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Chemical composition, glycemic index and risk assessment of selected bean species

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Abstract

Beans (*Vigna Spp*) is one of the most important legumes consumed in Nigeria due to their high nutritional value and affordability. These studies aimed to investigate the chemical composition, glycemic index and risk assessment of selected bean species, Honey beans (*vigna unguiculata*) and Kampala beans (*Phaseolus lunatus*) using standard analytical methods. Honey beans contained higher moisture, carbohydrate, potassium, calcium, zinc, phenols, tannins, and glycosides, while Kampala beans had higher protein, fibre, fat, ash, sodium, flavonoids, and saponins. Both varieties showed low glycemic index values (48.69 and 48.21), making them suitable for healthy diets. Glutamic acid was the predominant amino acid in both samples, with Honey beans richer in leucine and Kampala beans higher in lysine. Lead was not detected, and Target Hazard Quotient (THQ) values were below one, indicating no significant health risk. Overall, both bean varieties are safe and highly nutritious. Their complementary nutrient profiles can enhance dietary protein quality, mineral intake, antioxidant capacity, food security, and overall human health.

Key words: *Honey beans, Kampala beans, Chemical composition, Glycemic index, Risk assessment, Mineral composition*

1. Introductiion

1.1. Background of the study

The beans (Vigna unguiculata [Linn.] Walp varieties) are one of the most important food crops grown and eaten all over the world, especially in sub-Saharan Africa, which includes Nigeria. They are a significant dietary source of protein, carbohydrates, dietary fibre and micronutrients that greatly contribute to human nutrition and food security. Beans are also a significant source of income and livelihood in many households. The Food and Agriculture Organization (FAO) (2024), International Food Policy Research Institute (IFPRI) (2024) and the World Bank (2024) all show Nigeria as the top bean producer of West Africa with over half of

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the region's bean production. Although beans are nutritious foods, their chemical composition and mineral content is highly variable due to a number of factors, including soil characteristics, climatic conditions, farming practices, and the variety being grown (FAO, 2024; IFPRI, 2024). The cultivation of various bean types namely Kampala beans (*Phaseolus lunatus*), honey beans (*Vigna unguiculata*), is common in Nigeria, and regional differences are dependent on climatic conditions, soil types and farming practices (Saka *et al.*, 2004).

The very high consumption of beans in Nigeria, warrants the noteworthy research interest of their nutritive value, chemical composition and consumption suitability. The chemical composition and mineral content of these varieties of beans are important to understand in order to evaluate their nutritional value to humans. In addition, analyzing the potential risks associated with contaminants such as heavy metals (Ailenokhuoria and Omolekan, 2019).

1.2. Statement of problem

In fact, beans are eaten all over the world and are regarded as a nutritious and versatile food item. Although Kampala and honey beans are consumed in Nigeria, comprehensive data on their chemical composition, mineral content, and potential risk of heavy metal contamination are limited. The uncontrolled application of agrochemicals and environmental pollution has the risk of the accumulation of toxic elements (as in food crops: Pb, Cd). Consumer safety could be at risk if they are unaware of the safety of consuming these beans, which are commonly found in their diet. They are vulnerable to environmental contamination and therefore, to food safety and quality issues. Thus, this research project aims to address this knowledge gap regarding the deficiency of data and the effect of environmental factors on the various types of beans in Nigeria's markets.

1.3. Justification of the study

Beans are an integral part of the food supply in the world and a major source of key nutrients for millions of people around the world. Beans are an important food source and have a key role in the food security and public health. Hence, the need to research and minimize the impacts of environmental factors on beans to continue safe, healthy, and nutritious beans for global communities. To collect as much as possible of the various types of bean sold in our market, more important physicochemical data.

1.4. Scope

Types of beans: The study focuses on commonly consumed bean varieties in Nigeria, such as Kampala and honey beans.

Chemical composition analysis: The study will determine the proximate composition (moisture, protein, fat, fiber, and ash), mineral content (macro and micro elements), and anti-nutritional factors (phytates, tannins, and oxalates) phytochemicals, amino acid profile and glycemic index in the bean's samples.

Risk assessment: The study will evaluate the potential health risks associated with the consumption of the beans, considering factors like heavy metal contamination.

Statistical analysis: The study will apply statistical tools to determine correlations and significance levels between variables.

1.5. Aim

This study seeks to investigate the proximate composition, amino acid profile, phytochemical content, glycemic index, mineral and potential health risks associated with Kampala and honey bean varieties cultivated in Nigeria, with the goal of generating data that supports their nutritional evaluation and ensures their safety for human consumption.

1.6. Objectives of the study

The objectives of the research work on Kampala and honey beans are to carryout:

1. Proximate composition (moisture, protein, fat, fiber, and ash) and mineral content.
2. Phytochemicals and antioxidat properties.
3. Amino acid profile (essential amino profile, non-essential amino profile and protein quality).

4. The glycemic index (i.e., a measure of how quickly a carbonhydrate-containing food raises blood glucose level).
5. Risk assessment (such as heavy metals and anti- nutritional factors).

2. Materials and methods

2.1. *Materials and their preparation*

The kampala and honey beans seeds were purchased from Akure, Nigeria market. They were sorted out and sun-dried. The dried sample were ground into powder, kept in an airtight container and put in a refrigerator at 4 °C prior to analysis.

2.2. *Methods*

2.2.1. Determination of proximate composition

Analyzing the proximate composition of the beans involves determining the moisture content, ash content, crude fat, crude protein, crude fiber, and total carbohydrates. The total carbohydrates are calculated by subtracting the percentage sum of all other parameters from 100 (AOAC, 2005).

2.2.2. Determination of moisture content

About 5.0 g of the sample were weighed into pre-weighed crucible (W_1) and placed into a hot drying oven at 105 °C for 24 hours. The crucible was removed, cooled in a desiccator, and weighed. The process of drying, cooling, and weighing was repeated until a constant weight (W_2) was obtained and the weight loss due to moisture was obtained by the equation below.

$$Moisture\ Content(\%) = \frac{(Weight\ Loss)}{(Weight\ of\ Sample)} \times 100$$

$$= \frac{(W_1 - W_2)}{(5\ g)} \times 100$$

W_1 = Weight of fresh sample and empty crucible.

W_2 = Weight of dried sample and empty crucible (AOAC, 2005).

2.2.3. Determination of ash content

Marked crucibles were tarred in a muffle furnace at 500-550 °C for 2-3 hours. The temperature was lowered to 180 °C and the crucibles were transferred into a desiccator, cooled and weighed (W_1). About 2.0 g of sample each was weighed into the pre-weighed crucible and reweighed (W_2). The sample were charred over a hotplate in a fume cupboard until smoking ceased. The crucibles with their contents were transferred into the furnace and incinerated at 500 °C until the residue were uniformly white. The temperature of the furnace was decreased to 180 °C and the crucibles were transferred into a desiccator, cooled and reweighed (W_3). The process of heating in the furnace, cooling in a desiccator, and weighing on a chemical balance was repeated until a constant weight was obtained. The ash content was obtained thus:

$$\%Ash\ Content = \frac{(Weight\ of\ Ash)}{(Weight\ of\ Sample)} \times 100$$

$$= \frac{(W_3 - W_1)}{(2\ g)} \times 100$$

W_1 = Weight of empty crucible (g).

W_2 = Weight of empty crucible + sample before drying (g).

W_3 = Weight of empty crucible + ash (g) (AOAC, 2005).

