using external calibration curves.

## HPLC-MS for analysis of anthocyanins

HPLC-MS system was the same model as used above for phenolic acid analyses. However, a Symmetry C18 3.5µm column (4.6×75 mm) was used to separate the anthocyanins. The mobile phase consisted of A (0.5% acetic acid in water) and B (100% methanol), and the flow rate was 0.6 mL/min. The gradient was as follows: 0-2 min, 5% B, 2-40 min, 5-25% B, 40-50 min, 25-5% B. The anthocyanins were detected at a wavelength of 520 nm. The Q-TOF-MS was calibrated by using sodium iodide for positive mode through the mass range of 100-1000. MS parameters were set as follows: capillary voltage: 2100 V, sample cone voltage: 35 V, source temperature: 120 °C, desolvation temperature: 250 °C, desolvation gas (nitrogen gas) flow rate: 900 L/h.

## Statistical analysis

The results were presented as mean ± standard deviation of duplicated determinations. The data were analyzed by ANOVA using SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Duncan's multiple range test was used to evaluate significant difference among means. Pearson correlation test was used to evaluate the correlation among variables. Significance level was defined at  $p < 0.05$ .

## Results and Discussion

### Proximate composition of bean varieties

Results of proximate analysis are presented in Table 1. Dimeta bean had the highest (9.66 g/100g) moisture followed by Napirira bean (9.00 g/100g), and Nanyati bean had the lowest (8.58 g/100g). Nanyati bean had significantly higher ash and total carbohydrate content (3.77 g/100g and 67.14 g/100g, respectively) than the other two bean varieties. The highest fat content (2.99 g/100g) was detected in Dimeta bean; however, there was no significant difference between Napirira and Nanyati beans. Napirira bean had significantly higher protein content (21.32 g/100g) than Nanyati bean, whereas there was

no significant difference between Dimeta and Napirira beans. Prolla et al. [12] reported the nutritional quality of common beans from sixteen bean cultivars. The moisture, ash, crude fat and crude protein ranged from 12.21-13.91, 2.67-4.54, 22.50-27.96 and 0.92-4.43 g/100g, respectively. Similar results were found with the Malawian bean samples. The findings imply that the three bean varieties from Malawian have the same nutritional quality as common beans from other areas. However, the moisture was low. Moisture content is important to control post-harvest as it affects yield and most of the grain properties [13].

**Table 1:** Proximate composition, phytochemicals and antioxidant capacity of common beans.

Variable	Bean varieties		
	Dimeta	Napirira	Nanyati
Moisture (g/100g)	9.66 ± 0.03a	9.00 ± 0.09b	8.58 ± 0.11c
Ash (g/100g)	3.57 ± 0.03b	3.61 ± 0.01b	3.77 ± 0.00a
Crude fat (g/100g)	2.99 ± 0.16a	1.80 ± 0.04b	1.70 ± 0.12b
Crude protein (g/100g)	19.43 ± 0.58ab	21.32 ± 1.50a	18.82 ± 0.73b
Carbohydrate (g/100g)	64.35 ± 0.36b	64.28 ± 1.61b	67.14 ± 0.73a
Total anthocyanins (µg/g)	29.73 ± 0.08c	99.06 ± 1.39a	36.63 ± 1.84b
Total carotenoids (µg/g)	0.28 ± 0.00b	0.39 ± 0.01a	0.19 ± 0.00c
Total flavonoids (mg/g)	5.20 ± 0.21b	5.56 ± 0.19a	2.92 ± 0.01c
Free phenolic (mg/g)	1.28 ± 0.01a	1.20 ± 0.05a	0.79 ± 0.02b
Bound phenolic (mg/g)	0.65 ± 0.04a	0.62 ± 0.01a	0.50 ± 0.02b
ORAC (µmol/g)	24.13 ± 0.10b	29.45 ± 0.57a	21.23 ± 0.27c

Values in each line with different letters are significantly different ( $p < 0.05$ ), the same as follow.

