

# Nutritional properties and nutrients chemical analysis of common beans seed

## Abstract

This paper presents a revision on the nutritional properties and chemical methods used in nutrient analysis together with their main applications in food science research. The dissertation includes a review of literature on the distribution of common beans and their nutritional and importance develops knowledge about common beans and nutrient chemical analysis. In fact, nutrients are substances required by the body to perform its basic functions. Nutrients must be obtained from diet, since the human body does not synthesize them. Nutrients are used to produce energy, detect and respond to environmental surroundings, move, excrete wastes, respire (breathe), grow, and reproduce. There are six classes of nutrients required for the body to function and maintain overall health. These are carbohydrates, lipids, proteins, water, vitamins, and minerals. Separate methods are required to describe quantity, composition, and quality of nutrient in foods. Our work has to present both nutrients compositional in common beans and analysis methods.

**Keywords:** nutritional properties, nutrients, chemical analysis, common beans

Volume 3 Issue 2 - 2018

Kotue TC,<sup>1,2</sup> Marlyne Josephine M,<sup>1</sup> Wirba LY,<sup>1</sup> Amalene SRH,<sup>1</sup> Nkenmeni DC,<sup>1</sup> Kwuimgoin<sup>1</sup>,<sup>1</sup> Djote WNB,<sup>1</sup> Kansci G,<sup>1</sup> Fokou E,<sup>1</sup> Fokam DP<sup>2</sup>

<sup>1</sup>Department of Biochemistry, University of Yaounde I, Cameroon

<sup>2</sup>Laboratory of Biochemistry of Physiology and Pharmacology, Faculty of Medicine and Biomedical Science, Cameroon

**Correspondence:** Kotue TC, Faculty of Science, Laboratory of Food Science and Metabolism, Department of Biochemistry, University of Yaounde I, Cameroon, Email ctkotue\_bio@yahoo.fr, ctkotue@uy1.uninet.cm

**Received:** December 21, 2017 | **Published:** June 08, 2018

**Abbreviations:** RDI, recommendation daily intake; FAME, fatty acid methyl esters; BSA, bovine serum albumin; TIU, trypsin inhibitor units; A, absorbance; TCA, trichloroacetic acid; HPLC, high-performance liquid chromatography; IUPAC, international union of pure and applied chemistry

## Introduction

Nutrition is the science that interprets the interaction of nutrients and other substances in food (e.g. phytonutrients, anthocyanins, tannins, etc.) in relation to maintenance, growth, reproduction, health and disease of an organism. It includes food intake, absorption, assimilation, biosynthesis, catabolism and excretion.<sup>1</sup> Increasing interest in nutrition, fitness and beauty consciousness has enhanced concerns over a healthy diet. Nutritional properties have assumed the status of “functional” foods, capable of providing health benefits, like prevention or delaying onset of chronic diseases, as well as meeting basic nutritional requirements.<sup>2</sup>

Nutrients are at the base of the nutrition and can be grouped into six major categories: carbohydrate, protein, lipid (fat), water, vitamins, and minerals. Carbohydrate, protein, and fat are macronutrients because they make up the bulk of your diet. Vitamins and minerals are micronutrients because they are required in much smaller amounts. However, water is a micronutrient because it does not contain energy.<sup>3</sup> Appropriate intake of food ensures sufficient supply of nutrient. It is therefore important to analyse the composition of some foods like common beans to establish a data base necessary to know the nutritional contribution to our health.

## Common beans

Common beans (*Phaseolus vulgaris* L.) are an important legume used for human nutrition. It is an herbaceous plant that belongs to the

family of Fabaceae. Over 50 species of *Phaseolus* have reported from America and out of these only five namely common beans (*Phaseolus vulgaris* L.), year bean (*Phaseolus dumosus*), scarlet runner bean (*Phaseolus coccineus* L.), tepary bean (*Phaseolus acutifolius* A.) and lima bean (*Phaseolus lunatus* L.) are known to be domesticated.<sup>4</sup> The plant originated from the western area of Mexico and Guatemala. It is widely cultivated and distributed from Mexico to the southern ends of the southern Andes.<sup>5</sup> It is widely consumed throughout the world.<sup>6</sup> Common beans are one of the staple foods in Africa, India, and Latin America. Because of their nutritional composition, these economical foods have the potential to improve the diet quality and long-term health of those who consume beans regularly.<sup>7</sup>

Common beans are often a main source of protein, dietary fiber and minerals in diet, occupying a very important worldwide place in human alimentation, offering benefits for human health. The bean carbohydrates are composed primarily of starch, following by dietary fiber and  $\alpha$ -galactosyl derivatives of sucrose. The major proteins of bean are globulins (54-79%) and albumins (12-30%) and the presence of protease inhibitors ( $\alpha$ -amylase, chymotrypsin and trypsin), lectins and lipoxygenase were been verified.<sup>8</sup> But the presence of phytate, tannins and oxalate referred to anti-nutritive factors that affect the nutritional quality by interacting with intestinal tract and also reduce protein digestibility and amino acid absorption.<sup>9</sup> According to<sup>10</sup> unless these substances are destroyed by heat or other treatments, they can exert adverse physiological effects when utilized by animals and man.

