Heating Effect on Phytochemical and Proximate Content of Cooked Aqueous Extract of Phaseolus Vulgaris (Kidney Beans)

ABSTRACT
Plant chemicals and nutrients abound in different parts of plants and in different compositions. Phaseolus vulgaris (kidney beans) is a nourishing leguminous food commonly eaten by both human beings and animals in the world due to its health benefits and risk reduction of diseases. It is however, indispensible for plants’ food stuff to retain their phytonutrients for maximum benefits. This study therefore investigated the effect of heat on the phytochemicals and proximate contents in cooked Phaseolus vulgaris (kidney beans). Qualitative and quantitative phytochemical analysis and proximate (nutrient contents) analysis were determined on fresh kidney bean (FKB) and cooked kidney bean (CKB). Results of phytochemical quantification revealed a significant (p<0.05) increase of alkaloids and saponins in FKB than those in CKB, significant (p<0.05) increase of flavonoids, glycosides and tannins in CKB than those in FKB. While proximate analysis of cooked sample (CKB) showed significant (p<0.05) increase in protein content, crude ash content and carbohydrate content FKB. While moisture content, crude fibre and crude fat of FKB were significantly (p<0.05) higher than those in CKB of Phaseolus vulgaris. Obviously, the increased concentrations of phytochemicals in fresh Phaseolus vulgaris may be due to the absence of heat action and the heating effect on cooked Phaseolus vulgaris could unleash the high rich nutrients value and could supply its antioxidants roles, thereby improving healthy life when eaten cooked.

Keyword: Cooked bean, fresh bean, heating effect, Phaseolus vulgaris, phytochemicals, proximate content.

INTRODUCTION
Kidney bean (Phaseolus vulgaris) derived its name from the visual resemblance of the kidney in shape and colour. However, the red colour of the kidney beans should not be confused with other beans such as the pinto beans and adzuki beans, which are also red in colour. Phaseolus vulgaris beans plant strive best in subtropical and tropical weather. The worlds’ largest producer of beans is Brazil, having approximately five million hectares of land cultivated, with the production of 2.2 – 2.5 million tons. Researchers have evidently reported the various nutrients in beans and their health benefits, sample recipes and preparation tips. Beans contain several nutrients including mineral salts such as Mg, Ca, P, Fe and K; contain about 20 – 25% proteins, 50-60% complex carbohydrates and vitamins. Certain antinutrients are also found in beans which inhibit and limit absorption of other useful nutrients. They include phytic acid and oligosaccharides; they limit carbohydrate and protein absorption and trypsin, which inhibit
tannins absorption. Anti-nutritional contents in beans can modify the bioavailability of iron. Cooking was reported to improve the bioavailability, digestibility and hence the absorption of macro and micronutrients found in food due to a soften matrix of the food by heat treatment. Phytic acid and tannins present in beans may bind to proteins and some essential dietary minerals, thus affecting their absorption and bioavailability. Generally, food cooking has been resourcefully shown to destroy injurious microorganisms and bacteria that may result in food-borne disease due to improper handling. The digestion of protein and starch was found to be increased from 25-60% in raw beans to 85% in cooked beans. The sensory properties in beans, including soft texture, mushy texture, sweet, taste and flavor of cooked beans were found to be improved by cooking. Cooking can result in reduction of content of certain vitamins and volatile phytochemicals and also affect the composition of some nutrients such as amino acids and minerals. Soaking (quick, short or traditional long soaking) and cooking of beans can easily result in loss or reduction of folate during interaction with fibers. However, in order to take full advantage of the natural folate content in beans, it is recommended that the use of the slow-soak routine and a cooking method that prepares the beans in 150 minutes or less should be adopted. The folate vitamin B notably in beans or its synthetic equivalent, folic acid plays a very vital role in the synthesis of red blood cells in human body and also is also involved in embryonic nervous system development at early stages of pregnancy. Cooking was reported to increase the solubility and bioactivity of soluble iron in beans, lentils, legumes and chickpeas. The chemical composition of Phaseolus vulgaris (kidney beans) was reported to be affected by cooking. Cooking resulted in destruction of antinutrients, alteration of distribution or bioavailability of iron (in glutelins, globulins, albumins and prolamins) and protein denaturation. Beans generally contain high concentration of health-rich nutrients and consuming more beans diets could better in general, the healthiness of a person and eliminate risk of obesity, cancers and heart diseases. Thus, this study investigated the effect of heat on the phytochemicals and proximate contents in cooked Phaseolus vulgaris (kidney beans).

MATERIALS AND METHODS

Collection and Authentication of Bean Seeds

Phaseolus vulgaris (kidney beans) seeds were purchased from a commercial market (Ogbete main market) in Enugu south local government area, Enugu state, Nigeria. The sample was identified and authenticated and a voucher number of UNH no 452 (UNH stands for University of Nigeria Herbarium), was given by Mr. Onyeukwu Chijioke John a plant Taxonomist, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state.

