

Phenolic Compound Profiles of Two Common Beans Consumed by Rwandans

Owino Joseph^{1*}, Mukashyaka Phelomene¹, Ndayisaba Helene¹, Habimana Valens¹, Ongol Martin Patrick², Dil Thavarajah³, Pushparajah Thavarajah³

¹Department of Applied Chemistry, College of Science and Technology, University of Rwanda, Kigali, Rwanda

²School of Food Science and Technology, College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda, Kigali, Rwanda

³School of Food Systems, College of Agriculture, Food Systems and Natural Resources, North Dakota State University, Fargo, ND, USA

Email: [*owinoj@gmail.com](mailto:owinoj@gmail.com)

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Abstract

Legumes are high-protein, medium-energy and micronutrient-rich food consumed in many parts of the world including Africa. This study evaluated the levels of specific phenolic compounds in three legumes. Two varieties of the common bean, (*Phaseolus vulgaris* L.) soybeans (*Glycine max* L.), and peas (*Pisum sativum* L.) from Rwanda were analyzed using high performance liquid chromatography with diode array detection. The phenolic compounds were identified by comparison to the chromatographic retention times and UV spectra of known reference compounds. This study results clearly shows the presence of 11 different phenolic compounds in common beans: gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, o-coumaric acid, chlorogenic acid, quercetin, 4-hydrobenzoic acid, syringic acid, ferulic acid and vanillic acid. The concentration ranged from 0.59 to 2.27 mg/kg for epicatechin. High levels of catechin (13.5 to 57.9 mg/kg) ferulic acid (26.1 to 47.6 mg/kg) were also observed. Therefore, the results of this study show that Rwandan common beans are a good source of phenolic acids in particular catechins and ferulic acid.

Keywords

Common Bean, Phenolic Compounds, Rwanda, Identification and Quantification

1. Introduction

Legumes are a source of proteins, minerals, and vitamins for millions of world populations. The common bean

*Corresponding author.

(*Phaseolus vulgaris* L.), one of the food legumes, is a part of daily diets of African, Asian and South American populations. Common beans being a member of *Leguminacea* family fixing atmospheric nitrogen provide significant crop rotational advantages, and a contributor towards agricultural sustainability in many parts of the world [1].

Common beans are also a rich source of phytochemicals. Consumption of common beans rich in phytochemicals promotes human health. Among phytochemicals the phenolic class of compounds such as epicatechin, epigallocatechin gallate and catechin flavonoids protect against neurotoxic oxidative stresses. Epicatechins are also able to cross the blood-brain barrier [2] to reduce lipid peroxidation and inhibit platelet aggregation. In diabetic red blood cells, epicatechins increase acetylcholinesterase activity [3]. In addition, other phenolic compounds such as flavonoids have been shown to protect against lung diseases and a range of cancers [4].

Besides positive nutritional benefits of phenolic compounds, polymeric forms of phenolic compound or polyphenols exert negative health effects on protein digestibility and α -amylase activities. For example, heat-stable tannins inhibit pectinases, cellulase, amylases, β -galactosidases, lipases and several proteolytic enzyme activities. Iron (Fe) and zinc (Zn) mineral micronutrient bi-availabilities have been reduced in common beans with high concentration of certain phenolic compounds [5]. Polyphenols are also a contributor towards off colors and undesirable flavors in beans and other foods.

Common bean is a part of daily diet of majority of Rwandans. It is estimated that Rwandan consumes 60 kg common beans per person per year [6]. With significant common bean consumption by Rwandans this study was carried out to determine type and concentrations of phenolic compounds in most widely consumed bean varieties. In addition, common bean phenolic compounds types and concentrations were compared with those of soybean and peas grown in Rwanda.

2. Materials and Methods

2.1. Study Samples

The legumes varieties of pink and blue common beans of one kg were purchased in April 2013 from the local market within two km radius of the University of Rwanda Campus in Kigali, Rwanda. They were washed, sorted, and powdered using a domestic grinder (Philips, Model No. 2161, Netherlands). The powdered samples kept in refrigerator 4°C until sample analysis.

2.2. Chemicals and Reagents

All chemicals and reagents used were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Ultra-pure water was purchased from Milli-Q plus system from Millipore (Billerica, MA, USA).

2.3. Extraction

Six ml of 95% ethanol was added to 0.6 g of finely ground dry samples, followed by rigorously mixing (2500 rpm) using a vortex for about 2 minutes. Then the samples were centrifuged at 1400 rpm for 10 minutes at room temperature and the supernatant was taken for further analysis.

