



Research Article

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Phenolic Compound, Antioxidant Activity and Nutritional Components of Five Legume Seed

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Abstract

Legume is a major source of antioxidant and nutritional component that is very helpful for human health. In recent years, the functional properties of legume seed have received attention, particularly with respect to antioxidant, antitumor, anti-diabetic and anti-cholesterol effects. The objective of this study was to investigate the phenolic compound, flavonoid, antioxidant capacity and nutritional profiles of five legume seeds. The phenolic content determined according to the Folin Ciocalteu method, for five legume samples varied from 13.68 to 35.5 mg Tannic acid equivalent/g of extract. Flavonoid content was measured using 2% aluminum chloride varied from 1.21 to 4.81 mg quercetin equivalent/g of extract. Red bean contained maximum phenolic (35.5 mg/g extract) and flavonoid (4.81 mg/g extract) content that was statistically different from other legume seed ($p < 0.05$). Antioxidant activities were comparatively assessed by ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical decolorization assay method. Red bean, soybean and black bean showed good free radical scavenging capacity by ABTS and DPPH. Red bean contained highest amount of protein (262.73 ± 8.87 mg/g), free amino acid content (27.19 ± 0.40 mg/g) and reducing sugars (35.78 ± 0.76 mg/g) whereas black bean contained highest amount of total soluble sugar content (570.52 ± 5.44 mg/g). Obtained results suggest that red bean, soybean and black bean can be regarded as promising candidates for natural plant sources of antioxidants with high nutritional value.

Keywords: Phenolic compound; Antioxidant; Nutrition; Legume seed

Introduction

The term legumes refer to the plants whose fruit is enclosed in a pod. A legume is a plant in the family Leguminosae (or Fabaceae). The Leguminosae contain more than 700 genera and 25,000 species, yet fewer than 20 of these are grown as major world crops. Grain legumes 'the poor man's meat' are widely recognized as important sources of food and feed proteins. Being an important plant source of protein, legumes provide vital building blocks in the growth and development.

Recent studies conducted on potential health benefits of legume due to the presence of some bioactive phenolic constituents. These bioactive constituents are able to keep from reactive oxygen species (ROS), which are capable of some reactions causing many serious diseases [1]. Legume seed Phenolics are secondary metabolites involved in the defense mechanisms against microbial pathogens, various environmental stresses and insect herbivores [2]. These

bioactive constituents of grain legumes make them suitable for creating new functional foods [3]. Antioxidant activity of phenolic compounds present in edible grain legume seeds have been investigated in recent studies [4-6]. These components have played an important role in the treatment and avoid human diseases. A high intake of fruits, vegetables, whole grains, legumes (beans), nuts, and seeds is linked to significantly lower risks of heart disease, high blood pressure, stroke, and type 2 diabetes [7-8]. Experimental, epidemiological, and clinical studies show correlations between the consumption of food legumes and decreasing incidence of several diseases, such as cancer, cardiovascular diseases, obesity, and diabetes [9-11]. The antioxidant capacity [12] and the antimutagenic [13,14], apoptosis-related [15] and antiproliferative effects of legumes are associated with the presence of phenolic compounds [16,17].



The nutritional value of legume seeds is frequently less than ideal, however, because their proteins contain lower concentrations of certain "essential" amino acids than do animal proteins. The fact that "free" amino acids frequently constitute more than 10% of the weight of legume seeds is often overlooked when considering their nutritional value, as free amino acids tend to be lost in traditional methods of cooking.

The objective of this study was to investigate the phenolic compound, antioxidant activity and nutritional compound in five legume seed.

Materials Method

Plant Materials

Five legume seeds were selected for the study namely Soybean (*Glycine max*), Red adzuki bean (*Vigna angularis*), Black bean (*Phaseolus vulgaris L.*), Lentil (*Lens culinaris*) and Chickpea (*Cicer arietinum*) belonging to the same family Fabaceae. All seed sample were collected from South Korea seed market.

Chemicals and Other Materials

Tannic acid, Folin & Ciocalteu's phenol, 2,2'-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS), DPPH (1,1-diphenyl-2-picrylhydrazyl) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Quercetin, sulfuric acid, Sodium carbonate (Na_2CO_3), Sodium hydroxide, Rochelle salt, Sodium sulfate, Phenol, 3,5 Dinitro salicylic acid, Bovine serum albumin fraction V, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Potassium sodium tartrate tetrahydrate, sucrose, L-tyrosine, glucose (dextrose) were also from Sigma Aldrich (St. Louis, MO, USA).