Because of their high concentration of health-promoting nutrients, consuming more beans in diet could improve overall health and also decrease the risk of developing certain diseases, including heart disease, obesity and many types of cancers. The 2010 Dietary Guidelines for Americans recommend consuming 1.5 cups of beans per week to take advantage of this potential health benefits.<sup>11</sup>

Dry beans are very good source of low fat protein. They contain between 21 to 25% protein by weight, which is much higher than other vegetable products. In many parts of the world, they provide a substantial proportion of the total protein intake for the population. The intake of dried beans as a protein source is extremely important worldwide as they provide a good source of protein at minimal cost relative to the production of animal protein sources.

Beans are one of the most of nutritionally complete foods available. In fact, no other food comes close to beans in providing protein, iron, magnesium, zinc, potassium and fibers together in high amounts. Following are some of reasons why beans are a key ingredient in a healthy diet of all ages: high in complex carbohydrate, in protein, in

dietary fiber, in folate; low in fat, in sodium, cholesterol free; rich in vitamins and minerals.

The new food pyramid now uses the term ounce-equivalents in place of the traditional ounce serving size for the meat and beans group. A ¼ cup serving of cooked dry beans equals an ounce-equivalent. In the vegetable group, the serving is based directly on a measured amount of beans. For example, ½ cup of cooked beans equal ½ cup of vegetables.<sup>12</sup>

The United States Department of Agriculture<sup>13</sup> has an extensive nutritional profile for edible beans (Table 1) in the National Nutrient Database for Standard Reference.

**Table 1** Nutrient profile of beans.<sup>13</sup>

½cup (85-89 g) cooked beans	Black	Cranberry	Great northern	Dark red kidney	Light red kidney	Navy	pink	pinto	Small red
%calories	114	120	104	109	110	127	126	123	112
%calories from fat	4	3	3	1	1	4	3	4	1
Fat(g)	0.5	0.4	0.4	0.2	0.1	0.6	0.4	0.5	0.2
Protein(g)	8	8	7	8	8	8	8	8	8
Carbohydrates(g)	21	22	19	19	20	24	24	22	20
Dietary fiber(g)	8	9	6	8	8	9	5	8	6
Calcium(mg)	23	43	60	39	58	63	44	40	25
Magnesium(mg)	60	44	44	40	40	48	56	43	40
Iron(mg)	2	2	2	2	3	2	2	2	3
Potassium(mg)	306	342	346	335	371	354	329	373	357
Sodium(mg)	1	1	2	4	4	0	2	1	2
Riboflavin (mg)	0.05	0.06	0.05	0.05	0.05	0.06	0.04	0.05	0.05
Vitamine K(µg)	0	0	0	7	6	0.5	3	3	3
Folate	128	183	90	65	65	127	142	147	115

## Carbohydrate

Carbohydrate is considered an energy (calorie) yielding nutrient. However, not all carbohydrate provides energy. Both simple and complex forms of carbohydrates exist in beans. The simple form includes sugars such as glucose. In contrast, complex forms of carbohydrates such as starches and dietary fiber are not always a source of energy. Starches can be digested to produce glucose; which is the used as an energy source. Fiber does not digest and therefore cannot produce any energy.<sup>14</sup> Thus consumption of beans provides multiple form of carbohydrate. Dietary fiber is composed of both soluble and insoluble forms. Beans vary in the composition of soluble to insoluble fibers. As general rule, the fiber in beans is composed of between 60-85% insoluble and 40-55% soluble. A 60:40 ratio is most common in cooked beans. Approximately 75-80% of the fiber is found in the beans hull.<sup>15</sup>

## Proteins content

Proteins content in kidney (29.45%), pinto (26.8%), black (25.31%) and navy (24.48%) beans are just a few examples of the valuable protein source bean provide. Consuming beans are a great

way to get a low fat source of protein.<sup>13</sup> We must consume proteins as a source of amino acid which the body in turn uses makes proteins necessary for life. There are approximately nine essential amino acids. The three most important essential amino acids in beans include lysine, cysteine and methionine.<sup>16</sup>