### Phytochemicals and antioxidant capacity in bean varieties

Common beans are rich in antioxidants, which may include a variety of flavonoids such as anthocyanins, flavonols, proanthocyanidins, tannins, glycosides as well as a wide range of phenolic acids [3]. The phytochemicals and antioxidant capacity are shown in Table 1. Napirira had a significantly higher content (99.06 µg/g) of total anthocyanins than Nanyati (36.63 µg/g). Dimeta had the lowest total anthocyanin content (29.73 µg/g) among the beans. Previous studies indicated that the total anthocyanin content in bean coats ranged from 0.20 to 1.29 mg/g [14] and 0.13 to 2.10 mg/g [15]; however, there are no reports on the total anthocyanin content of whole beans. The results illustrated that the anthocyanins are primarily concentrated on the bean coats and therefore, the bean coats should be included during consumption of the beans. The highest content of total carotenoids and total flavonoids were detected in Napirira (0.39 µg/g and 5.56 µg/g) followed by Dimeta (0.28 µg/g and 5.20 µg/g), and the lowest was found in Nanyati beans (0.19 µg/g and 2.92 µg/g). Dimeta and Napirira had significantly higher free and bound phenolic compounds than Nanyati; however, there was no significant difference between Dimeta and Napirira beans. The total phenolic content in cranberry beans ranged between 0.67 to 4.15 µg/g [16]; however, there are no reports on free

and bound phenolic content of common beans. Napirira had the highest (29.45  $\mu\text{mol/g}$ ) ORAC value followed by Dimeta (24.13  $\mu\text{mol/g}$ ). Nanyati bean had the lowest (21.23  $\mu\text{mol/g}$ ). Similar results were also found in black bean [17] which suggested that the three bean varieties are likely good sources of food antioxidant components.

### Phenolic compounds in bean varieties

The phenolic compounds existing in beans can be classified as free, free/conjugated and bound. Since very small peaks were found in bound fractions (data not shown), only free phenolic compounds were detected and quantified in bean varieties (Table 2). A total of six phenolic compounds were found in these bean varieties. Dimeta bean had a highest content (36.68  $\mu\text{g/g}$ ) of protocatechuic acid and Napirira had the lowest. The highest content of p-hydroxybenzoic acid was found in Napirira bean. There was no significant difference between Dimeta and Nanyati beans. Dimeta and Napirira beans had higher content of p-coumaric acid than Nanyati bean. Napirira bean had the highest content of ferulic acid and Dimeta had the lowest. The highest content of sinapinic acid was also found in Napirira bean. Protocatechuic acid was the most predominant followed by p-coumaric acid and ferulic acid. This contrasted with previous reports. Gallic, chlorogenic and caffeic acid were found to be the major phenolic compounds in black bean and brown bean [18]. Meanwhile, the catechin, a flavanol was only detected in Napirira bean. Catechin and p-coumaric acid were the major phenolic compounds in cranberry beans [16]. The differences might possibly be attributed to different bean varieties, as well as environment growth conditions.

**Table 2:** Phenolic compounds in free phenolic extract of common beans.

Phenolic compounds	Bean varieties		
	Dimeta	Napirira	Nanyati
Protocatechuic acid ( $\mu\text{g/g}$ )	36.86 $\pm$ 0.08a	35.71 $\pm$ 0.10c	35.97 $\pm$ 0.04b
p-Hydroxybenzoic acid ( $\mu\text{g/g}$ )	2.00 $\pm$ 0.11b	15.91 $\pm$ 0.21a	1.98 $\pm$ 0.05b
Catechin ( $\mu\text{g/g}$ )	ND	25.18 $\pm$ 0.48a	ND
p-Coumaric acid ( $\mu\text{g/g}$ )	16.53 $\pm$ 0.08a	16.44 $\pm$ 0.01a	16.17 $\pm$ 0.09b
Ferulic acid ( $\mu\text{g/g}$ )	18.74 $\pm$ 0.07c	20.64 $\pm$ 0.02a	20.40 $\pm$ 0.08b
Sinapinic acid ( $\mu\text{g/g}$ )	3.01 $\pm$ 0.03b	3.05 $\pm$ 0.01a	2.86 $\pm$ 0.01c