2.4. Chromatographic Conditions

The sample analysis was performed on a high performance liquid chromatography (HPLC) with PDA detection (Shimadzu Scientific Instruments, Kyoto, Japan). The analyte separation column was Shim-Pack VP-ODS C18 column (250 mm \times 4.6 mm, 5 μ m) Shim-Pack VP, Shimadzu Scientific Instruments, Kyoto, Japan. The DAD detector was applied to scan the phenolic compounds of interest to ascertain their maximum absorbance wavelengths and acquire other spectral information within a range of 200 to 400 nm. A gradient solvent system was employed with solvent A being water-acetic acid (97:3, v/v) and solvent B being acetonitrile. The elution profile had the following proportions (v/v) of solvent B: 0.00 - 5.00 min, 0% - 8.5%; 5.00 - 16.50 min, 8.5% - 2.0%; 16.50 - 35.00 min, 2.0% - 18%; 35.00 - 50.00 min, 18% - 20%; 50.00 - 65.00 min, 20% - 30%; 65.00 - 70.00 min, 0% - 30%. The separated peaks were identified at 255, 260, 275, 280, 304, 324 and 360 nm. The column temperature was maintained at 30°C with a flow rate of 0.8 ml/min. All the prepared solutions and samples were filtered through 0.25 μ m membranes (Minisart, Sartorium Stedim Biotech, Russia) prior to analysis.

2.5. Calibration Curves

Solutions of the phenolic standards were prepared in the mobile phase. Separate calibration curves were used for phenolic compounds at four different concentrations. Quantifications were validated using inter laboratory analysis of selected phenolic compounds at both at University of Rwanda (UOR), Kigali, Rwanda and North Dakota State University (NDSU), ND, USA. All samples were analyzed in triplicate.

2.6. Statistical Analysis

The results were analyzed by analysis of variance and calculation of correlations and regression with SPSS 16.0 statistical software (IBM Corporation, USA). Differences between means were evaluated using the Duncan's multiple range test. The difference was considered significant $p < 0.05$.

3. Results and Discussion

3.1. Phenolic Compounds Separation and Identification

The chromatographic conditions of the phenolic compounds were optimized prior to sample analysis. The peaks of all analytes were well resolved, and a representative chromatogram is shown in **Figure 1**. The standard curves of all phenolic compounds correlations were 0.99 or higher. Therefore, we are certain that peak identification and sample quantification are accurate. Inter laboratory validation between the UOR and NDSU also confirmed selected peak identification and quantification. Chromatographic identification and confirmation of phenolic compounds were based on comparing retention times with authentic standards. The relative concentrations at different wavelengths were calculated from the calibration curves [7].

3.2. Bean Phenolic Compound Quantification

Beans are good source of phenolic compounds. In this study sample phenolic compound detailed results are shown in **Table 1**. The levels of phenolic compound varied from 16.78 - 25.01 mg/kg for gallic acid, 18.18 - 29.64 mg/kg for 4 hydroxy benzoic acid, 10.69 - 13.33 mg/kg for vanillic acid, 23.17 - 24.06 mg/kg for chlorogenic acid, 11.8 - 41.9 mg/kg for caffeic acid, 14.1 - 31.3 mg/kg for syringic acid, 13.5 - 57.9 mg/kg for catechin, 0.59 - 2.27 mg/kg for epicatechin, 12.57 - 27.1 mg/kg for o-coumaric acid, 26.1 - 47.6 for ferulic acid and 16.73 - 19.23 mg/kg for quercetin. From the results there was no significant difference ($p > 0.05$) in epicatechin, quercetin, catechin and ferulic acid in the four samples analyzed implying that consumption of the samples will result in the same amount of the named phenolic compounds. In blue dry beans, catechin had the highest concen-