Preparation of Aqueous Extract of Fresh Kidney Bean (FKB) and Cooked Kidney Bean (CKB)

Phaseolus vulgaris (kidney beans) seeds were prepared by winnowing, hand picking of stones and removal of dirt and then lightly washed to remove dust and air dried.

Preparation of Fresh Sample (FKB)

Five hundred gram (500g) of the dried bean seeds was weighed and ground/homogenized into powder. After which it was stored in a clean grease free airtight container with proper labeling for proximate and phytochemicals analysis.
Preparation of Cooked Sample (CKB)
The cooked *Phaseolus vulgaris* (kidney beans) sample was prepared appropriately by hand picking to remove all foreign particles followed by washing and cooked with enough water until soft and without broth to prevent the loss of some phytochemicals in the bean broth. This was dried under mild sunlight for two weeks under strict supervision. Five hundred gram (500g) of the dried bean seeds was weighed grinded/homogenized into powder. After which it was stored in a clean grease free airtight container with proper labeling for proximate and phytochemical analysis.

Preparation of Dry Extract from Samples
From the powder samples (CKB and FKB), 200g was weighed and soaked in 700ml of distilled water, carefully sealed and allowed to stand for 48 hours (for thorough extraction), before filtering with whatman filter paper. The filtrate was concentrated in water bath at temperature of 70ºC.

Qualitative Phytochemical Screening of Fresh Bean (FKB) and Cooked Kidney Bean (CKB) samples
Alkaloids, flavonoids, saponins, glycosides, phenols, steroids, tannins, reducing sugars and anthraquinones were identified in cooked and fresh kidney beans samples using standard methods described by 14,18 with some modifications.

Quantitative Phytochemical Screening of Fresh Kidney Bean (FKB) and Cooked Kidney Bean (CKB) samples

**Determination of Alkaloids**
Alkaloids in the bean samples were determined by the method described by 14. Five gram (5g) of the sample was weighed into a 250mls beaker and 200mls of 10% acetic acid in ethanol was added and it was covered and allowed to stand for 4 hours at room temperature, after which it was filtered. The filtrate was concentrated on a water bath to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous ammonium solution until the alkaloid was precipitated. Alkaloid precipitated was received in a weighed filter paper (W1). It was washed with 1% NH₃ solution and dried in the oven at 80ºC. The filter paper and residue was cooled in a desiccator and recorded as (W2). The alkaloid content was calculated and expressed as a percentage of the weight of the sample.

Percentage alkaloid content was calculated using the formula:

\[
\text{Alkaloid\%} = \frac{\text{weight of filter paper} + \text{sample after drying} - \text{weight of filter paper}}{\text{weight of filter paper}}
\]

**Determination of Flavonoid**
Flavonoid in samples was determined by the method described by 15. Ten gram (10g) of sample was weighed into a 250mls conical flask. 100mls of 80% aqueous methanol added, the mixture
was shaken for 3 hours by the aid of electrical shaker. The mixture was filtered into a previously weighed beaker, and evaporated to dryness over a water bath and weighed to a constant. Percentage flavonoid content was calculated using the formula:

\[
\text{Flavonoid\%} = \frac{W_2 - W_1}{W_2} \times 100
\]

were \( W_1 \) = weight of empty beaker and \( W_2 \) = weight of residue (weight of empty beaker + sample after drying).

**Determination of Saponin**

Saponin in both samples was determined using \(^{16}\) method. About 10g of grinded sample was put into a conical flask and 100mls of 20% aqueous ethanol was added. The mixture was thoroughly mixed with for about 20 to 30 minutes and it was immediately transferred into a 250mls conical flask. Then, the mixture was covered properly and heated over a hot water bath at 90°C for 4 hours with continuous stirring. The mixture was filtered with whatman filter paper and the supernatant separated. The solid residue was mixed with 100mls of another 20% ethanol and heated in a similar way for 4hours. The solution was then filtered and mixed with the previously filtered solution. The combined filtered solution was placed on a hot water bath at 90°C and heated to 20% of the original volume. The concentrated solution was then transferred into a 250mls separating funnel and 10mls of diethyl ether was added to it and vigorously mixed. The diethyl layer was discarded carefully after settling down the solution. The purification process was repeated again. 60mls of n-butanol was added and two layer was formed, then the bottom layer was discarded and the upper layer recovered. The combined n-butanol extract was washed twice with 10mls of 5% NaCl solution. The remaining solution (i.e upper layer) was heated in a water bath at 50°C until the solvent evaporated and the solution turned into semi-dried form. Percentage saponin content was calculated using the formula:

\[
\text{Saponin\%} = W_2 - W_1 \times 100
\]

were \( W_1 \) = weight of empty beaker and \( w_2 \) = weight of beaker + sample after drying