2.2.4. Determination of crude fat content

Fat was determined using Soxhlet extraction method. About 3.0 g of each of the samples (W_1) was weighed into a previously dried and weighed filter paper and then weighed (W_2). The filter papers with their contents were carefully placed into a clean and dry thimble in the Soxhlet apparatus. This was connected to a clean and dry 500 ml round bottom flask containing 300 ml of n-Hexane. Then the condenser was connected and the flask was heated on the heating mantle to the boiling (60 °C). The whole system was allowed to reflux for 6 hours, after which the solvent was distilled off and collected for reuse. While the defatted samples were collected, dried in the oven at 60 °C and then re-weighed (W_3). The percentage crude fat was calculated from the weight loss as follows.

$$\%Crude\ Fat\ Content = \frac{(Weight\ of\ Oil\ Obtained)}{(Weight\ of\ Sample)} \times 100$$

$$= \frac{(W_2 - W_3)}{W_1} \times 100$$

W_1 = Weight of sample (g).

W_2 = Weight of filter paper + sample before extraction (g).

W_3 = Weight of filter paper + defected sample after extraction (g) (AOAC, 2005).

2.2.5. Determination of crude fibre

The defatted samples obtained in Section 3.2.1.3 were transferred into 250 ml conical flasks, 200 ml of 1.25% H_2SO_4 was added, and the mixture was boiled under reflux for 30 minutes. The solution was filtered through a poplin cloth; the residue was rinsed thoroughly with hot distilled water until it was no more acidic, then tested using pH paper. The residue was transferred back into a 250 ml conical flask, and 200 ml of 1.25% NaOH was added and boiled under reflux for 30 minutes after which it was filtered and rinsed with hot distilled water until the filtrate was neutral, when tested with pH paper. The residue was rinsed once with 10% HCl and four times with hot distilled water. Finally, the residue was carefully salvaged into previously tarred and weighed crucible (W_1), and placed in electric oven at 100 °C for 4 hours to dry. It was then removed and placed in a desiccator to cool before re-weighing (W_2). After weighing, the residue was incinerated in the muffle furnace for 4 hours at 500 °C, cooled in a desiccator, and re-weighed (W_3). The percentage crude fiber was calculated as follows.

$$\%Crude\ Fibre\ Content = \frac{(Weight\ of\ Oil\ Organic\ Mattrer\ Left)}{(Weight\ of\ Sample)} \times 100$$

$$= \frac{(W_2 - W_3)}{Weight\ of\ Sample} \times 100$$

W_2 = weight of fibre + ash.

W_3 = weight of ash.

2.2.6. Determination of crude protein

Protein content of the sample was determined using the Kjeldahl method. The method is based on the digestion of protein and other organic food components in the sample with sulfuric acid in the presence of catalysts, e.g., sodium or potassium sulfate to release nitrogen from protein and retain it as ammonium salt. Ammonial gas was liberated upon addition of excess alkali (concentrated sodium hydroxide) and is distilled into a boric acid solution to form ammonium-borate complex. The ammonia liberated from the complex was titrated with standardized hydrochloric acid. The amount of nitrogen in the sample was determined from the milligram

equivalent of the acid used. Crude protein was determined by multiplying the nitrogen content with a conversion factor of 6.25 to obtain the protein content. The determination was carried out in three stages.

A. Digestion: 0.5 g of each sample was weighed into a clean and dried Kjeldahl digestion flask. A tablet of Kjeldahl catalyst was added to it. 10 ml of concentrate H_2SO_4, was also added to the flask and digested electro thermally inside the fume cupboard until the sample was completely digested and a clear greenish blue solution was obtained which was allowed to cool. The solution was carefully removed and then diluted to 100 ml distilled water in a 100 ml volumetric flask.

Sample

$$N_{(s)} + \text{excess } H_2SO_{4(aq)} \longrightarrow (NH4)_2SO_{4(aq)}$$

B. Distillation: Exactly 5 ml of 2% boric acid was transferred into a clean 100 ml Erlenmeyer flask, and 2 drops of mixed indicator was added. This was connected to the delivery tube with the tip of the tube just below the surface of the boric acid-indicator mixture. Then 5 ml digest was measured into the Kjeldahl distillation apparatus. 10 ml of 40% NaOH was added (to neutralize the excess acid) and the mixture was distilled until a total of 50 ml distillate was collected into the receiving flask containing boric acid. The condenser was rinsed with distilled water into the receiver before titration was carried out.

C. Titration: The distillate collected was titrated with standardized 0.01 M HCl. The end point of the titration was observed when the color of the distillate changed to the initial color of the mixture of boric acid and mixed indicator (green to wine red). The volume of acid used was recorded. The whole process was carried out for each of the sample in replicates, and blank. Percentage nitrogen in each sample was calculated from the relation

Nitrogen (%) = M/W × T × 14 × 0.014 × V1/V2 × 100,

where

M = Molarity of acid.

T = Titer value = volume of acid used for sample-volume of acid used for the blank.

V1 = Final volume of digest.

V2 = Volume of the digest distilled.

W = Weight of sample digested.

$$NH_{4^+(aq)} + H_2BO_{3^-(aq)} + HCl_{(aq)} \longrightarrow NH_4Cl_{(aq)} + H_3BO_{3(aq)}$$

The protein content was obtained by multiplying the percentage nitrogen obtained above by 6.25.

2.2.7. Determination of carbohydrate

Carbohydrate content for each sample was calculated by difference.

Total carbohydrate (%) = 100 - (% moisture + % crude protein + % crude lipid + % crude fiber + % ash) (AOAC, 2005).

2.2.8. Determination of mineral composition of bean seeds

The standard procedures recommended by the Association of Official Analytical Chemists (AOAC, 2025), was employed in the analysis of minerals. The ashes from the proximate analysis were digested with 10% HNO_3, the resultant solutions were each fitted and made up to 100 ml with deionized water. Then Na and K were determined using flame emission spectrometer. Other minerals such as Ca, Zn and Pb were carried out by the use of Atomic Absorption Spectrophotometer (AAS).

2.2.8.1. Phytochemical screening analysis (qualitative)

Phytochemicals present in the samples were determined by standard methods. Qualitative chemical tests were carried out on each sample to determine the presence of phytochemicals constituents as described by Harborne (1984), Trease and Evans (1989) and Sofowora (2006).

Test for steroids: A red color produced in the lower chloroform layer, when 0.5 g each of the sample was dissolved in 2 ml of chloroform and 2 ml concentrated sulfuric acid added indicates the presence of steroids.

Test for terpenoids: Exactly 0.5 g each of the samples was dissolved in 2 ml of chloroform and it was evaporated to dryness. 2 ml of concentrated sulfuric acid was then added and heated for about 2 minutes. A grayish color indicates the presence of terpenoids.

Test for flavonoids: To 0.5 g each of the samples was added 10 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for tannins: Exactly 0.5 g each of the samples was stirred with 20 ml of distilled water and few drops of $FeCl_3$ solution was added. The formation of a green precipitate was an indication for the presence of tannins.

Test for saponins: About 0.5 g each of the sample was shaken rigorously with 20 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

Test for alkaloids: About 0.5 g each of the sample was stirred with 5 ml of 1% HCl on the steam bath. Mayer's and Wagner's reagent were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for glycosides: Exactly 0.5 g each of the sample was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution placed in ice. Sulfuric acid was then added carefully. A color change from violet to blue to green indicates the presence of steroidal nucleus (that is a glycone portion of glycoside).