## Vitamins and minerals

Beans are excellent sources of iron, magnesium, zinc potassium and folic acid.<sup>13</sup> The recommendation daily intake (RDI) for iron falls between 8 and 11mg for men and women, respectively. A ½ cup serving of beans provides approximately 11% RDI for iron. A ½ cup serving of beans provide 10 to 15% of the 320 to 42 mg RDI for magnesium. Beans provide approximately 300 to 43 mg of potassium per ½ cup serving or 6-10% of the RDI for potassium. Approximately, beans provide 0.75-0.95mg of zinc per ½ cup serving or approximately 10-11% of the RDI for women and 6-8% of RDI for men. The RDI for folic acid (or folate) is 400µg per day. A ½ cup serving of cooked beans provide 65-183µg or 16-46% of RDI. Beans also provide 8-15% of the RDI for copper 8% of the RDI for selenium, and 2-6% of the RDI for calcium. Beans also provide 19-26% of the adequate intake value for manganese.

Nutritional properties of food depending upon its composition and physical properties. Separate methods are required to describe quantity, composition, and quality of nutrients in foods. Our work has to present also nutrient compositional in common beans and analysis method.

### Nutrient composition

This exhaustive check-list has presented parameters of beans.<sup>17</sup>

### Proximate component

The term proximate component refers to those macronutrients that include water (moisture), crude protein, crude lipid (fat), carbohydrate, crude fiber and ash<sup>18</sup>. The other parameters are:

- i. Soluble and insoluble fiber
- ii. Amino acids: Essential and non-essential amino acids.
- iii. Fatty acids and Phytostérols
- iv. Mineral composition and trace elements: It is about: Na; K; Ca; P; Mg; Fe; Mn; Cu; Zn; S; I and Se; Cl; Pb; Co; Cr; Cd; Hg.

### Vitamins

#### a. Fat soluble vitamins

Vitamin A (retinol); vitamin D3 (cholecalciferol); vitamin D2 (ergocalciferol); vitamin E ( $\alpha$ -tocopherol); vitamin K (phylloquinone);  $\beta$ -carotene.

#### b. Water soluble vitamins

Vitamins B1 (thiamin); vitamin B2 (riboflavin), vitamin B6 (pyridoxine); niacin; pantothenic acid; folic acid; biotin; vitamin B12 (cobalamin); vitamin C.

### Anti-nutrients factors

Tannins; phytate, proteases inhibitors; lectins or hemagglutinins.

### Analytical methods

Reference method must be used. The choice of the analytical method is crucial for the validation and the signification of the result.

### Proximate component analysis

The dry beans must used in the study and must finely ground into flour and kept at 4–6°C in sealed polyethylene bags until analysis.

Moisture, ash, crude fat, crude protein, and carbohydrate will be analyse according to AOAC<sup>19</sup> methods as described below. Samples were analyzed in triplicate.

### Determination of moisture

Water content is determined by removing moisture and then by measuring weight loss;

3g of bean powder is accurately weighed in a pre-weighed petri-dish and dried in a hot air oven for 6-12h at 100±2°C. The dish with sample is cooled in desiccators and weighed. This exercise is repeated until the difference in weight between two successive weighing becomes constant. From the weight loss during drying, amount of moisture is calculated using the following formula and the moisture can be represented in percentage.

$$\text{Moisture}(\%) = \frac{(W_1 - W_2)}{W} \times 100$$

$W_1$  = Weight of sample with petridish before drying

$W_2$  = Weight of sample with petridish after drying

W = Weight of sample

### Determination of ash content

1g of dried sample is accurately weighed into pre-weighed, clean crucible. The crucible is heated to the point of charring of the sample on a hot plate. The crucible with the carbon residue obtained as a result of ignition, is placed in muffle furnace at temperature of 650°C until the carbon residue disappears. The sample is allowed to cool and then weighed. From the difference in weight obtained the ash content is calculated using the formula:

$$\text{Total ash content} (\%) = \frac{\text{Weight of crucible with ash (g)}}{\text{Weight of crucible with sample (g)}} \times 100$$

### Crude fat estimation

Take 10g of sample in a thimble and plug the top of the thimble with a wad of fat-free cotton. Drop the thimble into the fat extraction tube of a Soxhlet apparatus. Attach the bottom the extraction tube to a Soxhlet flask. Pour approximately 75mL or more of hexane through the sample in the tube into the flask. Attach the top of fat extraction tube to the condenser. Extract the sample for 6h or longer on a heating mantle at 40°C. At the end of the extraction period, remove the thimble from the apparatus and concentrate the extract at rotavapor at 40°C. Dry at 100°C for 1h, cool and weigh. The difference in weights gives the ether soluble material present in the sample.