**Determination of Glycosides**

Glycosides contents in samples were determined by the method of \(^{17}\). Five gram (5g) of the sample was soaked into 100mls of distilled water in a 250mls conical flask and agitated for 3 hours. The sample was filtered and the total extract was measured and noted. Into a test tube was 2mls of the extract measured and 2mls of 10% DNS reagent was added. Then, the test tube was boiled for 20 minutes in a beaker of boiling water. The test tube was cooled in cold water. The absorbance was read at 540nm using UV-Vis Spectrophotometer, DHG-9101 machine. Percentage glycoside content was calculated using the formula:
% Glycoside = \frac{Absorbance \times \text{total volume of extract} \times 100}{1000 \times \text{weight of sample used}}

**Determination of Tannin**

Tannin in the bean samples was determined using the method described by \textsuperscript{17} with some modification. 0.5g of the sample was weighed into a conical flask and 50mls of distilled water was added. The flask was shaken for 1 hour and filtered. About 5ml of the filtrate was pipetted into a 50ml volumetric flask followed by addition of 5ml of 0.1% tannic acid. The blank was prepared using 5ml of distilled water in a 50ml volumetric flask. The three flasks were incubated for one and half hour at 20\(^\circ\)C using a water bath and the flasks were made up to 50ml mark with distilled water. The concentration was determined at 760nm using UV-Vis Spectrophotometer, DHG-9101.

The concentration of tannin was calculated using the formula:

\[
\text{Tannin (mg/l)} = \frac{x - y}{z - y}
\]

where \(x\) = concentration of extract; \(y\) = concentration = standard (tannic); \(z\) = concentration of blank.

**Proximate Analysis**

The proximate analysis of the bean samples was done using standard prescription as described by \textsuperscript{19}. The proximate composition determined include; the moisture content, ash content, crude fat content, crude protein content, crude fibre content and carbohydrate content of the samples.

**Determination of Moisture Content**

The moisture content of the samples was determined according to the standard prescription as described by \textsuperscript{19}, with the use of hot air oven. The sample was thoroughly homogenized in a domestic mixer. About 2-10g of the homogenized sample was weighed in a clean dried petri-dish pre-dried at 98\(^\circ\)C for 60 minutes. The sample was dried by heating for a period of 2 to 3 hours to overnight in a hot air oven at 100\(^\circ\)C. The sample was weighed periodically until it reaches a constant weight. The percent moisture content was calculated from the difference between the initial sample weight (\(W_I\)) and the final sample weight after drying (\(W_D\)).

\[
\% \text{ Moisture} = \frac{W_I - W_D}{W_I} \times 100
\]

Where, \(W_I\) – Initial sample weight
\(W_D\) – Final sample weight

**Determination of Crude Ash Content**

Ash content was determined by the standard prescription as described by \textsuperscript{19}, using muffle furnace. A platinum crucible was heated to 600\(^\circ\)C in a muffle furnace for 1 hour, and was cooled
in a desiccator and weighed ($W_1$). About 2g of the dried sample was weighed ($W_2$) into a crucible and heated at low flame by keeping on a clay triangle to char the organic matter. The charred material was kept inside the previously set muffle furnace and heat for 6 to 8 hours to greyish white ash and the crucible was cooled in a desiccator and weighed ($W_3$). The crucible was heated again for further 30 minutes to confirm completion of ashing, cooled and weighed. Percentage of ash content was calculated using the formula:

$$\text{% of ash content (g/100g)} = \frac{(W_3-W_1) \times 100}{(W_2-W_1)}$$

Where, $W_1$ – Weight of crucible
$W_2$ - Weight of dry matter with crucible taken for ashing
$W_3$ – Weight of crucible with ash

**Determination of Total Protein**

The total protein of the samples was determined by the standard prescription as described by $^{19}$, with the use of biuret. Series of dilution of 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard was pipetted into different test tubes. About 0.5ml and 1ml of the sample extract was pipetted into two other test tubes. The test tubes were made up to 2mls with distilled water along with the blank tubes. About 3mls of the Biuret reagent was added in all tubes and were mixed properly and incubated at 37ºC for 15minutes. The colour complex was measured spectrophotometrically at 520nm.

$$\text{Concentration of the protein (mg %)} = \frac{OD \ (test)}{OD \ (std)} \times \frac{Conc \ (std)}{Aliquot \ (test)} \times 100$$

**Determination of Crude Fat**

Crude fat of samples was determined by the standard prescription as described by $^{20,21}$, using soxhlet apparatus. About 5-10g ($W_1$) of the dry sample was weighed into a thimble and a cotton plug was kept on top of it. The thimble was placed in a soxhlet apparatus and $1\frac{1}{2}$ volumes of ether was added into a pre-weighed flat-bottom flask ($W_2$) and distilled for 16hours the apparatus was cooled and the solvent was filtered into a pre-weighed conical flask ($W_2$). The flask of the apparatus was rinsed with small quantities of ether and then added washings to the above flask. The ether was removed by evaporation and the flask was dried with the fat at 80-100ºC, cooled in a desiccator and weighed ($W_3$).

