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# Effect of processing methods on polyphenols content of red, white and black kidney beans

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**ABSTRACT**

This study was aimed to evaluate the effects of processing methods on total phenolic compositions of red, white and black kidney beans cultivated in Ethiopia by UV-Visible spectrophotometry. The polyphenol contents were found to be 48, 99 and 193 mg/100 g in the raw, soaked and cooked red kidney bean samples, respectively. It showed 51.3% increment during soaking followed by 48.5% increment upon cooking. The polyphenol contents were found to be 45, 83 and 182 mg/100 g in the raw, soaked and cooked white kidney bean samples, respectively, which showed 45.6-54.2% gaining of polyphenol content during soaking followed by cooking. The polyphenol contents of black kidney beans were found to be 43, 95 and 190 mg/100 g in the raw, soaked and cooked samples, respectively, showing the increment of polyphenol during soaking 50.0% followed by 45.2% upon cooking. The results were analyzed using an ANOVA and Tukey's test. There were significant differences in the polyphenols content between bean cultivars and treatments processes. These results suggest that soaking and cooking favored the extraction of polyphenols, important for human health as kidney beans are an excellent source of phenolic compounds with antioxidant activity.



**Capsule Summary:** The polyphenols content of raw, soaked and cooked red, white and black kidney beans cultivated in Ethiopia determined as a function of processing methods was assessed. The soaking and cooking of kidney beans considerably increased the polyphenol contents which are beneficial for human health.

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## INTRODUCTION

Kidney bean is one of the major food and cash crops in Ethiopia and it has national economic significance and also traditionally certain food security in Ethiopia (Asfaw et al., 2009; PABRA, 2014). It takes the third position in an export commodity in Ethiopia, contributing about 9.5% of total export value from agriculture. It is consumed as a main traditional dish in many parts of Ethiopia. It is mainly cultivated in Eastern, Southern, South Western and the rift valley areas of Ethiopia. Principally produced in Oromia, South Nations Nationality People and Amhara regions with their area coverage of 146,452.41 ha (41%), 117,969.97 ha (33%) and 81,235.07 (22.74%) ha, respectively. The remaining 3.25% is produced in other regions of Ethiopia (CSA, 2016). Negash (2007) reported that in the southern part of the country, Sidama region and Gamo Gofa zones produce red and

speckled types mainly for home consumption. In the eastern part; mainly Hararghe highlands, where low land pulses are being preferred for food and they are mostly speckled food beans and small whites. Kidney bean is usually grown in small and medium sized farms due to small land holding of most of the farmers in Ethiopia (CSA, 2016).

Kidney bean, *Pharsalus vulgaris*, green bean or common bean, is an annual, climbing plant in the *Fabaceae* (legume or bean family) in the genus *Pharsalus* L. that originated in Central and South America and is currently cultivated in many parts of the world for its beans, that can be harvested and consumed immature, still in the edible pod, or when mature, shelled, and dried (Boateng et al., 2008). They are adapted to a wide range of climatic conditions ranging from sea level to nearly 3000 meters above sea level (m.a.s.l.) depending on variety. However, it does not comfortably grow below 600 meters as a result of poor pod set caused by high temperature (Freytag and Debouck, 2002). Red, white and black kidney beans are some of the popular varieties of kidney beans and are good source of important nutrients with high amount of protein, essential and non-essential minerals, crude fiber, and carbohydrates.

Phenolic compounds are secondary metabolites widely distributed in food. Due to their structural complexity, they can be classified as phenolic acids, flavonoids, stilbenes, lignans, and tannins (Belščak-Cvitanović et al., 2018; Dias et al., 2020; Los et al., 2018; Mojica et al., 2015; Telles et al., 2017). Polyphenols are the predominant bioactive components with multifold bioactivities in diverse kidney bean cultivars (Los et al., 2018; Singh et al., 2017). Phenolic acids, flavonoids and proanthocyanidins are the main polyphenols in kidney beans and colorful common beans are overall rich in polyphenols, mainly in their pigmented seed coats (Los et al., 2018; Singh et al., 2017). In addition, factors of influence, such as genotype, environmental conditions, storage, and processing methods, play a critical role in the content and composition of kidney bean polyphenols (Caprioli et al., 2018; Los et al., 2019; Madrera and Valles, 2020). Besides, analytical methods, including extraction, separation, and identification, are of importance for precise and comparable evaluation of polyphenols in kidney beans (Caprioli et al., 2018; Madrera and Valles, 2020). Therefore, in order to provide a comprehensive and updated understanding of polyphenols in kidney beans, this study summarizes the amount of polyphenols in kidney beans. The amount of polyphenols in foods can vary widely depending on where they are grown, how they are cooked, and whether they are organic or produced conventionally (Yang et al., 2018; Yang et al., 2019). For optimal health, individual person needs consistent consumption of very high levels of fruits and vegetables every day to maintain adequate intakes of polyphenols.