Preparation of Extraction

Dried seed samples were ground with a laboratory grinder to make powder or flour and sieved with a 100-mesh sieve. One gram of sample was extracted by 10mL 70% ethanol for three hours shaking at 180 rpm, and then centrifuged 10 min on 3000 rpm. Extract sample filtered by syringe and preserved in refrigerator for chemical analysis.

Total Phenolic Compounds Content

Total phenolic content (TPC) of legume seed extract was determined by the reported method [18] with slight modification. Mixed 100 μL of each sample and 100 μL of Folin-Denis reagent firstly, after 3 minutes, added 1 mL of 0.7 M Sodium carbonate in the mixture and then reacted at room temperature for 1 h. Absorbance of sample was measured at 750 nm. Total phenolic compounds in the legume seed extracts were determined using an equation obtained from a standard curve of tannic acid (0–500 $\mu\text{g}/\text{mL}$, $Y = 0.0024x - 0.0071$, $R^2 = 0.9993$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis.

The results are expressed as mg Tannic acid equivalents (TA eq) per g of extract of legume seeds. All determinations were carried out in triplicate.

Estimation of Flavonoid

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample [19-22]. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 10 mg quercetin in 1.0 mL methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (0–500 $\mu\text{g}/\text{mL}$). An amount of 100 μL diluted standard quercetin solutions and all sample extracts was separately mixed with 500 μL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with spectrophotometer. The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0039x + 0.0079$, $R^2 = 0.9915$) and expressed as mg quercetin equivalent (QE)/g of extract legume seeds. All the determinations were carried out in triplicate.

Free Radical-Scavenging Ability by the use of ABTS Radical

Antioxidant activity was determined by the ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) free radical decolorization assay method developed by Re [23]. The ABTS positive (+) radical cation was progenerated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16 h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the ABTS. + solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature, then 100 μL was mixed with 50 μL of the test sample and the absorbance was measured at 734 nm after 6 min. All experiments were repeated three times. The radical scavenging activities of legume sample were calculated by the following equation:

$$\text{ABTS radical scavenging or inhibition activity(\%)} = \left(1 - \frac{\text{sample absorbance}}{\text{control absorbance}} \right) \times 100$$

Free Radical-Scavenging Ability by the use of DPPH Radical

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was determined using the method described by Brand-Williams [24]. DPPH reagent was pre-prepared by dissolving in 99.9% ethanol. After DPPH reacts with antioxidants, the color would turn purple into a light-yellow color of diphenylpicrylhydrazine. In this study, 50 μL of DPPH solution was reacted with 100 μL of samples at different concentrations. A control (Abs Control)

containing methanol and DPPH solution was also realized. The blank control was treated with sample diluting and DPPH reagent diluting solvent. All solutions obtained were then incubated for 10 minutes at room temperature. Ascorbic acid used as standard. Absorbances were measured at 517 nm. The radical scavenging capacity using the free DPPH radical was evaluated by measuring the decrease of absorbance at 517 nm. When the reading was complete, then the radical scavenging activities of samples were calculated as following equation:

$$\text{DPPH radical scavenging or inhibition activity(\%)} = \left(1 - \frac{\text{sample absorbance}}{\text{control absorbance}}\right) \times 100$$

Then, curves were constructed by plotting percentage of inhibition against concentration in $\mu\text{g/mL}$. The equation of this curve allowed to calculate the IC₅₀ corresponding to the sample concentration that reduced the initial ABTS and DPPH absorbance of 50 %. A smaller IC₅₀ value corresponds to a higher antioxidant activity. All test analyses were realized in triplicate.

Determination of Protein Contents

The protein content was measured by Lowry Assay [25] with minor modification. For protein content determination, Bovine serum albumin (BSA) was used to make the standard calibration curve. Stock Bovine serum albumin (BSA) solution was prepared by dissolving 10 mg BSA powder in 1.0 mL distilled water, then the standard solutions of BSA were prepared by serial dilutions using distilled water (0–500 $\mu\text{g/mL}$). An amount of 100 μL diluted standard BSA solutions and all sample extracts was separately mixed with 1mL Lowry reagent. Lowry reagent was prepared by mixing 0.5 ml of 1% cupric sulfate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2% sodium carbonate in 0.1 N NaOH. Each sample reacted with Lowry reagent for 10 minutes then react with Folin's reagent. Color was allowed to develop for 30 minutes at room temperature and the absorbance measured at 750 nm and blanked on the water only control. Protein content in the legume seed extracts were determined using an equation obtained from a standard curve of BSA (0–500 $\mu\text{g/mL}$, $Y = 0.0004x + 0.0135$, $R^2 = 0.999$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg BSA equivalents per g of extract or per 100 g of seeds. All determinations were carried out in triplicate.