Test for phenolic content: About 0.5 g each of the samples was stirred with 5 ml 10% $FeCl_3$ solution. The formation of greenish-black color indicated the presence of phenolics.

2.2.8.2. Phytochemical screening analysis (quantitative)

Extraction of sample: About 10 grams of the prepared sample were extracted with 50 ml solution of 1 M HCl and methanol (1:1 v/v) and then filtered under vacuum. The filtrate was concentrated using rotary evaporator at 45 °C. Then 1 g each of the extract was reconstituted with 100 ml of distilled water and stored in a refrigerator prior to analysis.

2.2.9. Determination of total phenol

The total phenolic content of the extract was determined using the Folin–Ciocalteu method as described by Singleton and Rossi (1965). Briefly, 0.2 ml of the fat-free extract was mixed with 2.5 ml of 10% Folin–Ciocalteu reagent and 2.0 ml of 7.5% (w/v) sodium carbonate solution. The reaction mixture was incubated at 45 °C for 40 min, after which the absorbance was measured at 760 nm using a UV–Visible spectrophotometer. Gallic acid was used as the standard, and the total phenolic content was determined from the gallic acid calibration curve.

2.2.10. Determination of tannins

The spectrophotometric determination of tannin was carried out according to the method of Makkar *et al*. (1993) by extracting 0.2 g of the powder sample (W) with 70% (v/v) acetone. The mixture was placed in an ice bath to prevent acetone from evaporating. The mixture was placed on a mechanical shaker for 12 minutes and then filtered. 0.5 ml of the sample extract (V), aliquots were mixed with equal volume of water and 2.5 ml folin ciocaltau reagent (diluted 1:1 with distilled water) was added followed by 2.0 ml of 10% (w/v) sodium carbonate solution and the mixture were incubated at room temperature for 40 minutes. The absorbance was measured at 700 nm using a spectrophotometer to obtain the value (R) from the calibration curve prepared using standard tannic acid solution 0.5 mg/ml. From the calibration curve, the concentration of tannin in the sample were obtained from the equation:

$$\text{mg/g TA} = \frac{R \times 10}{W \times V}$$

Where:

R = mg/ml of tanning from the calibration curve.

V = volume of sample extract analyzed = 0.5 ml.

W = weight of sample extracted.

10 ml = total volume of extract.

2.2.11. Determination of saponin

Saponin was determined using the procedure according to Oloyede (2005). Exactly 2.0 g each of the sample (W) in a conical flask was added 100 ml of 20% (v/v) aqueous ethanol. The samples were heated over a hot water bath for 4 hours at 55 °C with constant shaking. The mixtures were filtered and the residue were re-extracted using 100 ml of 20% aqueous ethanol. The combined extract of each sample was reduced to 40 ml over a water bath at about 90 °C. Each of the concentrates was transferred into 250 ml separatory funnel and 20 ml of diethyl ether was added, shook vigorously and allowed to separate. The aqueous layer (lower portion) was recovered while the ether layer was discarded. The purification process was repeated. The combined aqueous extracts were extracted using 60 ml of n-butanol. Then the combined n-butanol extract (upper portion) were washed twice with 10ml of 5% aqueous NaCl. Finally, the remaining solution was heated over hot water bath to evaporate to dryness and then dried in the oven to constant weight (W_e). The saponin content was calculated as percentage from the equation.

$$\text{Saponin}(\%) = \frac{W_e}{W} \times 100$$

Where, We = Weight of Saponin residue.

W = Weight of sample used.

2.2.12. Determination of total flavonoid

About 5.0 g of each sample (W_t) was placed in a 250 ml beaker, and 200 ml of 10% (v/v) acetic acid (CH_3COOH) in ethanol (C_2H_5OH) was added. The mixture was covered and allowed to stand for 4 hours at 25 °C. It was then filtered with filter paper (W_1) and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added drop-wise until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a previously dried and weighed filter paper (W_1). The residue was washed with NH_4OH. The precipitate, called alkaloid, was dried and weighed (W_2). The percentage alkaloid was calculated by difference from:

$$\text{Alkaloids}(\%) = \frac{W_2 - W_1}{W_t \text{ of Sample}} \times 100$$

W_1 = Weight of empty (previously dried) filter paper.

W_2 = Weight of filter paper + dried precipitate (alkaloid).

Wt = Weight of sample used (5.0 g).

2.2.13. Determination of glycemic index

The *in vitro* method of Oboh *et al.* (2015) as modified by Amoo *et al.* (2023), was used. This approach is designed to replicate how starch is digested in the gastrointestinal tract (GIT). The oral phase was stimulated by mechanically breaking down 10 grams of the food sample. The gastric phase was developed for 60 minutes at 40 °C with 10 ml of HCl-KCl buffer (pH 1.5) and 0.2 mg of pepsin. The intestinal phase was carried out in 7.5 ml of sodium-potassium-phosphate buffer (0.05 M, pH 6.9) 2.5 ml of alpha-amylase solution (0.05 g/10 ml) was added and incubated at 37 °C in a shaking water bath for 60 minutes. On expiration of the time, 0.2 ml aliquot was taken from each of the tube at 0, 30, 60, 90, and 120-minute interval and boiled on a water bath at 100 °C for 5 minutes to inactivate the enzymes. After boiling, 0.5 ml of sodium-acetate buffer (0.4 M, pH 4.7) was added and the residue starch digested to glucose by adding 7 ml of alpha-glucosidase solution extracted from swine gut in phosphate buffer (1:4). The mixture was incubated for 45 minutes at 60 °C, after which 0.2 ml of 3,5-dinitrosalicyclic acid (D-NSA) was added. The mixture was boiled again at 100 °C on a water bath for 5 minutes. About 2 ml of distilled water was added and the mixture centrifuged at 2000 rpm for 10 minutes. Finally, the absorbance of the supernatant was taken at 450 nm using spectrophotometer. Glucose-D from

Evans Nigerian Limited was also analyzed as reference product. The rate of starch digestion was expressed as the percentage of starch hydrolyzer per time using glucose standard curve. A non-linear model established by Goni *et al.* (1997) was applied to describe the kinetic of starch hydrolysis. Valued for the Area Under Curve (AUC) were obtained for each of the starch hydrolysis curves and standard from which the Glycemic Index (GI) was calculated using equation.

$$\mathrm{GI}(\%) = \frac{\text{AUC of Sample}}{\text{Average AUC of Std}} \times 100$$

2.2.14. Determining the amino acid profile

The Amino Acid profile in the samples were determined using the methods described by AOAC (2006). However, the method was slightly modified. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting sample: The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 5 g of the sample was put in extraction thimble (or filter paper) and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen determination: A small amount (150 mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put in the Kjelcihal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 M hydrochloric acid to grey colored end point.

$$\text{Percentage Nitrogen} = \frac{(a\text{-}b) \times 0.01 \times 14 \times V}{W \times C} \times 100$$

Where:

a – Titre value of the digested sample.

b – Titre value of blank sample.

V – Volume after dilution (100 ml).

W – Weight of dried sample (mg).

C – Aliquot of the sample used (10 ml).

14 – Nitrogen constant in mg.

2.3. Hydrolysis of the sample

About 5.0 g of the defatted sample was weighed into glass ampoule. 7 ml of 6 M HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis, e.g., methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 °C ± 5 °C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6 M HCl. During acidic hydrolysis, asparagine and glutamine are transformed into aspartic and glutamic acid respectively, and cysteine and methionine are partially oxidised (Peace and Gilani, 2005).