The percentage of fat content was calculated using the formula:

$$\text{Fat content (g/100%) } = \frac{(W_3-W_2) \times 100}{W_1}$$

Where, $W_1$ – Weight of dry matter taken for extraction
$W_2$ – Weight of flask bottom flask
$W_3$ – Weight of flask with flat
Determination of Total Carbohydrate

The carbohydrate contents of the bean samples were determined by the standard prescription as described by 19 and by the nitrogen free extractive (NFE) method described by 20. Series of dilution of 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard were prepared and pipetted into different test tubes. About 0.1ml and 0.2 ml of the sample solution was pipetted into two separate test tubes and each of the test tubes was made up to 1ml with distilled water. A blank test tube was set with distilled water. About 1ml of phenol solution was added to each tube followed by 5ml of 96% Sulphuric acid and was mixed very well. After 10minutes, the contents in the test tubes was mixed and placed in a water bath at 25 to 30ºC for 20minutes. The colour was read at 490nm. The amount of carbohydrate present was calculated using a standard graph. The percentage of total carbohydrate present was calculated using the formula:

Absorbance corresponding to 0.1ml of the test = X mg of glucose

100ml of the sample solution contains = \( \frac{X}{0.1} \times 100 \) mg of glucose = % of total carbohydrate present

Determination of Crude Fibre

The crude fiber of the samples was determined by the prescription as described by 19. Two gram (2g) of the dried sample was boiled with 200ml of Sulphuric acid for 30minutes with bumping chips and filtered through muslin and washed with boiling water until washings are no longer acidic. The residue was boiled with 200ml of Sodium hydroxide solution for 30minutes and filtered through a muslin cloth again and washed with 25ml of boiling 1.25% H₂SO₄, three 50ml portions of water and 25ml alcohol (Ethanol). The residue was removed and transferred to a pre weighed ashing dish (W₁) and was dried for 2 hours at 130 ± 2ºC. The dish was cooled in a desiccator and weighed (W₂). The dish was heated again for 30 minutes at 600 ± 15ºC, cooled in a desiccator and reweighed (W₃).

The percentage content of crude fibre in sample was calculated using the formula:

% Crude fibre in sample = \( \frac{\text{Loss in weight on ignition (W₂-W₁)-(W₃-W₂)}}{\text{Weight of the sample}} \times 100 \)

Statistical Analysis

All the analyses were performed in triplicate and average values calculated were expressed according to required units. Data collected were analysed by analysis of variance (ANOVA) with the IBM statistical package for social sciences (SPSS) for Windows version 23. The Bonferroni post hoc test was used to identify the means that differ significantly at p<0.05. Results were expressed as mean ± standard deviation.
RESULTS

Results of qualitative phytochemical analysis of aqueous extracts of fresh kidney bean (FKB) and cooked kidney bean (CKB) samples.

The phytochemical qualitative screening (Table 1) of aqueous extracts of both samples showed that alkaloids were very deeply present (+++) in FKB and deeply present (++) in CKB; both frothing and emulsion saponins were present (+) only in FKB; Cyanogenic glycosides were very deeply present (+++) in FKB and deeply present (+) in CKB; Phenols were very deeply present (+++) in both FKB and CKB; Tannins were deeply present (+) in both FKB and CKB; Tertioids were present (+) in CKB.

Table 1: Results of Qualitative Phytochemical Analysis of FKB and CKB Samples

| S/N | PARAMETER          | FKB   | CKB   |
|-----|--------------------|-------|-------|
| 1   | Alkaloids          | +++   | ++    |
| 2   | Flavonoids         | ND    | ND    |
| 3   | Glycoside (a)      | +++   | ++    |
|     | Cyanogenic         |       |       |
|     | (b) Cardiac        | ND    | ND    |
| 4   | Phenols            | +++   | +++   |
| 5   | Steroid            | ND    | ND    |
| 6   | Tannins            | ++    | ++    |
| 7   | Reducing Sugar     | ND    | ND    |
| 8   | Anthraquinone      | ND    | ND    |
| 9   | Tertioids          | ND    | +     |
| 10  | Saponins           |       |       |
|     | a) For frothing    | +     | ND    |
|     | b) For emulsion    | +     | ND    |

Keywords: Very deeply present (+++), deeply present (++), present (+), and not detected (ND), Fresh kidney beans (FKB) and Cooked kidney beans (CKB).