Detection of Free Amino Acid Contents

Free amino acid content of legume seed was detected by the method described by Setsuro Matsushita [26] with minor modification. For free amino acid content determination, L-tyrosine was used to make the standard calibration curve. Stock L-tyrosine solution was prepared by dissolving 10 mg L-tyrosine powder in 400 μL , 1N HCl then add 600 μL distilled water. Standard solutions of L-tyrosine were prepared by serial dilutions using distilled water (0–500 $\mu\text{g/mL}$). An amount of 100 μL diluted standard L-tyrosine

solutions and all sample extracts was separately mixed with 1ml of 0.55M Na_2CO_3 for 5 minutes then react with 100 μL of 2N Folin & Ciocalteu's phenol reagent. Color was allowed to develop for 30 minutes at room temperature and the absorbance measured at 750 nm and blanked on the water only control. Free amino acid content in the legume seed extracts were determined using an equation obtained from a standard curve of L-tyrosine (0–500 $\mu\text{g/mL}$, $Y = 0.0019x + 0.0516$, $R^2 = 0.99989$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg L-tyrosine equivalents per g of extract or per 100 g of seeds. All determinations were carried out in triplicate.

Determination of Total Sugar

Total sugar content was determined by the phenol-sulfuric acid method described by DuBois [27] with minor modification. For total sugar content determination, Sucrose was used to make the standard calibration curve. Stock sucrose solution was prepared by dissolving 10 mg sucrose powder in 1mL distilled water. Standard solutions of sucrose were prepared by serial dilutions using distilled water (0–500 $\mu\text{g/mL}$). An amount of 100 μL diluted standard sucrose solutions and all sample extracts was separately mixed with 100 μL of 5% phenol solution. Then 500 μL of sulfuric acid (highly concentrated) was added in this mixture. This mixture needed 10 minutes for reaction in boiling water and then cooled in ice bath for another 15 minutes. Finally, the absorbance of each sample was measured at 490 nm and blanked on the water only control. Total sugar content in the legume seed extracts were determined using an equation obtained from a standard curve of Sucrose (0–500 $\mu\text{g/mL}$, $Y = 0.0027x + 0.0585$, $R^2 = 0.9998$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg Sucrose equivalents per g of extract or per 100 g of seeds. All determinations were carried out in triplicate.

Determination of Reducing Sugar

Reducing sugars were measured by the method of Somogyi & Nelson [28,29] with minor modification. For reducing sugar content determination, Glucose (Dextrose) was used to make the standard calibration curve. Stock glucose solution was prepared by dissolving 10 mg glucose in 1mL distilled water. Standard solutions of glucose were prepared by serial dilutions using distilled water (0–500 $\mu\text{g/mL}$). An amount of 100 μL diluted standard glucose solutions and all sample extracts was separately mixed with 300 μL of DNS reagent. DNS reagent was prepared by mixing 1% Sodium hydroxide, 20% Rochelle salt, 0.05 % Sodium sulfate, 1% 3,5 Dinitro salicylic acid with 0.2ml Phenol. DNS reagent mixing all sample put in water bath at 95°C for 5 minutes for reaction and then cooled in ice bath for another 5 minutes. Finally, the absorbance of each sample was measured at 540 nm and blanked on the water only control. Reducing sugar content in the legume seed extracts were

determined using an equation obtained from a standard curve of glucose (0–500 µg/mL, $Y = 0.0021x + 0.0605$, $R^2 = 0.9920$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg Glucose equivalents per g of extract or per 100 g of seeds. All determinations were carried out in triplicate.

Statistical Analysis

All the experiments for determination of total phenolics, total

flavonoids, and antioxidant properties using ABTS and DPPH and all nutritional value were conducted in triplicates. The values are expressed as the mean ± standard deviation (SD). The statistical analysis of the results was done by using R-statistical software package version 2.2.1 [30]. Analysis of variance and significance of difference among means were tested by one-way ANOVA and least significant difference (LSD) on mean values. Correlation coefficients (r) and coefficients of determination (r^2) were calculated using Microsoft Excel 2010 (Figure 1).