When it comes to how much polyphenols one should consume, there are benefits ranging from intakes of 500 mg to 1500 mg per day (Yang et al., 2019). The concentration of polyphenols in vegetables (0.1% by weight) and fruits (0.2% by weight) can be low, requiring high levels of consumption. To give a sense of what 1000 mg of polyphenols per day looks like, it could be approximately 12 cups of cauliflower, 1.5 cups of blueberries, or 3 glasses (~5 fluid ounces each) of red wine or some combination of various fruits and vegetables (Yang et al., 2019).

The current knowledge of the antioxidant properties of phenols is reviewed, with particular emphasis on the role of the solvent (Dias et al., 2020). Phenols are familiar to reduce the rates of oxidation of organic matter by transferring a hydrogen atom (from their OH groups) to the chain-carrying $ROO^{\cdot}$ radicals, a mechanism that most likely including a concerted transfer of the hydrogen as a proton and of one electron between the two oxygen atoms, proton-coupled electron transfer mechanism (Yang et al., 2019). The antioxidant capabilities of phenols are strongly diminished by hydrogen-bond accepting solvents since the hydrogen-bonded molecules ArOH---S are virtually unreactive toward $ROO^{\cdot}$ radicals (Dias et al., 2020). The magnitude of these kinetic solvent effects is determined by the solute acidity and solvent basicity. Solvents such as alcohols have a double effect on ArOH. On the other hand, they act as hydrogen-bond accepting solvents and reduce the canonical rates of the ArOH + $ROO^{\cdot}$ reaction (Dias et al., 2020). On the one hand, these solvents favor the ionization of ArOH into their phenoxide anions $ArO^{-}$, which may react with $ROO^{\cdot}$ very quickly by electron transfer (Dias et al., 2020).

Polyphenols can help control blood pressure levels and retain the blood vessels healthy and flexible, promoting good circulation (Dias et al., 2020). They also help demote chronic inflammation and other risk factor for heart disease. Extraction method, the type of solvent, the analytical technique, and the variety of beans are the factors that affect the amount of total phenols (Singh et al., 2017; Yang et al., 2018; Yang et al., 2019). Several studies have quantified the phenolic compounds in different types of food in Ethiopia (Mehari et al., 2016; Debebe et al., 2016; Haile et al., 2016; Seifu et al., 2017; Shewakena et al., 2017; Mehari et al., 2020; Yisak et al., 2022). However, there is no study on the quantification of polyphenols in kidney beans in Ethiopia. Considering the recent surge in interest in the use of grain legumes including kidney beans, it is worthwhile to determine polyphenols content of raw, soaked and cooked red, white and black kidney beans cultivated in Ethiopia and to assess the effect of processing methods on polyphenol contents of beans.

## MATERIAL AND METHODS

### Instruments

Hotplate (Wadtech, Hotplate SH3, UK), analytical balance (item ARZ140 N315: b Max. capacity 210 g C20379705, reliability 0.0001 g SNR 1203290469, Ohaus Corp., NS USA), mechanical shaker, vortex mixer and UV-Visible spectrophotometer (Hitachi-U 1800 UV-Vis), centrifuge

(DYNAC II centrifuge, clay adams, division of Becton Dikinson and company, USA) were used.

**Chemicals**

Analytical reagent-grade chemicals were employed for the preparation of all solutions. HCl (37%, Riedel-Dehaen, Sigma-Aldrich Chemicals GmbH, Germany), 3 M $H_2SO_4$ (98%, (Riedel-DeHaen, Germany), methanol (30%, Riedel-DeHaen, Germany) and freshly prepared deionized water were used in this study.

**Sample collection**

Red, white and black kidney bean samples were collected from three different regions of Ethiopia namely Sidama (Bansa Daye), Amhara (Gojam, Bure) and Oromia (Arsi Negele) and taken in to the laboratory, washed, dried and kept in airtight sealed polyethylene bags for further preparation.

**Sample preparation**

About 1 kg of red, white and black kidney beans were manually washed, dried in an open-air condition and divided into two groups [raw and processed]. The beans in the processed group were further divided into two equal groups just after soaking for overnight (16 h) and soaking water is drained off. For cooking, half of the soaked samples were cooked in distilled water at 100 °C for 60±5 min and allowed to air dry (at room temperature for 72 h). The dried samples (raw, soaked and cooked) were ground to fine powder by laboratory electrical grinder and sieved to 0.425 mm mesh size, packed into airtight sealed polyethylene bags and left for later analysis. The flours of the kidney beans samples were subsequently analyzed for their total phenol content.