Results of quantitative phytochemical analysis of aqueous extracts of fresh kidney bean (FKB) and cooked kidney bean (CKB) samples.

The results of quantitative analysis of CKB and FKB in Table 2 revealed a significant (p<0.05) difference in the following amount of phytochemical in a decreasing order distribution: Alkaloids in FKB (27.17±0.17%) > CKB (5.8±0.01%); Flavonoids in CKB (18.27±0.24%) > FKB (10.68±0.33%); Glycosides in CKB (1.36±0.01%) > FKB (1.18±0.02%); Saponins in FKB (1.17±0.01%) > CKB (ND) and Tannins in CKB (1.04±0.01%) > FKB (0.56±0.11%).

Table 2: Results of Quantitative Phytochemical Analysis of RBEB and CBEB Samples

| S/N | PARAMETER | FKB       | CKB   |
|-----|-----------|-----------|-------|
| 1   | Alkaloids % | 27.17±0.17 | 5.8±0.01 |
2. Flavonoids % 10.68±0.33 18.27±0.24
3. Glycosides % 1.18±0.02 1.36±0.01
4. Saponins % 1.17±0.01 ND
5. Tannins % 0.56±0.11 1.04±0.01

Results are Mean ± Standard deviation for duplicate analysis; the mean difference is significant at P<0.05. Keywords: Fresh Kidney beans (FKB) and cooked kidney beans (CKB).

Results of proximate analysis of fresh kidney bean (FKB) and cooked kidney bean (CKD) samples

The proximate analysis of the bean samples is shown in Table 3 below. The results showed that moisture content of FKB (3.70±0.01%) was > CKB (3.41±0.01%); ash content of CKB (9.94±0.01%) was > FKB (9.72±0.02%); protein content of CKB (82.290%) was > FKB (22.28%); crude fibre of FKB (16.97±0.01%) was > CKB (6.58±0.01%); crude fat of FKB (14.02±0.01%) was > CKB (8.28±0.01%) and carbohydrate content of CKB (87.29±0.01%) was > FKB (82.90±0.01%).

Table 3: Results of proximate analysis of RBEB and CBEB Samples

| S/N | PARAMETER     | FKB          | CKB         |
|-----|---------------|--------------|-------------|
| 1.  | Moisture Content % | 3.70±0.01   | 3.41±0.01   |
| 2.  | Crude Ash Content % | 9.72±0.02   | 9.94±0.01   |
| 3.  | Protein Content %   | 22.28±0.01   | 82.29±0.01   |
| 4.  | Carbohydrate %      | 82.90±0.01   | 87.29±0.01   |
| 5.  | Crude fibre %        | 16.97±0.01   | 6.58±0.01   |
| 6.  | Crude fat %          | 14.02±0.01   | 8.28±0.01   |

Results are Mean ± Standard deviation for duplicate analysis; the mean difference is significant at P<0.05. Keywords: Fresh Kidney beans (FKB) and cooked kidney beans (CKB).

Keywords: Fresh kidney beans (FKB) and cooked kidney beans (CKB).

DISCUSSION

Results of phytochemical quantification revealed that alkaloids and saponins in FKB were higher than those in CKB, flavonoids, glycosides and tannins in CKB were higher than those in FKB. While proximate analysis of cooked sample (CKB) showed that protein content, crude ash content and carbohydrate content were higher than those in fresh sample (FKB). While moisture content, crude fibre and crude fat of fresh sample (FKB) were higher than those in cooked sample (CKB) Phaseolus vulgaris.