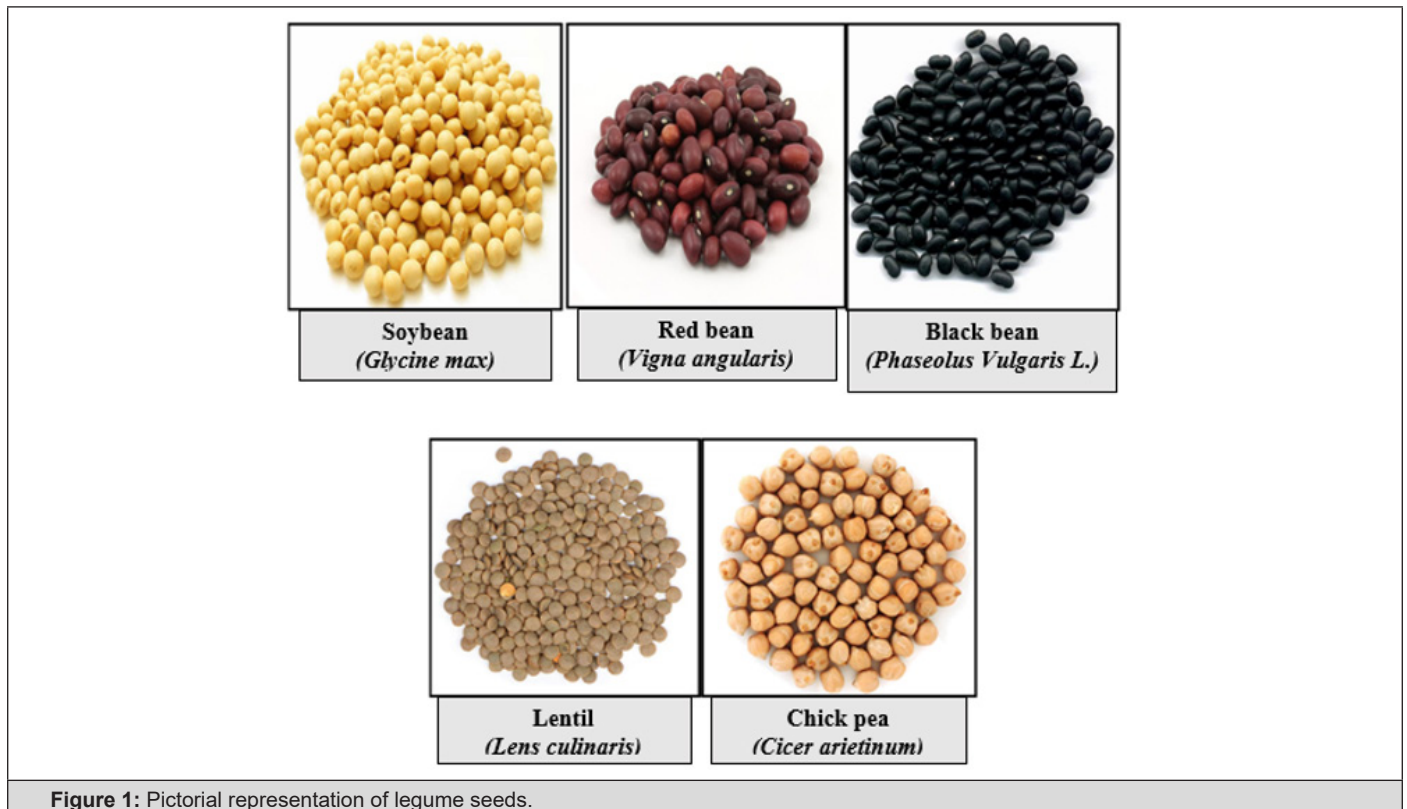


Figure 1: Pictorial representation of legume seeds.

Results and Discussion

The extraction yield of five legume seeds were shown in Table 1. Maximum extraction yield was observed in soybean (11.15%) then in chickpea (9.44 %) that was statistically similar with black bean (8.98%) and minimum was in red bean (5.30%). Hakime Hulya et al. [31] reported that the extraction yield of Mungbam was 10.70%.

Table 1: Ethanol Extraction yield of five legume seeds.