**Soaking**

All the red, white and black kidney bean seeds were soaked separately in the dark at room temperature for 16 h in distilled water then the soaking water was drained off. The seeds were classified in to two half parts in which one allowed to air-dried in open air for 72 h for complete drying. Samples were ground to pass through 30-mesh screen. The ground samples were kept in air-tight bottles and stored at 4 °C for subsequent analysis.

**Cooking**

The presoaked seeds of red, white and black kidney bean were cooked on a hot plate (about 60±5 min) until it get easily crushed by a gentle press between the thumb and index fingers. The cooking liquid and seeds were separated by filtration and the cooked seeds were dried in the same way as after the soaking process.

Each of the three sets of samples was analyzed in triplicate for their polyphenols. Analysis of samples was carried out according to the available standard/published methods. Hitachi-U 1800 UV-Vis were used for the determination of polyphenols.

**Calibration curve**

Gallic acid stock solution (1000 µg/mL) was prepared by dissolving 100 mg of gallic acid in 100 mL of ethanol. Various dilutions of standard gallic acid in the range 1–100 µg/mL were prepared from stock solution. Folin-Ciocalteau reagent was prepared by mixing Folin's reagent with Phenol reagent (1:1), and diluted in ratio 1:1 in distilled water. Calibration curve was plotted by mixing 1 mL aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/mL of gallic acid solutions with 5.0 mL of

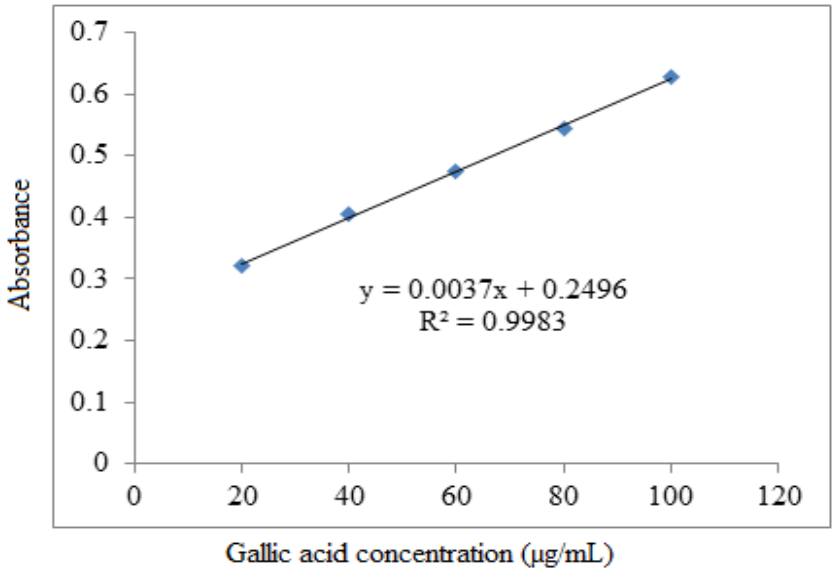


**Fig. 1:** Calibration curve of gallic acid standard solutions

Folin-Ciocalteu reagent and 4.0 mL of sodium carbonate solution (75 g/L). The absorbance was measured after 30 min at 20 °C at 765 nm. One mL of aqueous and methanol extract (1.0 g/100 mL) was mixed separately with the same reagents as in case of calibration curve, and after 1 hour, the absorbance was measured for the determination of total phenolic compound in both the extract separately (Kumar et al., 2006). The calibration curve is shown in Figure 1.

**Table 1**: Total polyphenol contents of raw, soaked and cooked red, white and black kidney bean samples (mean±SD of the triplicates, n = 3)

| Samples* | Total polyphenols (mg/100 g) |
|---|---|
| RR | 48±0.20 |
| SR | 99±0.32 |
| CR | 193±0.58 |
| RW | 45±0.28 |
| SW | 83±0.78 |
| CW | 182±0.10 |
| RB | 43±0.42 |
| SB | 95±0.71 |
| CB | 190±0.50 |

*Sample code: RR: Raw red, RW: Raw white, RB: Raw black, SR: Soaked red, SW: Soaked white, SB: Soaked black, CR: Cooked red, CW: Cooked white, CB: Cooked black kidney beans

### Determination of polyphenols

Total polyphenols were determined as described by Robu et al. (2012). One gram sample was mixed with 10 mL of 80% methanol, shaken for 2 h and filtrated. The color was developed by Folin-Ciocalteu reagent and sodium carbonate. 0.250 mL was mixed with 0.250 mL Folin–Ciocalteau reagent, 0.50 mL of 10% sodium carbonate ($Na_2CO_3$) and the volume was adjusted to 5 mL with distilled water. After incubation in dark at room temperature for 30 min, the absorbance of the reaction mixture was measured at 725 nm against blank on a spectrophotometer (Hitachi-U 1800 UV-Vis). Gallic acid was chosen as a standard to prepare the standard curve. Polyphenols were expressed as mg/100 g sample on dry weight basis.