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

2.4. Loading of the hydrolysate into analyzer

The amount loaded was 60 microlitre. Norleucine (10 microlitre) was added to the sample and amino acid standard mixture. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Norleucine is an internal standard. It corrects any variation that may occur in the sample and amino acids standard mixture.

2.5. Method of calculating amino acid values

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

2.5.1. Determination of tryptophan

Tryptophan is a difficult amino acid to determine in proteins and peptides because it chemically decomposes during acid hydrolysis. It should be noted that tryptophan is destroyed by 6 N HCL during hydrolysis.

Antioxidants such as thioglycolic acid or dodecanethiol have been used to preserve tryptophan. Alkaline hydrolysis has also been studied and was shown to produce higher tryptophan recovery than acid hydrolysis. The addition of phenol has also been reported. Alkaline hydrolysis was improved by using sodium hydroxide (NaOH) instead of barium hydroxide to prevent problems with both precipitation and adsorption of tryptophan.

Determination: Tryptophan was determined by alkaline hydrolysis using 4.2 M sodium hydroxide (NaOH) according to the method described by Yust *et al.* (2004). Approximately 5.0 g of the dried, defatted sample was hydrolysed with 4.2 M NaOH under the prescribed conditions. After hydrolysis, the hydrolysate was concentrated using a rotary evaporator and subsequently analysed using an Applied Biosystems PTH Amino Acid Analyzer for the determination of tryptophan content.

Defatting sample: About 5.0 g of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006) the extraction lasted for 15 hrs.

Nitrogen determination: A small amount (200 mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put in the Kjelcihal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ration of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 M hydrochloric acid to grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

$$\text{Percentage Nitrogen} = \frac{(a\text{-}b)\times 0.01\times 14\times V}{W\times C}\times 100$$

Where:

a – Titre value of the digested sample.

b – Titre value of blank sample.

V – Volume after dilution (100 ml).

W – Weight of dried sample (mg).

c – Aliquot of the sample used (10 ml).

14 – Nitrogen constant in mg.

2.6. Hydrolysis of the sample

About 5.0 g of the defatted sample was weighed into glass ampoule. 10 ml of 4.2 M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 °C ± 5 °C for 4 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of borate buffer (pH 9.0) and store in plastic specimen bottles, which were kept in the freezer.

2.7. Protein quality evaluation

The protein quality of the bean samples was evaluated using the Essential Amino Acid Index (EAAI), a method that was first introduced by Oser (1951) and later utilized in various nutritional studies (FAO/WHO, 2013). The EAAI works by comparing the Essential Amino Acid (EAA) profiles of our test protein against a reference protein, calculating the geometric mean of their ratios. The formula is presented as follows:

$$EAAI = \left(\prod_{i=1}^{n} \frac{a_i}{r_i} \right)^{\frac{1}{n}} \times 100$$

Where:

EAAI = Essential Amino Acid Index, expressed as a percentage (%) and used to estimate the overall protein quality.

Π = mathematical product notation, indicating the multiplication of all amino acid ratios.

i = the i^{th} essential amino acid, ranging from 1 to.

n = total number of essential amino acids considered. In human nutrition, these include nine: histidine, isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, threonine, tryptophan, and valine.

a_i = concentration of the i^{th} essential amino acid in the test protein (mg/g protein).

r_i = concentration of the essential amino acid in the FAO/WHO reference protein (mg/g protein).

$\frac{a_i}{r_i}$ = Theamino acid score, i.e., the adequacy of each essential amino acid relative to the reference standard.

$\left(\prod_{i=1}^{n} \frac{a_i}{r_i} \right)^{\frac{1}{n}}$ = The geometric mean of all amino acid scores, which balances very high and very low values.

× 100 = Scaling factor that expresses the result as a percentage.

Step 1: The protein content was converted from g/100 g to mg/g by multiplying the g/100 g protein value by 10, since 1 g equals 1000 mg.

Step 2: Each value (a_i) was taken and divided by the reference requirement (r_i), then the result was multiplied by 100.

Step 3: The geometric mean of the nine essential amino acid scores (% of Ref) for each bean sample was calculated. This was done by multiplying all the scores together and then taking the 9th root to find the EAAI for each sample. An EAAI value of 100% means the protein meets the FAO/WHO amino acid requirements perfectly. If the value is above 100%, it indicates that the protein offers more essential amino acids than the reference pattern, while a value below 100% points to a deficiency in one or more essential amino acids.

2.8. Determination of risk assessment

2.8.1. Health risk estimates

The Estimated Daily Intake (EDI) for each pesticide was calculated using the equation:

$$EDI = \frac{Beans\ consumption\left(kg/day\right) \times Pesticide\ concentration\ in\ the\ beans\left(mg/kg\right)}{Body\ weight}$$

2.8.2. Estimation of health risk from pesticides

The Health Risk Index (HRI) for non-carcinogenic chemicals was determined according to Akoto *et al.* (2015), using the formula:

$$HDI = \frac{EDI}{ADI}$$

where HRI stands for health risk index, EDI stands for estimated daily intake, and ADI stands for acceptable daily intake. According to Akoto *et al.* (2015), when HRI is greater than one, lifetime consumption of beans containing the measured level of pesticide could pose health risks.

2.8.3. Beans consumption rate

A standard questionnaire was used to collect data on beans consumption rates. A total of 100 people was randomly interviewed within the study area. Beans consumption rates was converted to kilograms, and the average estimated consumption rate was calculated.

2.8.4. Risk assessment of heavy metals

Risk assessment was conducted to evaluate potential health risks from consumption of beans contaminated with heavy metals (e.g., Pb). Parameters calculated include:

Estimated Daily Intake (EDI).

The estimated daily intake of metals was calculated using:

EDI = (mg/KgBw/day).

C = Concentration of metal (mg/kg).

IR = Ingestion rate (beans/day) = 0.12 kg/day (120 g/day).

BW = Average body weight of an adult (kg) = 60 kg.

EDI (mg/day person) = C × IR.

Target Hazard Quotient (THQ).

The non-carcinogenic risk was assessed using:

THQ = EDI (mg/kg bw/kg)/RfD.

RfD = Oral reference dose (mg/kg/day).

Na = 0.3 mg/kg/day (approx).

Ca = Not Established.

Zn = Not Established.

Pb = 0.0035 mg/kg/day (US-EPA).

K = Not Established.

RDA = Recommended dietary allowance (mg/day).

K = 3510 mg/day (WHO).

Zn = 11 mg/day (men), 8 mg/day (women).

Ca = 1000 mg/day.

Hazard Index (HI).

The overall non-carcinogenic risk was estimated as:

Hazard Index (HI) = Sum of all THQs.

Carcinogenic Risk (CR).

Carcinogenic risk was estimated using:

CR = EDI × SF

Percentage Recommended Dietary Allowance (%RDA).

%RDA = EDI (mg/day person) × 100/RDA.

RDA values were obtained from the World Health Organization.

A THQ or HI greater than 1 implies potential health risks, while values less than 1 indicate safe consumption.

3. Results of proximate analysis

The proximate composition (%) of the two bean varieties (Honey/Oloyin beans and Kampala beans) is presented in Table 1.