Name of sample	Extraction yield (%)
Soybean	11.15 ± 0.18 ^a
Red bean	5.30 ± 0.29 ^d
Black Bean	8.98 ± 0.31 ^b
Lentil	7.59 ± 0.87 ^c
Chickpea	9.44 ± 0.51 ^b

Data are reported as the mean ± standard deviation (n=3). Means with superscript different letters are differing significantly (P<0.05).

Phenolic compounds are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity and are likely that the activity of these extracts is due to this phenolic compound [32,33]. The result of phenolic content of legume seeds was presented in Table 2. The results showed that Red bean contain maximum phenolics (35.50 mg/g extract) content that was statistically different from other legume seed (p<0.05), while chickpea showed the minimum phenolic content (13.68 mg/g extract). Although the phenolic content of soybean and black bean were statically similar. Amarowicz & Troszynska [8] reported that red bean contained 55 mg /g extract phenolic content. Tijana M. Djordjevic et al. [34] described that Polyphenol content of lentil, red bean, soybean, mung bean was 21.9, 18.8, 18.7 and 17 mg/g extract respectively. Sucheta Sharma et al. [35] also reported that polyphenol content of soybean is 1.2 ± 0.20 mg/g.

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used

in plants to produce yellow and other pigments which play an important role in the color development. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities [36]. The result of flavonoid content of legume seeds were also presented in Table 2. The results showed that Red bean contain maximum flavonoid content (4.81 mg/g extract) that was statistically different from other legume seed ($p < 0.05$) followed by the soybean (2.77 mg/g extract). The lowest flavonoid content was observed in chick pea (1.21 mg/g extract). Flavonoid content of black bean (2.11 mg/g extract) and lentil (1.99 mg/g extract) were statistically similar (Table 2). Flavonoid content in 2010 varied from 0.433 to 0.659, and in 2011 from 0.428 to 0.580 mg/g of dry weight of soybean seed [37].

Table 2: Polyphenol and Flavonoid content of five legume seed.

Sample Name	Content (mg/ extract 1 gm)	
	Polyphenol	Flavonoid
Soybean	23.01 ± 0.84 ^b	2.77 ± 0.27 ^b
Red bean	35.50 ± 1.72 ^a	4.81 ± 0.39 ^a
Black Bean	23.36 ± 0.51 ^b	2.11 ± 0.30 ^c
Lentil	18.89 ± 0.38 ^c	1.99 ± 0.46 ^c
Chickpea	13.68 ± 1.66 ^d	1.21 ± 0.14 ^d

Data are reported as the mean ± standard deviation (n=3). Means with superscript different letters are differing significantly ($P < 0.05$).

Table 3: Radical scavenging activity of five legume seed by using ABTS and DPPH radical.

Sample Name	Radical Scavenging Activity IC ₅₀ (µg/mL)	
	ABTS	DPPH
Soybean	1132.65	7056.34
Red bean	375.64	500.29
Black Bean	1125.3	6123.22
Lentil	1302.64	10041.3
Chickpea	1335.05	N

IC₅₀: Inhibition concentration for 50% reduction of ABTS and DPPH radical.

Consumption of foods containing antioxidant phytoconstituents is beneficial to human health since they can protect the human body from detrimental free radicals and inhibit the progress of many chronic disease [38]. Previous studies by Schlesier et al. [39] showed that when analyzing the antioxidant activity, it is preferable to use at least two methods. In their experiments, the analysis of the antioxidant activity of legume seed was performed using two methods: ABTS and DPPH. The ABTS and DPPH radical is widely used to evaluate the free-radical scavenging capacity of antioxidants according to their hydrogen donating ability [40]. In addition to that, reactions involved in these methods are fully unaffected by side reactions [41]. These methods are distinguished

by their mechanism of action and would be complementary to the study of the antioxidant potential of legume seed. The antioxidant proprieties of extracts were measured in terms of their efficient IC₅₀ concentration corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%. These IC₅₀ values for ABTS and DPPH are given in Table 3.

In this study, it was investigated that the five legume extracts had different antioxidant activity levels that the IC₅₀ values for the test samples lie in the range between 375.64 to 1335.05 µg/ml for ABTS and 500.29 to 10041.30 µg/ml for DPPH (Table 3). Among the sample, red ben showed good free radical scavenging capacity by ABTS (IC₅₀ 375.64 µg/ml) and by DPPH (IC₅₀ 500.29 µg/ml). Radical scavenging activity of soybean and black bean was more or less similar. Red bean, soybean and black bean showed a stronger scavenging activity than another legume. Red bean, soybean and black bean might be a potential material for antioxidants. Yildirim et al. [42] reported that some potent antioxidants applied in food might cause a serious of side effects. Therefore, it is important to find more natural antioxidative ingredients for food industries. Tijana M. Djordjevic et al. [34] described that DPPH of lentil, red bean, soybean, mung bean was 143.7, 138, >200 and 152.3 µg/ml. The deviation in results can be due to the difference in extraction technique and assay method.