Qualitative and quantitative phytochemical results are shown in (tables 1 and 2). The results revealed higher percentages of flavonoids, glycosides and tannins in CKB than FKB and lower percentages of alkaloids and saponins in CKB than FKB. Higher concentrations flavonoids were
found in the extract of cooked bean. This was consistent with 22, who reported that cooking increases the concentrations of flavonoids fractions in *Phaseolus vulgaris* especially catechin, quercetin and quercetin-3-glucoside and kaempferol and kaempferol-3-rutinoside. Quercetin was reported to be very effective in the deterrence and treatment of cancer, inhibition of free radical generation by hydrogen peroxide, blockage of histamine generation and involved in the reversal of cognitive insufficiency 23. On the contrary, 24 assert that cooking and soaking reduce the concentrations flavonoid in *Phaseolus vulgaris*. Cooking boosted the phenolic compounds’ antioxidant ability of beans and increases its nutritive values 22. It is reported that flavonoids are the most abundant secondary metabolites in the plant and their presence in beans are appreciated as they can enhance tast, color and flavor in most beans specie when they are well cooked 22. Cooking enhanced the concentration of glycosides in the cooked extract than in the fresh. The level of flavonol glycoside in *Phaseolus vulgaris* was reported to be high in cooked *Phaseolus vulgaris* 25. Cyanogenic glycoside is classified as a significant antinutrient factor in plants which must be reduced or destroyed for human and animal food consumption 26. The tannin concentration of cooked *Phaseolus vulgaris* is higher than the fresh sample. Like other dark colour legumes, *Phaseolus vulgaris* (red kidney beans) is known to have high content of tannins and phytic acid than white beans 27. This could support the high level of tannins in the cooked sample, that even after cooking, the percentage was still high. However, 28 reported contrary reduced tannin content in *Phaseolus vulgaris* after cooking. Tannins are included as antinutrients in legumes which inhibit the digestion of protein but are destroyed by cooking to increase the functions of amylase and protease and better protein digestion 29. Dietary tannins, flavonoids and saponins found in *Phaseolus vulgaris* like other plant sources, are known for their medicinal importance in the preparation of herbal formulation as composite active ingredients to partake in cancer prevention, function as antioxidants and possess anti-inflammatory ability 30,31. Cooking reduced the content of alkaloids in cooked extract of *Phaseolus vulgaris*. Different toxic alkaloids are said to be present in beans especially lupines spp, such as pyrrolizidine and piperidine alkaloids 32. However on the contrary, 33 reported that alkaloids applications in medicine are suited for their non toxicity for spectacular physiological functions. The low alkaloid content in the cooked extract of *Phaseolus vulgaris* was in agreement with a previous work reported by 32, that after the treatment of soaking and cooking, the alkaloid content of Lupin Bean was significantly reduced. On the contrary, 34 reported high level of alkaloid in *Balanites aegyptiaca* kernel.

Proximate analysis of cooked sample (CKB) showed that protein content, crude ash content and carbohydrate content were higher than those in fresh sample (FKB). While moisture content, crude fibre and crude fat of fresh sample (FKB) were higher than those in cooked sample (CKB) *Phaseolus vulgaris*. It was observed that cooking increased the concentration of protein. By contrast, 35 reported that heat treatment of pressure cooking and boiling had reductive effect of amino acids including lysine, methionine and tryptophan in *Vigna unguiculata* and protein was reported to be significantly reduced by boiling in *Vigna Sesquipedalis* 36. In a diet combination experiment, the rich protein content in beans was reported to induce the higher weight loss in an 8 week period among 35 obese men 37. Protein consumption in legumes was found to be associated to decrease in blood pressure, total cholesterol, waist circumference and body fat mass 37. Similarly, a slightly higher crude ash was found in the cooked extract of *Phaseolus vulgaris*. This finding is in contrast to that reported by 35, that boiling reduced the content of ash in beans. The amount of mineral present in plant is attributed to its ash content, thus increased crude ash
could suggest high mineral contents in *Phaseolus vulgaris* extract. Higher amount of carbohydrate was found cooked extract than in fresh extract. This is in agreement with, who reported that boiling for 40 minutes significantly increased the carbohydrate content of *Phaseolus vulgaris* 58.70 ± 0.7% to 63.37 ± 0.11% (8% increase), a value which is well lower than the one reported here, indicating an effect of cooking on carbohydrate content in *Phaseolus vulgaris*. Carbohydrates contain resistant starch and cooking of legumes in boiling water is reported to decreases the resistant starch content because of destruction of a huge part of the crystalline regions. However, due to great amount of amylase in legumes and a stable form of crystalline amylase, an infinitesimal amount of resistant starch is eventually destroyed by cooking legumes. Because the metabolism of resistant starch does not occur in the small intestine, glucose are unconfined into the blood, therefore the need for insulin and food calorific density, which may tend to obesity are reduced. Furthermore, resistant starch in beans increases satiety, reduced glycemic index, reduced systolic blood pressure, decreased risk of coronary heart disease and prevent rise in blood glucose level. The moisture content of this finding was higher in fresh extract than in cooked extract of *Phaseolus vulgaris*. This is consistent with, who reported that heat treatment by boiling and roasting significantly reduced the moisture content of *Vigna Sesquipedalis* but in contrast to, who reported that boiling increased the moisture content of *Vigna unguiculata*. Reduced moisture content in legumes plays a role in food storage and preservation as it guarantees a microbial growth inhibition. The moisture content was reduced possibly because of the high temperature subjection which favors evaporation of the cooked extract for the period it was cooked. Crude fiber of cooked *Phaseolus vulgaris* extract was found to be reduced. This is in agreement to, who reported that boiling and roasting reduced crude fiber content in *Vigna Sesquipedalis* and, were crude fiber was reported to be reduced after boiling. Beans generally are rich sources of both soluble and insoluble fiber which play a very important role in bowel movement function. The presence of fiber in food helps to keep proper functioning of digestive system and provides satiety or fullness. It was observed that cooking significantly reduced the crude fat. The work of is in agreement with this finding as it was reported that boiling and roasting reduced the fat content as time increases in *Vigna Sesquipedalis*. Similarly, reported reduced content of crude fat after boiling of *Vigna unguiculata*. Beans are reported to contain little or no total fat, trans-fat, sodium and cholesterol. Little or no saturated fat content in cooked beans is recommended for the reduction of cardiovascular risk factors such as raised blood levels of triacylglycerol and low density lipoprotein cholesterol.