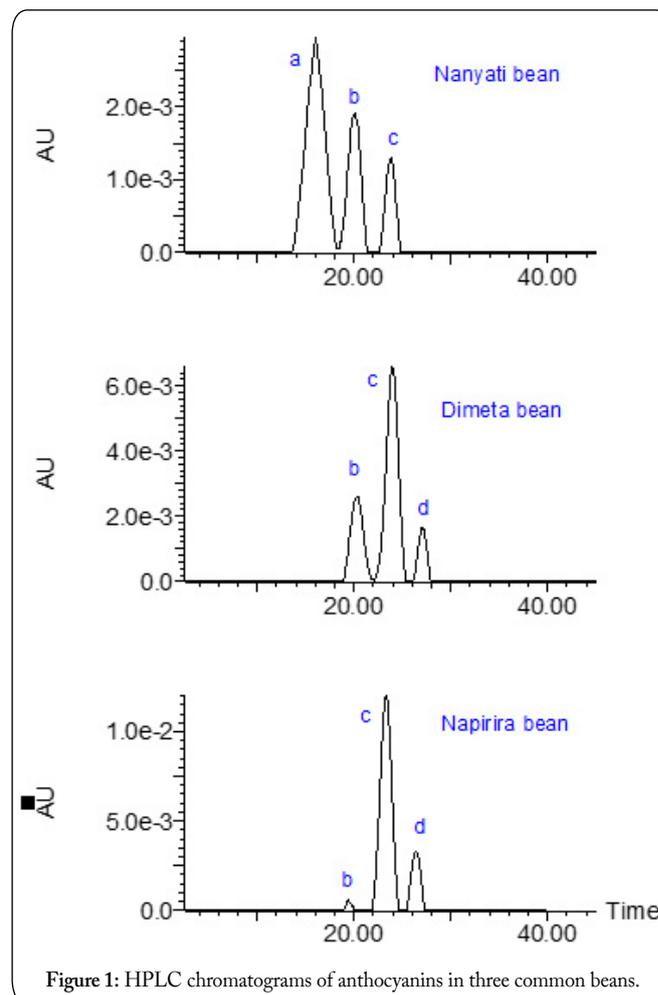
ND = not detected.

**Table 3:** Retention time and UV/Vis and mass spectra of anthocyanin in common beans.

Peak	Anthocyanin	Retention time (min)	[M+H] <sup>+</sup>	Major fragment	$\lambda_{\text{max}}$ (nm)
a	Delphinidin 3-O-glucoside	16.02	465	303	278, 524
b	Cyanidin 3-O-glucoside	20.08	449	287	278, 516
c	Pelargonidin 3-O-glucoside	23.82	433	271	274, 504
d	Pelargonidin derivative	27.08	565	271, 473	278, 510

### Anthocyanins in bean varieties

There is a large variety of anthocyanins found in nature, but only five aglycones or Anthocyanidins, namely pelargonidin, cyanidin, malvidin, petunidin, and delphinidin are present in common bean seed. The profiles of anthocyanins were determined by HPLC/MS. The chromatographic analysis of the purified extract registered at 520 nm, shows the existence of four principal anthocyanins (Figure 1). Peak a presented an [M + H]<sup>+</sup> ion at m/z 465 and a major fragment at m/z 303 (loss of a hexose). Peak b presented an [M+H]<sup>+</sup> ion at m/z 449 and a major fragment at m/z 287 (loss of a hexose). Peak c presented an [M+H]<sup>+</sup> ion at m/z 433 and a major fragment at m/z 271 (loss of a hexose). Peak d presented an [M+H]<sup>+</sup> ion at m/z 565 and two fragments at m/z 271 and 473 (Figure 2 and Table 3). As previously reported [5, 19], these four anthocyanins were identified as delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, pelargonidin 3-O-glucoside and pelargonidin derivative. However, the bean varieties affected the anthocyanin composition. Delphinidin 3-O-glucoside was only detected in Nanyati, while a new pelargonidin derivative was found in both Dimeta and Napirira beans. Delphinidin glucoside, petunidin glucoside and malvidin glucoside were found as the major anthocyanins in fifteen common beans from Mexico and Brazil [5]. Two anthocyanin compounds, peonidin-3-rutinoside and malvidin-3-O-glucoside were recently identified for the first time in the adzuki bean [20]. However, the content of each anthocyanin in the three bean



**Figure 1:** HPLC chromatograms of anthocyanins in three common beans.

varieties should be determined in future research.