Table 1: Proximate Composition (% w/w) of Honey (Oloyin) Beans and Kampala Beans

Parameter	Honey (Oloyin) Beans	Kampala Beans
Moisture	9.89	8.28
Ash	0.11	3.83
Fat (n-hexane Extract)	14.00	16.29
Crude Fibre	15.80	45.60
Crude Protein	11.30	14.00
Carbohydrate	48.90	12.00

The moisture content of Honey beans was 9.89, slightly higher than that of Kampala beans (8.28). These values fall within the recommended safe range of 8-12 for legumes, which ensures prolonged shelf life and reduced microbial attack (FAO, 2012). Adeyeye and Ayejuyo (2018) reported higher moisture values of 11.50 for brown beans and 12.20 for white beans, while Olapade and Adetuyi (2007) also recorded values in the range of 10-12 for Nigerian beans. Compared to these, the beans in this study have lower moisture contents, particularly Kampala beans, suggesting greater suitability for long-term storage. Ash content reflects the mineral composition of food. In this study, Honey beans contained 0.11, while Kampala beans contained 3.83. These values indicate a wide variation between the two samples. The ash content of Kampala beans agrees with the 2.80-4.00 range reported by Akinyele and Shokunbi (2015) and Aletor and Aladetimi (1989), whereas the value for Honey beans is significantly lower. The unusually low ash content of Honey beans may be attributed to soil variation or genetic differences between the varieties. Percentage fat content in the two investigated samples as presented in Table 1 are 14.00 fat for honey beans, whereas Kampala beans had a slightly higher fat content of 16.29. These results are within the range of 12-18 reported by Enwere (1998) for Nigerian beans and agree with Aremu *et al.* (2006), who noted that legumes generally contain moderate levels of fat. The higher fat in Kampala beans could enhance their energy value, but also makes them more prone to rancidity compared to Honey beans. The crude fibre levels were markedly different 15.80 in Honey beans compared to 45.60 in Kampala beans. The value for Honey beans is within the 10-20 range reported by Aremu *et al.* (2006), but the very high crude fibre content of Kampala beans exceeds the 40 upper limit reported for most legumes (FAO, 2012). This suggests that Kampala beans may provide enhanced digestive benefits and cholesterol reduction, though excessive fibre may reduce bioavailability of some nutrients. The protein content of the two samples Kampala beans and honey beans is 11.30 in Honey beans and 14.00 in Kampala beans. These values are within the 10-25 range reported for legumes (FAO, 2012). They also agree with Ailenokhuoria and Omolekan (2019), who reported 20.5-25 protein for Nigerian beans, though slightly lower. The higher protein content of Kampala beans suggests it is a better dietary protein source than Honey beans. The carbohydrate content of Honey beans is 48.90, much higher than that of Kampala beans (12.00). These findings agree with Olapade and Adetuyi (2007), who reported high carbohydrate contents in Oloyin beans, making them sweeter and more energy-dense. The value for Honey beans also falls within the 40-60 range typical for legumes (FAO, 2012). However, the low carbohydrate content of Kampala beans makes it less energy-rich, but potentially suitable for diabetic diets.

4. Mineral composition of Honey and Kampala beans

The mineral analysis of honey beans (Phaseolus vulgaris) and kampala beans (Vigna unguiculata) showed significant differences in both macro- and microelement content (Table 2). In this study, potassium was 28685.37 mg/kg in Honey beans and 4154.79 mg/kg in Kampala beans. This finding supports the report of Adamu *et al.* (2016) who reported potassium values ranging from 21,000 to 23,500 mg/kg in Nigerian cowpeas, while Owade *et al.* (2019) recorded slightly lower values of 15,000-22,000 mg/kg in cowpea varieties. The potassium concentration obtained for Honey beans in this study (28685.37 mg/kg) is higher than both literature ranges, while that of Kampala beans (4154.79 mg/kg) is significantly lower. These results confirm the assertion of Owade *et al.* (2019) that mineral content in beans varies widely depending on genetic factors, soil type, and agronomic practices. The calcium content was 2775.03 mg/kg in Honey beans and 2412.20 mg/kg in Kampala beans. These results agree with the findings of Ailenokhuoria and Omolekan (2019), who reported cowpea calcium levels within 2,000-3,000 mg/kg. Similarly, Nwadike *et al.* (2018) reported comparable calcium concentrations in Nigerian beans. The slightly higher calcium content in Honey beans suggests a better contribution to bone development and nerve function than Kampala beans. Sodium levels were 3861.39 mg/kg in Honey beans and 4079.82 mg/kg in Kampala beans. These values align with the report of Adamu *et al.* (2016), who observed sodium in the range of 3,500-4,200 mg/kg in Nigerian cowpeas. The slightly higher sodium concentration in Kampala beans compared with Honey beans may be linked to soil or environmental variations as suggested by Olapade and Adetuyi (2007). Zinc concentration was 65.46 mg/kg in Honey beans and 52.28 mg/kg in Kampala beans. These results are comparable with the report of Ene-Obong and Obiziba (2016), who found zinc values between 50-70 mg/kg in Nigerian beans. The higher zinc content in Honey beans indicates a greater potential in boosting immunity and enzyme activity compared with Kampala beans. Lead was Not Detected (ND) in either honey or Kampala beans, which means these samples are free from toxic heavy metal contamination. The absence of lead confirms that the beans in this study are safe regarding heavy metal toxicity and are well below the Codex Alimentarius maximum permissible limit of 0.3 mg/kg (FAO/WHO, 2011).

Table 2: Mineral composition of Honey and Kampala beans (mg/kg)

Metals	Honey Beans (mg/Kg)	Kampala Beans (mg/Kg)
K	28,685.37	4,154.79
Pb	ND	ND
Ca	2,775.03	2,412.20
Na	3,861.39	4,079.82
Zn	65.46	52.28

Note: ND = Not detected.

5. Results of phytochemical screening

The phytochemical analysis of the bean samples focused on anti-nutritional factors like flavonoids, saponins, tannins, phenols, alkaloids, and glycosides, along with qualitative screening tests for major secondary metabolites. This section will delve into the results for both Kampala beans and honey beans.

Table 3 depicts the results of the qualitative analysis of honey and Kampala beans. From the results, honey beans contain saponins, tannin, flavonoids, Alkaloids, glycosides, steroids and Phenols while Kampala beans contain saponins, tannin, flavonoids, terpenoids, alkaloids, glycosides and Phenols.

The flavonoids content of Kampala beans (21.08%) is higher than that of honey beans (16.85%). The result of the Kampala bean is higher than the (15-18%) range reported by Igwe *et al.* (2020) but that of honey beans (16.85%) fall within the range. Saponins content of Kampala beans were found at (19.31%) which is higher than that of honey beans (17.09%). The result of Kampala beans is within the (18-22%) range reported by Okwu (2004) and slightly higher than the 16.5% reported by Ajayi *et al.* (2016). But that of honey beans (17.09%) is lower that the (18-22%) range reported by Okwu (2004) and slightly higher than the 16.5% reported by Ajayi

Table 3: Qualitative phytochemical screening of Honey and Kampala beans

Phytochemical	Honey Beans	Kampala Beans
Saponins	+VE	+VE
Tannins	+VE	+VE
Flavonoids	+VE	+VE
Terpenoids	–VE	+VE
Alkaloids	+VE	+VE
Glycosides	+VE	+VE
Steroids	+VE	–VE
Phenols.	+VE	+VE
Anthraquinones	–VE	–VE

Note: +VE = Present; –VE = Absent.