**CONCLUSION**

Cooking affected the nutrient contents of *Phaseolus vulgaris* in this study. Cooking increased the levels of flavonoids, carbohydrate and protein contents. Thus consumption of cooked beans may boost the antioxidant potential of the body, reduce blood pressure, total cholesterol, waist circumference, glycemic index, systolic blood pressure, decreased risk of coronary heart disease, prevent rise in blood glucose level and increase satiety.

**CONFLICT OF INTEREST**

Authors have declared that no conflict of interest is linked with this work.

**References**
1. Gentry HS. Origin of the Common Bean, Phaseolus vulgaris. Economic Botany. New York: New York Botanical Garden Press 1999; 23 (1): 55–69.
2. Brágida P, Canniatti-Brazaca SG. Antinutrients and in vitro availability of iron in irradiated common beans (Phaseolus vulgaris). Food Chemistry 2006; 98: 85-89.
3. Julie G, Krystle M. All About Beans Nutrition, Health Benefits, Preparation and Use in Menus. North Dakota State University, Fargo, North Dakota 2019;1-16.
4. Slupski, J. Effect of cooking and sterilisation on the composition of amino acids in immature seeds of flageolet bean (Phaseolus vulgaris L.) cultivars. Food Chemistry 2010; 121(4):1171-1176.
5. Wang N, Hatcher DW, Tyler RT, Toews R, Gawalko EJ. Effect of cooking on the composition of beans (Phaseolus vulgaris L.) and chickpeas (Cicer arietinum L.). Food Res. Int 2010; 43(2): 589-594.
6. Garcia JS, Magalhães CS, Arruda MAZ. Trends in metal-binding and metalloprotein analysis. Talanta 2006;15;69(1):1-15.
7. Bressani R. Grain quality of common beans. Food Rev. Int 1993; 9(2): 237-297
8. Juliana N, Pedro VO. Cooking effects on iron and proteins content of beans (Phaseolus Vulgaris L.) by GF AAS and MALDI-TOF MS. J. Braz. Chem. Soc 2012;23(1):0103-5053.
9. Ramírez-Cárdenas L, Leonel AJ, Costa NMB, Reis FP. Zinc bioavailability in Legumes. Food Res. Int 2010; 43:573.
10. Mercy JN. Food Safety; Take life easy; eat, drink and be merry. Luke 12: 19b. Ghana Med J 2005;39(2): 44–45.
11. Hoppner K, Lampi B. Folate retention in dried legumes after different methods of meal preparation. Food Res Int 1993;26:45-48.
12. United States Food and Drug Administration, Health and Human Services: Code of Federal Regulations. Section 101.79 — Health claims: folate and neural tube defects. 2012:2.
13. Quinteros A, Farre R, Lagarda MJ. Optimization of iron speciation (soluble, ferrous and ferric) in beans, chickpeas and lentils. Food Chemistry 2001; 75:365.
14. Harborne JB. Phytochemical Methods, London Chapman and Hall Limited. 1973; 49-188.
15. Bohm BA, Koupaï-Abiyazani MR. Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium reticulatum and V. calycinum (Ericaceae). Pac Sci 1994;48(4): 458-463.
16. Obadoni BO, Ochiko PO. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science 2001; 8: 203-208.
17. Amadi ES, Nwakpu K, Nworie O, Ogbu O. Evaluation of the in vitro effects of some medicinal plants on Escherichia coli. J. Health Visual Sci 2004; 6:176-179.
18. Trease GE, Evans WC. Pharmacognosy. A physician guide to herbal medicine, 11edn. Ballere Tindal, London, United Kingdom 1989; 530.
19. Association of Official Analytical Chemists (AOAC). Official methods of Analysis, (18th edn). 18th ed. Washington, DC USA 2005
20. Pearson DA. The clinical analysis of food, (7th edn.) Churchill Livingstone, Edinburgh 1976.
21. James CS. Analytical Chemistry of Foods. Backie Academic and Professional New York 1996.
22. Huber K, Brágida P, Bretas EB, Canniatti-Brazaca SG. Phenolic Acid, Flavonoids and Antioxidant Activity of Common Brown Beans (Phaseolus vulgaris L.) Before and After Cooking. J Nutr Food Sci 2016; 6: 551. doi:10.4172/2155-9600.1000551.
23. Rauter AP, Dias C, Martins A, Branco I, Neng NR, et al. Non-toxic Salvia sclareaoides Brot. Extracts as a source of functional food ingredients: phenolic profile, antioxidant activity and príon binding properties. Food Chem 2012;132: 1930-1935.