The different nutritional parameters were found in varying concentrations in all samples taken in present studies. Proteins represent the major storage compound which is a common trait of most legume seeds. Red bean seed is a rich source of protein content. As compared to different legume seed samples, protein content was highest in red bean seeds (262.73 ± 8.87 mg/g) whereas lowest in chickpea seeds (71.75 ± 4.34 mg/g). Protein content of soybean was 153.25 mg/g which was statistically similar with black bean protein content (145.33mg/g) (Table 4). Sucheta Sharma et al. [35] described that protein content of soybean is 41.4 ± 1.82 %. Amresh Kumar et al. [43] reported that protein content of faba bean is with the range of 20–32 %.

Amino acid is the main components in forming protein. Amino acids content of red bean is much higher than other legume seed. The free amino acid content of red bean was 27.19 ± 0.40 mg/g which was significantly different from other legume seed. Soybean showed the lowest amount of free amino acid (4.09 ± 1.09 mg/g) (Table 4). Although free amino acids have little effect on the nutritional value of the seeds [44], but as seeds mature, these stored free amino acids are converted to storage proteins and/or non protein constituents. Amresh Kumar et al. [43] reported that free amino acid content of faba bean is with the range of 188–348 mg/ 100 g. Sucheta Sharma et al. [35] described that free amino acid content of soybean is 0.55 ± 0.17 %.

Table 4: Nutritional profiles (protein, free amino acid, total sugar and reducing sugar content) of five legumes.

Sample Name	Contents (mg/g)			
	Protein	Free amino acid	Total Sugar	Reducing Sugar
Soybean	153.25 ± 2.4 ^b	4.09 ± 1.09 ^d	513 ± 3.58 ^b	30.49 ± 1.53 ^b
Red bean	262.73 ± 8.87 ^a	27.19 ± 0.40 ^a	346.70 ± 8.64 ^d	35.78 ± .76 ^a
Black Bean	145.33 ± 8.33 ^b	9.09 ± 1.77 ^b	570.52 ± 5.44 ^a	28.95 ± 0.07 ^b
Lentil	98.67 ± 6.93 ^c	6.26 ± 0.42 ^c	345.65 ± 6.76 ^d	31.24 ± 1.94 ^b
Chickpea	71.75 ± 4.34 ^d	8.75 ± 0.57 ^b	367.31 ± 2.19 ^c	30.79 ± 2.16 ^b

Data are reported as the mean ± standard deviation (n=3). Means with superscript different letters are differ ing significantly (P<0.05).

The total soluble sugars were found to be highest in black bean seed (570.52 ± 5.44 mg/g) while the lowest in red bean seeds (346.70 ± 8.64 mg/g) which was statistically similar with total sugar content of lentil (345.65 ± 6.76 mg/g) (Table 4). Amresh Kumar et al. [43] reported that total sugar content of faba bean with the range of 0.80 to 1.90 %. Sucheta Sharma [35] described that total soluble sugar content of soybean is 6.7±0.69 %. Cowpea seed contain 22.31 ± 8.95 mg/g total soluble sugar content described by Maina Antoine N et al. [45].

With respect to reducing sugars, red bean (35.78 ± .76 mg/g) and black bean (28.95 ± 0.07 mg/g) were found to contain highest and lowest amount respectively though reducing sugar content of all legume seeds were statistically similar except red bean (Table 4). Amresh Kumar et al. [43] reported that reducing sugar content of faba bean with the range of 85–188 mg/100 g. Sucheta Sharma et al. [35] described that reducing sugar content of soybean is 0.27±0.04 %. Cowpea seed contain 9.11 ± 2.42 mg/g reducing sugar content described by Maina Antoine N et al. [45].

It is hypothesized that carbon from starch is used either for lipid synthesis or for protein synthesis especially in pulses seed where proteins are by far the major storage compounds. Amino acids needed for reserve protein synthesis. Although different soluble sugars were not determined separately in present study but major soluble sugars present in seeds are sucrose and raffinose family oligosaccharides (RFOs).