Table 4: Phytochemical composition of Kampala and Honey beans

Parameters	Honey Bean	Kampala Bean
Flavonoids (%)	16.85 ± 0.041	21.08 ± 0.080
Saponins (%)	17.09 ± 0.004	19.31 ± 0.224
Tannins (mg/g)	1.81 ± 0.010	1.49 ± 0.081
Phenols (%)	45.16 ± 0.045	36.02 ± 0.294
Alkaloids (%)	19.92 ± 0.737	18.49 ± 0.097
Glycosides (mg/kg)	27.65 ± 0.778	16.25 ± 0.099

Note: Values are expressed as mean ± standard deviation (SD) of duplicate determinations.

et al. (2016). Saponins are beneficial in lowering plasma cholesterol and boosting immunity (Okwu, 2004), their relatively high concentration in this study may contribute to a bitter taste and reduced nutrient absorption, as also noted by Owade *et al.* (2019).

The tannin content in Kampala beans (1.49 mg/g) is lower than that of honey beans (1.81 mg/g). The results are lower than the (2.1-2.5 mg/g) reported by Akande *et al.* (2019) and (2.0-2.3 mg/g) reported by Soetan and Oyewole (2009) for other Nigerian bean varieties. Tannins are known to bind proteins and minerals, reducing their bioavailability, but when present in moderate amounts, they provide antimicrobial benefits (Doss, 2009). The relatively lower tannin level found in this study may therefore improve nutrient digestibility while still retaining protective functions (Table 4).

The phenolic content of Kampala beans (36.02%) is lower than the phenolic content of honey beans (45.16%). The results are substantially higher than the (28-32%) reported by Ajayi *et al.* (2016). According to Dykes and Rooney (2007), phenolic compounds are powerful antioxidants, suggesting that both bean types, Kampala and honey beans, may provide strong protection against oxidative stress (Table 4).

The alkaloids content in Kampala beans as evaluated in this study is (18.49%) which is lower than that of honey beans (19.92%). The results are consistent with the (17-20%) range reported by Edeoga *et al.* (2005) and slightly higher than the 16.8% reported by Ajayi *et al.* (2016). Alkaloids are pharmacologically important, with reported anti-inflammatory and analgesic benefits (Edeoga *et al.*, 2005). However, as emphasized by Owade *et al.* (2019), excessive intake may result in toxicity, underscoring the need for moderation in consumption.

The glycoside content of Kampala beans as evaluated in this study is (16.25 mg/kg) which is lower than that of the honey beans (27.65 mg/kg). The result of the Kampala beans align with the (15-18 mg/kg) reported by Ajayi *et al.* (2016) but that of the honey beans (27.65 mg/kg) is above (18-22 mg/kg) range reported Okwu and Omodamiro (2005). Glycosides have been associated with beneficial bioactivity, particularly in cardiovascular health (El-Seedi *et al.*, 2019). The honey beans have stronger cardio protective and antimicrobial potential compared to Kampala beans (Table 4).

6. Comparative glycemic properties of Honey beans and Kampala beans

The glycemic index of honey beans and Kampala beans was evaluated by looking at Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), Resistant Starch (RS), and Glycemic Index (GI).

The Rapidly Digested Starch (RDS) content in Honey beans (22.20%) was slightly lower than that of Kampala beans (23.56%). Higher RDS values indicate faster glucose release and therefore higher glycemic response (Englyst *et al.*, 1992). This result is in agreement with the findings of Owade *et al.* (2019), who reported that bean varieties with higher RDS values tend to elicit a quicker postprandial glucose rise. Both varieties in this study had relatively higher RDS values which makes them less favorable for individuals requiring a slower glucose release, such as diabetic patients. Honey beans contained a higher proportion of SDS (50.06%) compared to Kampala beans (46.74%). This suggests that Honey beans may provide a more gradual release of glucose, thereby contributing to better glycemic control. Jenkins *et al.* (2002) emphasized that foods with higher SDS reduce postprandial spikes and sustain energy release. This observation also agrees with Truswell (2002), who highlighted the role of SDS in lowering glycemic index and improving metabolic health. Therefore, even though the RDS values of both beans are similar, the higher SDS in Honey beans may confer a slight nutritional advantage. The resistant starch content was also higher in Honey beans (30.46%) compared to Kampala beans (29.07%). Resistant starch is not digested in the small intestine but ferments in the colon, where it contributes to gut health and reduces glycemic response (Nugent, 2005). These results are consistent with Wolever (2006), who reported that legumes rich in resistant starch act like dietary fiber and may reduce the risk of type 2 diabetes. This further supports the notion that Honey beans may offer slightly greater health benefits than Kampala beans (Table 5).

The glycemic index values for Honey beans (48.69%) and Kampala beans (48.21%) were very close, both falling within the low to medium GI range (40-55). This indicates that both varieties are suitable for moderate glycemic control. However, compared with Sokoto white beans, which had a much lower GI (≈ 30, as reported by Adamu *et al.*, 2016), both Honey and Kampala beans show a relatively higher glycemic response. This agrees with earlier reports by Englyst *et al.* (1992) and Jenkins *et al.* (2002), who observed that bean varieties differ significantly in their glycemic properties due to starch composition and processing factors (Table 5).

Table 5: Glycemic index of Honey and Kampala beans

Bean Variety	RDS (%)	SDS (%)	RS (%)	GI
Honey Beans	22.20	50.06	30.46	48.69
Kampala Beans	23.56	46.74	29.07	48.21

7. Amino acid profile of Honey beans

The amino acid compositions of Honey beans and Kampala beans are presented in Table 6. The results show that both varieties share similar amino acid patterns, with glutamic acid and aspartic acid being the most abundant in each. In Honey beans, glutamic acid (14.21 g/100 g protein) recorded the highest concentration, followed by aspartic acid (10.27 g/100 g protein) and leucine (7.99 g/100 g protein). Similarly, in Kampala beans, glutamic acid (12.60 g/100 g protein) and aspartic acid (9.94 g/100 g protein) were predominant. Among the essential amino acids, Honey beans exhibited higher levels of leucine (7.99 g/100 g protein), whereas Kampala beans contained a slightly higher amount of lysine (6.17 g/100 g protein) compared to Honey beans (5.40 g/100 g protein). The relatively higher lysine content in Kampala beans suggests a better

Table 6: Amino acid composition of Honey and Kampala beans (g/100 g protein)

Amino Acid	Honey Beans (g/100 g Protein)	Kampala Beans (g/100 g Protein)
Glutamic acid	14.21	12.60
Aspartic acid	10.27	9.94
Leucine	7.99	6.13
Lysine	5.40	6.17
Phenylalanine	4.73	4.51
Proline	4.59	3.87
Glycine	3.90	3.52
Arginine	3.66	3.41
Valine	3.35	3.16
Serine	2.79	2.61
Alanine	2.51	2.43
Histidine	1.82	1.92
Tyrosine	1.77	1.84
Methionine	1.32	1.2
Cystine	1.24	1.25
Tryptophan	1.15	1.09

complementary potential for cereal-based diets such as maize and rice, which are typically deficient in lysine (FAO/WHO, 2013). In both bean varieties, the sulfur containing amino acids methionine and cystine were the least represented. Honey beans recorded methionine and cystine contents of 1.32 g and 1.24 g/100 g protein, respectively, while Kampala beans contained 1.2 g and 1.25 g/100 g protein, respectively. This observation aligns with the reports of Adeyeye (2019), Nwokolo (1996) and Olaofe *et al.* (1994), who highlighted that legumes are generally rich in acidic amino acids (glutamic and aspartic acids) but limited in sulfur amino acids, which are often the primary factors affecting their overall protein quality (Table 6).