24. Xu BJ, Chang SKC. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (Phaseolus vulgaris L.) as affected by thermal processing. J Agric Food Chem 2009; 57: 4754-4764.

25. Pitura K, Arntfield SD. Characteristics of flavonol glycosides in bean (Phaseolus vulgaris L.) seed coats. Food Chemistry 2019; 272:26-32.

26. Soetan KO, Oyewole OE. The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. African Journal of Food Science 2009; 3(9): 223-232.

27. Mamiro PS, Mwanri AW, Mongi RJ, Chivaghula TJ, Nyagaya M, Ntwenya J. Effect of cooking on tannin and phytate content in different bean (Phaseolus vulgaris L.) varieties grown in Tanzania. African Journal of Biotechnology 2017; 16(20):1186-1191

28. Nuzhat H, Muhammad A, Samreen S, Muhammad IK, Shahzad H. Effect of soaking and cooking on nutritional quality and safety of legumes. Nutrition and Food Science 2008; 38(6):570-577

29. Bressani R. Bean grain quality—a review. Arch Latinoam Nutr 1989;39:19–42.

30. Nwali ON, Idoko A, Okolie JE, Ezeh E, Ugwudike PO, Rita ON, Ezenwali MO, Odo IA, Ani PN and Okolie SO. Comparative analysis of the phytochemical compositions of leaf, stem-bark and root of Azadirachta Indica (neem). Universal Journal of Pharmaceutical Research 2018; 3(5): 46-50.

31. Oskouiean E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Ho YW. Antioxidant, anti-inflammatory and anti-cancer activities of methanolic extracts from Jatropha curcas Linn. J Med Plants Res. 2011; 5: 49–57.

32. Yadesa A, Biadge K. Effect of some traditional Processing Methods on Nutritional Composition and Alkaloid Content of Lupin Bean. International Journal of Bioorganic Chemistry 2017;2(4):174-179

33. Olaofe O, Sanni CO. Nutritional Component of some non-conventional leaf Vegetable consumed in Cameroon. Food Chemistry 1988; 30:73-77.

34. Alhassan AJ, Muhammad IU, Idi A, Danagmbo MA, Ramatu Y, Mohammad A, Nasir A, Yaradua AI, Adamu SM, Alexander I. Phytochemical Screening and Proximate Analysis of Balanites aegyptiaca Kernel. Food Science and Quality Management 2018; 74

35. Omenna EC, Olampekin OT, Kolade RO. Effect of boiling, pressure cooking and germination on the nutritional and antinutrients content of cowpea (Vigna unguiculata). ISABB Journal of Food and Agriculture Science 2016; 6(1): 1-8. DOI: 10.5897/ISABB-JFAS2016.0036.

36. Nzewi D, Egbuonu ACC. Effect of boiling and roasting on the proximate properties of asparagus bean (Vigna Sesquipedalis). African Journal of Biotechnology 2011; 10(54): 11239–11244. DOI: 10.5897/AJB11.452.

37. Abete I, Parra D, Martinez JA. Legume-, fish-, or high-protein-based hypocaloric diets: effects on weight loss and mitochondrial oxidation in obese men. J Med Food 2009;12(1):100-108.

38. Guy C. Ask the Expert: Legumes and Resistant Starch. The Nutrition Source 2015. Retrieved from www.hsph.harvard.edu/guy-crosbyl/

39. Behall KM, Howe JC. Contribution of Fiber and Resistant Starch to Metabolizable Energy. Am. J. Clin. Nutr 1995; 62: 1158S-1160S.

40. Jenkins DJA, Kendall CWC, Augustin LSA, Mitchell S, Sahye-Pudaruth S, Mejia SB, et al. Effect of legumes as part of a low glycemic index diet on glycemic control and cardiovascular risk factors in type 2 diabetes mellitus. Arch Intern Med 2012; Epubdoi:10.1001/2013.jamainternmed.70
41. Ejikeme PM, Obasi LN, Egbuonu ACC. Physico-chemical and toxicological studies on afzelia Africana seed and oil. Afr. J. Biotechnol 2010; 9(13): 1959-1963.

42. United States Department of Agriculture, Health and Human Services. Chapter 4 in Dietary Guidelines for Americans, 7th Edn, Washington, DC: U.S. Government Printing Office 2010.

43. United States Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page 2012.