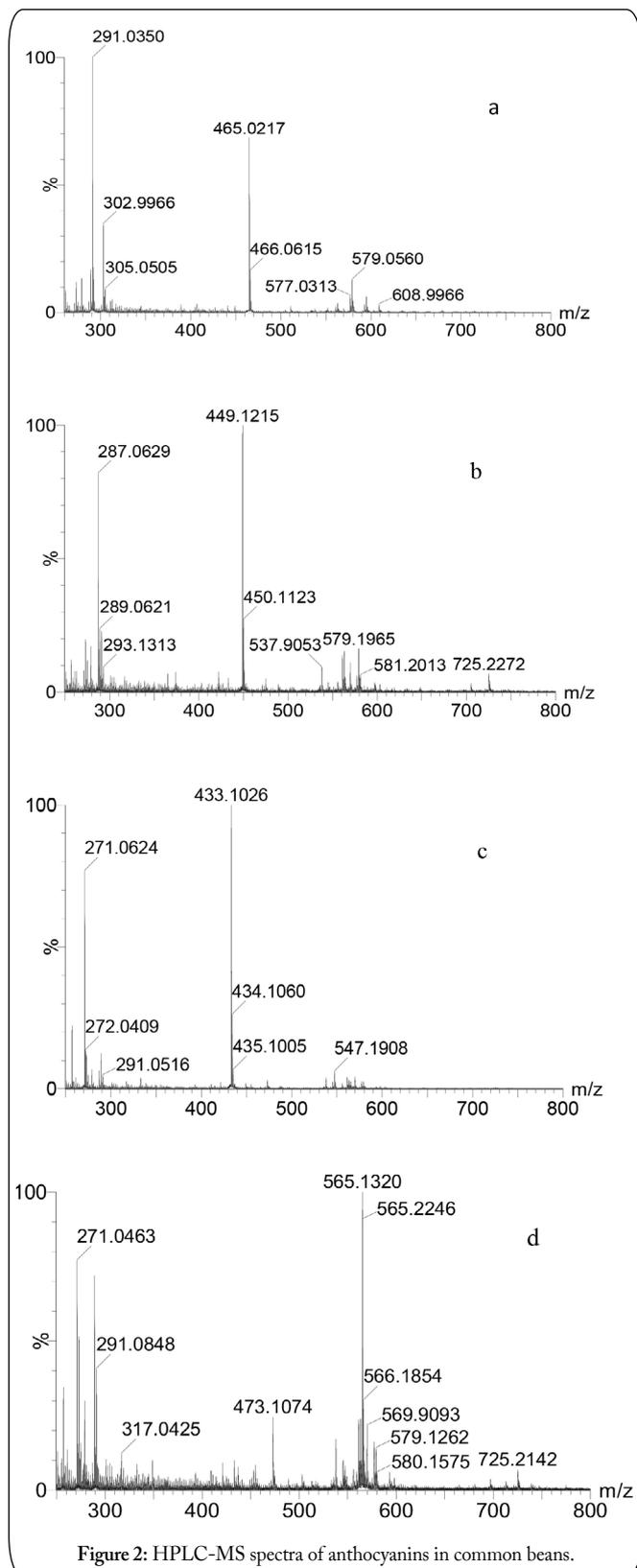


Figure 2: HPLC-MS spectra of anthocyanins in common beans.

## Conclusions

In conclusion, significant differences ( $p < 0.05$ ) in phytochemicals, nutritional and antioxidant properties were found in these three bean varieties. Napirira bean had

the highest content of all phytochemicals as well as high ORAC values, an indication that this bean variety is the most promising in terms of functional potential. This study also has demonstrated that even though proximate composition and phenolic profiles varied among varieties, these factors did not affect the potential health benefits associated with antioxidant capacity of beans. Bean varieties and their phenolic compounds are good natural sources of antioxidants.

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## Conflict of Interest

The authors declare no conflict of interest.

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