Overall, the two varieties display similar amino acid profiles, with slight variations that could be nutritionally significant. The higher glutamic and aspartic acid levels underscore their strong flavor and potential role in enhancing taste, while the complementary balance of lysine in Kampala beans may improve dietary protein quality when combined with cereals.

8. Protein quality based on amino acid profile

Table 7 presents the essential amino acid composition, amino acid scores, and Essential Amino Acid Index (EAAI) of Honey beans and Kampala beans based on the FAO/WHO (2013) reference amino acid scoring pattern. As shown in Table 7, both bean varieties contained all the essential amino acids required for human nutrition, although differences were observed in their amino acid profiles. The protein quality of Honey beans and Kampala beans was assessed using the FAO/WHO (2013) amino acid scoring pattern for older children, adolescents, and adults. The amino acid scores were determined by comparing each Essential Amino Acid (EAA) in the sample to the FAO reference pattern (mg/g protein × 100).

The high EAAI values (>100%) indicate that both Honey and Kampala beans have favorable amino acid

Table 7: Amino acid scores of Honey and Kampala beans compared with FAO/WHO reference pattern

Amino Acid	FAO/WHO Ref. (mg/g Protein) (r_i)	Honey Beans (mg/g) (a_i)	Honey % of Ref	Kampala Beans (mg/g) (a_i)	Kampala % of Ref
Histidine	16.0	28.5	178.1	27.3	170.6
Isoleucine	30.0	32.8	109.3	47.1	157.0
Leucine	61.0	79.9	131.0	61.3	100.5
Lysine	48.0	54.0	112.5	61.7	128.5
Methionine + Cystine	23.0	25.6	111.3	24.5	106.5
Phenylalanine + Tyrosine	41.0	90.1	219.7	84.1	205.1
Threonine	25.0	33.6	134.4	36.5	146.0
Tryptophan	6.6	11.5	174.2	10.3	156.1
Valine	40.0	46.1	115.3	35.6	89.0

Note: Scores below 100% indicate the limiting amino acid. Reference pattern from FAO/WHO (2013). EAAI (Essential Amino Acid Index); Honey beans: 138.7%; Kampala beans: 135.4%.

profiles relative to human requirements. Honey beans exhibited superior leucine and overall Branched-Chain Amino Acid (BCAA) content, which are important for muscle protein synthesis and growth (Wu, 2013). Kampala beans were richer in lysine, which complements cereal-based diets often deficient in lysine.

However, Kampala beans were limited in valine, with an amino acid score of only 89% of the FAO requirement. This makes valine the first limiting amino acid, reducing its PDCAAS to 0.85. By contrast, Honey beans exceeded the reference requirements for all EAAs, giving it a theoretical PDCAAS of 1.0 under assumed digestibility. These findings are consistent with earlier reports that legumes, although valuable, often have limiting amino acids that reduce overall protein quality (Olaofe *et al.*, 1994; FAO/WHO, 2013).

The results highlight the importance of dietary complementation. Consuming beans alongside cereals (rich in sulfur amino acids and valine) or animal protein can help overcome limiting amino acids and improve the biological value of the protein consumed.

9. Risk assessment

Table 8 presents the estimated daily intake (EDI), target hazard quotient (THQ), recommended dietary allowance (%RDA), and health risk assessment of selected minerals and lead (Pb) in Honey beans and Kampala beans. The assessment was based on the measured mineral concentrations, an average daily ingestion rate of 0.12 kg/person/day, and an average adult body weight of 60 kg. The results were used to evaluate the nutritional contribution and potential non-carcinogenic health risks associated with the consumption of both bean varieties. Risk assessment evaluates whether the consumption of honey and Kampala beans poses any health hazards or provides nutritional benefits, based on their mineral and phytochemical composition. The assessment considers both toxicological risks (heavy metals, anti-nutrients) and nutritional safety (essential minerals and phytochemicals). The health risk assessment was conducted based on the detected mineral levels. Macronutrients (K, Ca, Na). The high potassium content in honey beans suggests beneficial effects in preventing hypertension, provided overall dietary balance is maintained. Calcium levels contribute significantly to daily requirements, supporting bone health. Sodium values, though not excessive, require caution for individuals with cardiovascular risks.

Micronutrient (Zn): Both types of beans offer sufficient zinc, which helps lower the chances of deficiency-related issues like weakened immunity and slow wound healing.

Toxic element (Pb): Since lead wasn't detected, there's no health risk from heavy metal exposure. This alleviates

Table 8: Risk assessment of Honey and Kampala beans

Element	Variety	C (mg/kg)	IR (kg/day)	BW (kg)	EDI (mg/kg bw/day)	EDI (mg/day Person)	RfD (mg /kg/day)	THQ	RDA (mg /day)	% RDA
K	Honey	28685.37	0.12	60	57.37	3442.24	-	-	3510	98.07
	Kampala	4154.79	0.12	60	8.31	498.57	-	-	3510	14.20
Na	Honey	3861.39	0.12	60	7.72	463.37	-	-	1500	30.81
	Kampala	4154.71	0.12	60	8.31	498.57	-	-	1500	33.24
Ca	Honey	2775.03	0.12	60	5.55	333.00	-	-	1000	33.30
	Kampala	2412.20	0.12	60	4.82	289.46	-	-	1000	28.95
Zn	Honey	65.46	0.12	60	0.13	7.86	0.3	0.44	M=11, F=8	71.41, 98.19
	Kampala	52.28	0.12	60	0.10	6.27	0.3	0.35	M=11, F=8	57.03, 78.42
Pb	Honey	ND	0.12	60	ND	ND	0.0035	ND	-	-
	Kampala	ND	0.12	60	ND	ND	0.0035	ND	-	-

Notes: M stands for male, while F represents female; EDI (mg/kg bw/day) = (C × IR)/BW; EDI (mg/day person) = C × IR; THQ = EDI/RfD (values less than 1 suggest a low non-carcinogenic risk); %RDA = (EDI mg/day/RDA) × 100; IR refers to ingestion rate; BW is body weight; RfD means reference dose; RDA stands for recommended dietary allowance.

worries about nephrotoxicity, neurotoxicity, or anemia linked to dietary lead (ATSDR, 2007). Overall, these beans are not only nutritionally beneficial but also safe, with no toxicological worries.

The mineral content of the two bean varieties is outlined in Table 9. Honey beans had a significantly higher potassium level (28685.37 mg/kg) compared to Kampala beans (4154.79 mg/kg). Calcium was also a bit higher in honey beans (2775.03 mg/kg) than in Kampala beans (2412.20 mg/kg). Sodium levels were quite similar in both samples (3861.39 mg/kg in honey beans versus 4079.82 mg/kg in Kampala beans). Zinc levels were moderate, with honey beans (65.46 mg/kg) slightly surpassing Kampala beans (52.28 mg/kg). Lead (Pb) was Not Detected (ND) in either sample, indicating no contamination.

10. Estimated Dietary Intake (EDI)

The Estimated Dietary Intake (EDI) values, based on a daily consumption of 120 g of beans by a 60 kg adult, are presented in Table 9. Potassium intake from Honey beans was 57.37 mg/kg bw/day, whereas Kampala beans provided 8.31 mg/kg bw/day. The EDI values for zinc were 0.13 mg/kg bw/day for Honey beans and 0.10 mg/kg bw/day for Kampala beans. The estimated daily intakes of sodium and calcium were 7.72 and 5.55 mg/kg bw/day, respectively, for Honey beans, and 8.31 and 4.82 mg/kg bw/day, respectively, for Kampala beans. These values indicate that both bean varieties can contribute to the dietary intake of essential minerals without exceeding established safety limits.