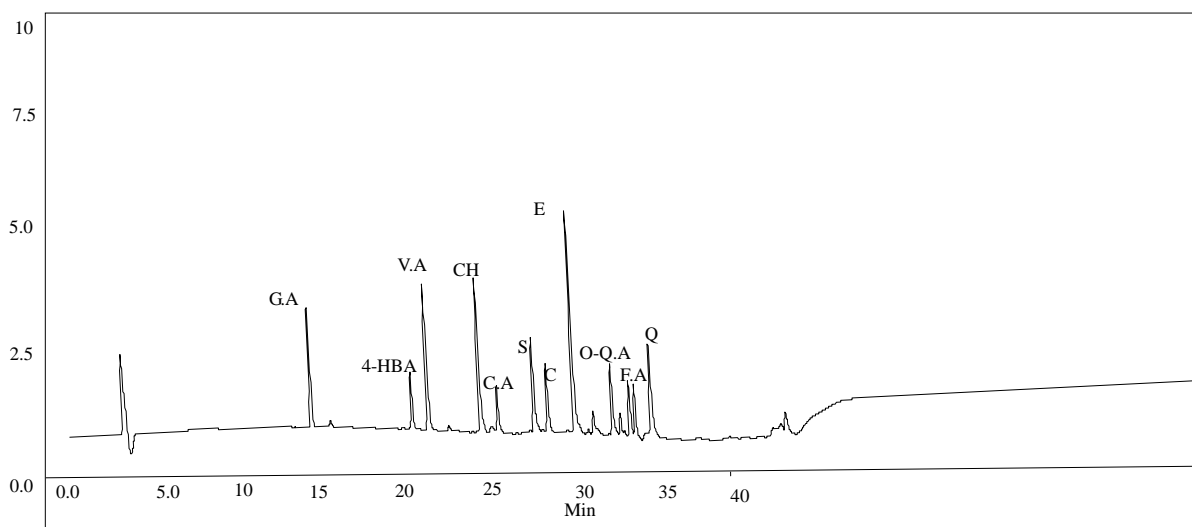


Figure 1. Representative HPLC chromatogram of 11 different phenolic compounds. GA: Gallic Acid, 4-HBA: 4-Hydroxy Benzoic Acid, V.A: Vanillic Acid, CH: Chlorogenic, C.A: Caffeic Acid, S: Syringic, C: Catechin, E: Epicatechin, O-Q.A: O-Qoumaric Acid, F.A: Ferulic Acid, Q: Quercetin.

Table 1. Composition of the phenolic compounds.

SAMPLES	Gallic acid	4-Hydroxy benzoic acid	Vanillic acid	Chlorogenic acid	Caffeic acid	Syringic acid	Catechin	Epicatechin	Coumaric acid	Ferulic acid	Quercetin
BLUE BEAN	16.78 ^a ± 0.24	26.84 ^a ± 1.15	10.69 ^a ± 0.07	23.36 ^a ± 0.53	41.9 ^c ± 1.04	14.4 ^a ± 1.11	57.0 ^c ± 0.80	1.10 ^b ± 0.00	12.57 ^a ± 0.37	26.1 ^a ± 0.01	16.77 ^a ± 0.24
PINK BEAN	25.01 ^c ± 2.77	27.17 ^a ± 0.54	11.54 ^a ± 0.08	23.59 ^a ± 0.08	25.5 ^b ± 3.20	14.1 ^a ± 0.34	13.5 ^a ± 0.50	2.27 ^c ± 0.04	16.66 ^a ± 0.65	36.0 ^b ± 1.44	17.21 ^a ± 0.15
SOYA	20.28 ^b ± 0.09	18.18 ^a ± 0.53	10.91 ^a ± 0.16	23.17 ^a ± 0.08	40.3 ^c ± 9.47	18.7 ^b ± 0.25	57.9 ^c ± 1.64	1.06 ^b ± 0.08	27.1 ^a ± 1.19	27.1 ^a ± 1.19	16.73 ^a ± 2.49
PEA	20.48 ^b ± 0.67	29.64 ^a ± 3.10	13.33 ^b ± 1.85	24.06 ^a ± 1.10	11.8 ^a ± 0.45	31.3 ^c ± 2.13	18.1 ^b ± 3.83	0.59 ^a ± 0.42	15.34 ^a ± 0.02	47.6 ^c ± 3.39	19.23 ^a ± 1.83

tration of 57 mg/kg while epicatechin had the lowest concentration 1.1 mg/kg. Similar trend was observed in a study of quantitation of phenolics from the seeds of green beans in which levels of catechin was 407.5 mg/kg while epicatechin was 207.6 mg/kg [8]. In pink dry beans ferulic acid had the highest concentration of 36 mg/kg while epicatechin had the lowest concentration of 2.7 mg/kg. In soybean, catechin had the highest concentration of 57.9 mg/kg while the lowest concentration was observed in epicatechin at 1.06 mg/kg. For pea, highest concentration was seen in ferulic acid at 47.6 mg/kg and the lowest was epicatechin at 0.59 mg/kg. The low levels of epicatechin could be attributed to an epimerization reaction which converts epicatechin to its epimer catechin during the extraction process [9].

Only p-coumaric and ferulic acid were reported in a study [10], from the flour and hull of the navy beans. In a study on phenolic acids content of fifteen dry edible bean varieties, caffeic acid was quantified at 1.1 mg/100mg in black beans, p-coumaric acid was 12.4 mg/100g, ferulic acid was 26.6 mg/100g and sinapic acid 9.4 mg/100mg [11]. The observed values are much higher for caffeic and ferulic acid compared to the study samples. This difference can be attributed to various factors such as variety, assay procedure, growing and storage conditions, agronomic practices (irrigation, fertilization, pest management), maturity at harvest, post harvest storage and climatic conditions [12]-[14].

Most studies in literature reports on the total phenolic content, which makes it not possible for, direct comparison with our study. However, this may be the first study from Rwanda reporting detailed phenolic compound results. Since polyphenols exert both beneficial and negative effects, further studies using common bean phenolic compounds on cell culture, animal models and human subjects will provide real human nutrition benefits.

4. Conclusion

Eleven phenolic compounds in common bean samples were analyzed and quantified using high-performance liquid chromatography-diode array detection. This study shows that Rwandan beans are a good source of phenolic compounds in particular catechins and ferulic acid.

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