Conclusion

Among five legume seeds, red bean contains maximum phenolic and flavonoid component. On the other hand, red bean, soybean and black bean showed a stronger scavenging activity than another legume seed. Red bean, soybean and black bean might be a potential material for antioxidants. Red bean also contains maximum nutritional value such as protein, free amino acid and reducing sugar content in this experiment. On the basis of the results obtained in the present experiment, it is concluded that soybean, black bean and red bean have maximum capacity of antioxidant activity and nutritional value among five legume sample. Obtained results suggest that red bean, black bean and

soybean can be used as functional ingredient with high antioxidant activity. More research is needed on red bean, black bean and soybean to adequately know their bioactive component and their functionality, which is needed in order to be able to produce food products with maximum health benefits.

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Conflict of Interest

The authors have no conflicts of interest relevant to this study to disclose.

References

1. Amarowicz R, Weidner S (2009) Biological activity of grapevine phenolic compounds. In KA Roubelakis Angelakis (Eds.), Grapevine molecular physiology & biotechnology pp. 389-405.
2. Kumar L, Mahatma MK, Kalariya KA, Bishi SK, Mann A (2014) Plant Phenolics: Important Bio-Weapon against Pathogens and Insect. Herbivores. Popular Kheti 2(3): 149-152.
3. Aguilera Y, Estrella I, Benitez V, Esteban RM, Martín-Cabrejas MA (2011) Bioactive phenolic compounds and functional properties of dehydrated bean flours. Food Research International 44(3): 774-780.
4. Karamać M, Amarowicz R, Weidner S, Shahidi F (2004) Antioxidant activity of phenolic fractions of white bean (*Phaseolus vulgaris* L.). J. Food Lipids 11(2): 165-177.
5. Amarowicz R, Estrella I, Hernández T, Troszyńska A (2008) Antioxidant activity of extract of adzuki bean and its fractions. J. Food Lipids 15: 119-136.
6. Orak HH, Karamać M, Orak A, Amarowicz R (2016) Antioxidant potential and phenolic compounds of some widely consumed Turkish white bean (*Phaseolus vulgaris* L.) varieties. Polish Journal of Food and Nutrition Science 66: 253-260.
7. Hu FB (2003) Plant-based foods and prevention of cardiovascular disease: an overview. Am J Clin Nutr 78(3 suppl): 544S-551S.
8. Orlich MJ, Fraser GE (2014) Vegetarian diets in the Adventist Health Study 2: a review of initial published findings. Am J Clin Nutr 100 Suppl 1(1): 353S-358S.
9. Boudjou S, Oomah BD, Zaidi F, Hosseinian F (2013) Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. Food Chemistry 138(2-3): 1543– 1550.