Table 9: Estimated daily intake (mg/kg-day) from Honey and Kampala beans

Mineral	Honey Beans	Kampala Beans
K	57.37	8.31
Na	7.72	8.31
Ca	5.55	4.82
Zn	0.13	0.10

11. Target Hazard Quotient (THQ)

Table 10 presents the THQ values calculated using the US. EPA Reference Dose (RfD). Lead was not detected; hence its THQ was 0. Zinc THQ was 0.44 for honey beans and 0.35 for Kampala beans, both below the threshold of 1, indicating no risk of adverse effects

Table 10: Target Hazard Quotient (THQ) of Honey and Kampala beans			
Element	**Honey Beans THQ**	**Kampala Beans THQ**	**Safe Limit (THQ < 1)**
Pb	0	0	<1
Zn	0.44	0.35	<1

12. Other risk metrics

Other health-relevant indices are shown in Table 11. Honey beans had a favorable Na/K ratio (0.13) compared to Kampala beans (1.00), indicating better potential for blood pressure regulation. Estimated tannin intake per 100 g was 181.9 mg/day in honey beans and 154.7 mg/day in Kampala beans. Glycosides were present in low concentrations (2.77 mg/day in honey beans; 1.63 mg/day in Kampala beans).

Table 11: Other risk metrics of Honey and Kampala beans		
Parameter	**Honey Beans**	**Kampala Beans**
Na/K Ratio	0.13	1.00
Tannins (mg/day)	181.9	154.7
Glycosides (mg/day)	2.77	1.63

13. Nutritional implications

The findings show that Honey beans are richer in potassium (28685.37 mg/kg) compared to Kampala beans (4154.79 mg/kg). A 100 g serving of Honey beans provides about 82% of the daily recommended potassium intake (3500 mg/day), while Kampala beans contribute only around 12%. Potassium plays a crucial role in cardiovascular function, muscle contraction, and fluid balance (WHO, 2012). These results are consistent with Ajayi *et al.* (2020), who also identified potassium as the dominant mineral in Nigerian cowpea varieties. Calcium levels were also appreciable in both Honey beans (2775.03 mg/kg) and Kampala beans (2412.20 mg/kg), contributing between 24-28% of the daily requirement (IOM, 2011). This observation is in line with Soetan *et al.* (2010), who emphasized that legumes are valuable sources of calcium essential for bone health. Zinc levels (65.46 mg/kg in Honey beans; 52.28 mg/kg in Kampala beans) provided 47-60% of daily requirements, supporting immune function and enzyme activity (Prasad, 2013). These values were comparable to those reported by Uwaifo *et al.* (2020), who found safe but nutritionally significant levels of zinc in Nigerian legumes.

14. Risk assessment of mineral content

No Detectable lead (ND) was found in either bean variety, which means both are free from heavy metal toxicity risks. This complies with Codex Alimentarius limits (0.3 mg/kg) for legumes (FAO/WHO, 2011). Similar findings were reported by Ajibola *et al.* (2022), who detected only negligible concentrations of toxic metals in Nigerian beans. Honey beans showed much higher potassium content (28685.37 mg/kg) compared to Kampala beans (4154.79 mg/kg). Although potassium supports blood pressure regulation (WHO, 2012), excessive intake can lead to hyperkalemia in individuals with impaired kidney function (Weiner and Wingo, 1998). Adamu *et al.* (2016) also highlighted potassium dominance in Nigerian beans, further confirming the high levels observed in Honey beans. Sodium values were moderate (3861.39 mg/kg in Honey beans; 4079.82 mg/kg in Kampala beans), aligning with the safe intake ranges reported by He and MacGregor (2009). The favorable potassium-to-sodium ratio in Honey beans makes them better suited for cardiovascular protection, a result comparable to Owade *et al.* (2019), who noted that Nigerian legumes typically exhibit beneficial K/Na ratios. Calcium levels in both Honey beans (2775.03 mg/kg) and Kampala beans (2412.20 mg/kg) were consistent with those reported by Olagunju *et al.* (2018), who also found legumes to be good calcium sources. These levels support bone health and reduce osteoporosis risk (Soetan *et al.*, 2010).

Zinc was present at safe but nutritionally valuable levels in both varieties (65.46 mg/kg in Honey beans and 52.28 mg/kg in Kampala beans). These results confirm earlier findings by Uwaifo *et al.* (2020), who also reported Target Hazard Quotient (THQ) values < 1 for zinc in Nigerian legumes, indicating no risk of toxicity.

15. Risk assessment of phytochemicals

Kampala beans contained higher flavonoid content (~21%), while Honey beans had higher phenolic content (~45%). Both are known antioxidants with protective roles against oxidative stress and chronic disease (Shahidi and Ambigaipalan, 2015). Similar phytochemical patterns were observed by Adepoju and Oyewole (2019), who reported flavonoids and phenols as dominant constituents in Nigerian beans. Kampala beans recorded higher saponins (~19%) than Honey beans (~17%). These findings are comparable to Amoo *et al.* (2023), who also observed considerable saponin content in Nigerian bean varieties. While excessive saponins may reduce nutrient absorption (Francis *et al.*, 2002), the levels detected here remain safe. Honey beans contained more tannins (~1.55 mg/g) compared to Kampala beans. This is in line with Akande *et al.* (2019), who reported similar tannin ranges in Nigerian beans. Although tannins can reduce protein and mineral bioavailability (Scalbert, 1991), the concentrations observed are considered low and are significantly reduced by processing methods such as soaking and boiling (Soetan and Oyewole, 2009). Both beans contained moderate alkaloid levels (~18-20%). These values are consistent with Edeoga *et al.* (2005), who noted that Nigerian legumes generally exhibit beneficial but safe alkaloid concentrations. Honey beans had higher glycosides (~27-28 mg/kg) compared to Kampala beans (~16-17 mg/kg). This agrees with findings by Okwu and Omodamiro (2005), who reported glycosides as common secondary metabolites in cowpeas, useful in heart protection when consumed in safe amounts.

16. Conclusion

This study establishes that both Honey beans and Kampala beans are nutritionally valuable, safe, and suitable for regular consumption in Nigeria. The findings show that each variety possesses unique nutritional strengths: Honey beans are richer in carbohydrates, essential minerals, and phenolic compounds, while Kampala beans provide higher protein, fibre, and certain phytochemicals. Both bean varieties exhibited low glycemic index values and similar glycemic responses, confirming their suitability for maintaining moderate blood glucose levels. However, the relatively higher slowly digestible and resistant starch fractions in Honey beans suggest a slightly better potential for sustained glucose release and improved metabolic health.

Mineral analysis and risk assessment further confirm that both beans are safe for human consumption, with no detectable heavy metal contamination and all Target Hazard Quotient values below the threshold of concern. Their contributions to essential mineral intake, particularly potassium, calcium, and zinc, highlight their importance in supporting vital physiological functions and overall health. Although anti-nutritional factors were present, their levels were within safe limits and can be effectively reduced through common processing methods. The complementary amino acid profiles of both beans also indicate the need for dietary combination with cereals to achieve optimal protein quality.

In conclusion, both Honey and Kampala beans are important, affordable food resources that can enhance nutritional status, support food security, and reduce the risk of diet-related diseases in Nigeria. However, Honey beans show a slight overall nutritional advantage. Further research is recommended to investigate the effects of processing methods on nutrient bioavailability and glycemic properties.

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