### Recovery

The features for the determination of polyphenols were evaluated in terms of LOD, LOQ, linear range, precision and accuracy (Ayele et al., 2015). 0.5 g of powdered kidney bean samples at three treatments (raw, soaked and cooked) were spiked as the method to be validated and the results were compared to the expected increase in the parameter to be analyzed relative to the raw. The recoveries were 91.3, 90.5 and 93.1% for the red, white and black kidney beans samples, respectively.

### Statistical analysis

Total phenol contents of the raw and processed samples of three different types of kidney bean were statistically compared using analysis of variance (ANOVA) and Tukey's test. The statistical package used was SPSS version 25. Significant differences were determined at $p < 0.05$ level. All the results were reported as mean values with their respective standard deviations. The results are reported as mg analyte/100 g of raw, soaked and cooked samples for consistency and comparison.

## RESULTS AND DISCUSSION

### Polyphenols contents of kidney beans

Cooking was previously reported to increase polyphenols (Teixeira-Guedes et al., 2019). In this study the effect of soaking and cooking showed significant difference ($p$<0.05).

**Table 2**: Comparison of total phenolic contents found in Ethiopian red kidney bean with other reported values mg/100 g

| Methods | Processing conditions | Total phenol (mg/100 g) | References |
|---|---|---|---|
| UV-VIS | Raw | 48 | This study |
| | Soaked | 99 | |
| | Cooked | 193 | |
| HPLC | Raw | 18 | Lomas-Soria et al. (2015) |
| | Soaked | NR | |
| | Cooked | 45 | |
| HPLC-UV/VIS | Raw | 1765 | Telles et al. (2017) |
| | Soaked | NR | |
| | Cooked | NR | |
| HPLC-DAD | Raw | 2505 | Brigide et al. (2018) |
| | Soaked | NR | |
| | Cooked | 3462 | |
| LC-MS/MS | Raw | 18 | Teixeira-Guedes et al. (2019) |
| | Soaked | NR | |
| | Cooked | 68 | |

NR = not reported

The amount of polyphenol showed 51.3, 45.6 and 50.0% increments for the red, white and black kidney beans samples respectively during soaking and 48.5, 54.2 and 45.2.7% increments for red, white and black kidney beans respectively up on cooking, which is relatively higher than the result reported by Teixeira-Guedes et al. (2019). The results of present study are almost closer with the results reported by Lomas-Soria et al. (2015) and much lower/incomparable with the results reported by Telles et al. (2017) and Brigide et al. (2018). The differences might be due to the fact that polyphenolic composition of food crop is directly related to its genetic origin, geographical source and soil conditions (Mathew et al., 2006).

The total phenolic contents of common beans have been extensively reported and the data are scattered across many research articles. Gan et al. (2017) have compiled the scattered information and summarized in their review showing that the total phenolic contents of common beans has been determined in the range of not detectable (ND) to 4871 mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW). The remarkable position of pinto kidney bean should be emphasized because its TPC in seed coats had the highest level (4871 mg GAE/100 g DW) among diverse common bean cultivars (Gan et al., 2017).

**Polyphenol contents comparison reported studies**

Polyphenols are the predominant bioactive components with multifold bioactivities in diverse common bean cultivars. Phenolic acids, flavonoids, and proanthocyanidins are the main polyphenols in common beans, and colorful common beans are overall rich in polyphenols, mainly in their pigmented seed coats. In addition, factors of influence, such as genotype, environmental conditions, storage, and processing methods, play a critical role in the content and composition of common bean polyphenols. Besides, analytical methods, including extraction, separation, and identification, are of importance for precise and comparable evaluation of polyphenols in common beans (Yang et al., 2018). It has, been noticed that the polyphenol composition of a food crop is directly related to its genetic origin, geographical source and soil conditions (Mathew et al., 2006). The comparison between this study using UV-VIS and other reported literatures by using HPLC, HPLC-UV/VIS, HPLC-DAD and LC-MS/MS are given in Table 2.

## CONCLUSIONS

This study provides data on polyphenol contents of red, white and black kidney beans cultivated in Ethiopia by UV-Visible spectrophotometry. The results obtained from the study showed that processing methods (soaking and cooking) significantly increase the total phenolic contents of all kinds of kidney beans. Since soaking and cooking are common practices to make the kidney beans edible which results in significantly increase the total phenolic contents of all kinds of kidney beans. This is very good for human health. All cultivars showed significant amounts of gallic acid. Discarding the cooking/soaking water did not result in loss of amounts of polyphenols. This is beneficial to human health.

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