10. Adebamowo CA, Cho EY, Sampson L, Katan MB, Spiegelman D, et al. (2005) Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *International Journal of Cancer* 114(4): 628-633.
11. Bhatena SJ, Velasquez MT (2002) Beneficial role of dietary phytoestrogens in obesity and diabetes. *The American Journal of Clinical Nutrition* 76(6): 1191-1201.
12. Heimler D, Vignolini P, Dini MG, Romani A (2005) Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. *Journal of Agricultural and Food Chemistry* 53(8): 3053-3056.
13. Cardador-Martínez A, Loarca-Piña G, Oomah BD (2002) Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* 50(24): 6975-6980.
14. De Mejía EG, Castano-Tostado E, Loarca-Piña G (1999) Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 441(1): 1-9.
15. Aparicio-Fernández X, Reynoso-Camacho R, Castaño-Tostado E, García-Gasca T, de Mejía EG, et al. (2008) Antiradical capacity and induction of apoptosis on HeLa cells by a *Phaseolus vulgaris* extract. *Plant Foods for Human Nutrition* 63(1): 35-40.
16. Segev A, Badani H, Kapulnik Y, Shomer I, Oren-Shamir M, et al. (2010) Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *Journal of Food Science* 75(2): S115-S119.
17. Dong M, He X, Liu R (2007) Phytochemicals of black bean seed coats: isolation, structure elucidation, and their antiproliferative and antioxidative activities. *Journal of Agricultural and Food Chemistry* 55(15): 6044-6051.
18. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin ciocalteu reagent. *Methods in Enzymology* 299: 152-178.
19. Marinova D, Ribarova F, Atanassova M (2005) Total phenolic and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy* 40(3): 255-260.
20. Chang CC, Ming HY, Hwei MW, Jiing CC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10(3): 178-182.
21. F Pourmorad, SJ Hosseinimehr, N Shahabimajd (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants." *African Journal of Biotechnology* 5(11): 1142-1145.
22. G Miliauskas, PR Venskutonis, T A van Beek (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts." *Food Chemistry* 85(2): 231-237.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Biol Med* 26(9-10): 1231-1237.
24. Brand-Williams W, Cuvelier ME, Berset CL (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology* 28(1): 25-30.
25. O H Lowry, N J Rosebrough, A L Farr, R J Randall (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193(1): 265-275.
26. Setsuro Matsushita, Nobuko Iwami, YukiNitta (1966) Colorimetric estimation of amino acids and peptides with the Folin phenol reagent. *Analytical Biochemistry* 16(2): 365-371.
27. DuBois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28(3): 350-356.
28. Somogyi M (1952) Notes on sugar determination. *Journal of Biological Chemistry* 195(1): 19-23.
29. Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* 153: 375-380.
30. R-Development-Core-Team, R: (2005) A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.
31. Hakime Hulya Orak, Magdalena Karamać, Adnan Orak, Ryszard Amarowicz, Michal Janiak (2018) Phenolics Content and Antioxidant Capacity of Mung Bean (*Vigna radiata* L.) Seed. *YYU J AGR SCI*, 28(özel sayı): 199-207.
32. Okudu T, Yoshida T, Hatano T (1994) Food phytochemicals for cancer prevention II. In Ho CT, et al. *Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs*. Washington, DC: American Chemical Society. pp. 132-143.
33. Tepe B, Sokmen M, Akpulat HA, Sokmen A (2006) Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem* 95(2): 200-204.
34. Tijana M Djordjevic, Slavica S Šiler-Marinkovic, Suzana I Dimitrijevic-Brankovic (2011) Antioxidant activity and total phenolic content in some cereals and legumes. *International Journal of Food Properties* 14: 175-184.
35. Sucheta Sharma, Manjot Kaur, Reeti Goyal, BS Gill (2014) Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. *J Food Sci Technol* 51(3): 551-557.
36. Crozier A, Ashihara H (2006) *Clifford MN. Plant Secondary Metabolites and the Human Diet*. Blackwell Publishing. Oxford. England.
37. Ana Josipović, Rezica Sudar, Aleksandra Sudarić, Vlatka Jurković, Maja Matoša Kočar, et al. (2016) Total phenolic and total flavonoid content variability of soybean genotypes in eastern Croatia. *Croat. J. Food Sci. Technol* 8(2): 60-65.
38. Khan Hamayun, Syed Aleem Jan, Mehwish Javed, Rabia Shaheen, Zahid Khan, et al. (2016) Nutritional composition, antioxidant and antimicrobial activities of selected wild edible plants. *J. Food Biochem* 40(1): 61-70.
39. Schlesier K, Harwat M, Böhm V, Bitsch R (2002) Assessment of Antioxidant Activity By Using Different In Vitro Methods. *Free rad Res* 36(2): 177-187.
40. Anna Maria Nuutila, RiittaPuupponen-Pimiä, Marjukka Aarni, Kirsi-MarjaOksman-Caldentey, et al. (2003) Comparasion of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity, *Food Chem* 81(4): 485-493.
41. Livani F, Ghorbanli M, Sateeyi A (2013) Changes in antioxidant activity and control of phenolic compounds during the ripening process of elm-leaved blackberry fruit. *Int J Agron Plant Prod* 4(1): 88-93.
42. Yildirim A, Oktay M, Bilaloglu V (2001) The antioxidant activity of the leaves of *Cydonia vulgaris*. *Turkish Journal of Medical Sciences* 31: 23-27.
43. Amresh Kumar, Nidhi, N Prasad, Subodh Kumar Sinha (2015) Nutritional and antinutritional attributes of faba bean (*Vicia faba* L.) germplasms growing in Bihar, India. *Physiol Mol Biol Plants* 21(1):159-162.
44. Kaldy M S, Kasting R (1974) Amino acid composition and protein quality of eight faba bean cultivars. *Can J Plant Sci* 54: 869-871.
45. Maina AntoineNassourou, Tchiagam Jean Baptiste Noubissiéa, Yanou Nicolas Njintang, Joseph Martin Bell (2017) Diallel analyses of soluble sugar content in cowpea (*Vigna unguiculata* L. Walp.). *The crop Journal* 5(6): 553-559.