$$\text{Crude Fat} (\%) = \frac{\text{Weight of hexane soluble material}}{\text{Weight of sample}} \times 100$$

### Determination of crude proteins

The conventional test for protein measurement is based on the nitrogen content (Kjeldahl method). 0.5g of sample and digestion mixture (copper sulphate + potassium sulphate) is weighed into a Kjeldahl flask and 10mL of concentrated H<sub>2</sub>SO<sub>4</sub> is added. The Kjeldahl flask will be then heated on a mantle (in slanting position) until colour of solution changes to pale blue green. This clear solution will made up to 25mL under cold conditions. The Kjeldahl apparatus is set up for protein estimation. 20mL of 4% boric acid and 1mL of mixed indicator (bromocresol green) is taken in conical flask and placed under condenser. 5mL of sample with 20mL of 40% NaOH and 10mL water are added to distillation tube through funnel. When water starts boiling inside the round bottom flask, steam produced then passes into distillation tube. NH<sub>3</sub> evolved in distillation tube is trapped in boric acid. Upon ammonia evolution, the colour of boric acid changes to blue. For maximum ammonia evolution, the process is continued for 20min. The solution is then titrated with standard HCl (0.01N) until blue colour of the solution disappears.

Amount of nitrogen in the samples is calculated by the following equation

$$\% \text{ of Nitrogen} = \frac{14 \times \text{Normality of HCl} \times \Delta V \times 100}{\text{Weight of Sample} \times 1000}$$

$$\% \text{ Protein} = \% \text{ of Nitrogen} \times 6.24$$

### Determination of Carbohydrate

Carbohydrate is found by difference method and expressed as percentage of carbohydrate.

$$\text{Carbohydrate} (\%) = 100 - [\text{Moisture} + \text{Ash} + \text{Fat} + \text{Protein}]$$

### Determination of fiber

#### Determination of Crude fiber

Crude fiber is determined as described by.<sup>19</sup>

Around 1g of sample is taken into the beaker. Added 60ml of boiling sulfuric acid, and connect it with the digestion apparatus. Boil for exactly 30minutes, filter through filtering cloth and wash with hot water until it is free from acid. Transfer the residue on the cloth into the flask with 200ml of boiling sodium hydroxide solution. Immediately connect the flask with the digestion apparatus and boil further for exactly 30minutes. Remove the flask and immediately filter through Gooch crucible or alundum crucible. Wash with hot water until it is free from alkali and then with 10ml of alcohol. Dry at 105-110°C in an air oven for about 2hours. Cool to room temperature in desiccator and weigh. Repeat the process of 30minute drying, cooling and weighing until the difference between two successive weightings is less than 1mg. Note the lowest weight which shall be considered as the weight of crucible and contents after drying.

Incinerate the contents and the crucible in the electric muffle furnace at 620°C for about 30minutes. Cool to room temperature in desiccator and weigh.

Repeat the process of 30minute incinerating, cooling and weighing until the difference between two successive weightings in less than 1mg. Note the lowest weight which shall be considered as the weight of crucible and ash after incinerating. The difference between the two weightings is the weight of crude fiber.

$$\text{Crude fibre, \% by weight} = \frac{(W_1 - W_2)}{W} \times 100$$

Where, W is weight of sample, g

$W_1$  is weight of crucible and contents after drying, g

$W_2$  is weight of crucible and ash after incinerating, g

### Insoluble and soluble dietary fibers

Insoluble dietary fiber content must be determined by enzymic-gravimetric methods and soluble dietary fiber content was calculated by difference using crude fiber result.<sup>20</sup> The enzymes employed for dietary fiber are:  $\alpha$ -amylase (Termamyl 120L), protease (Flavourzyme), and amyloglucosidase (AMG 300L).

The fiber was extracted enzymatically from fat-extracted samples, using Soxhlet's method. Dry sample was homogenized with 40ml

MES/TRIS (pH 8.2) solution and  $\alpha$ -amylase solution was added. Then heating with 95°C water bath was carried out. After which, the reactants was cooled at room temperature and washed with distilled water, adding protease solution in 60°C water bath. It is mixed with 5ml of 0.56N HCl solutions, adjusted at pH 4.0. After then, 300ul of amyloglucosidase solution is added and stirred at 60°C hot plate. To extract the insoluble fiber, the solution is filtered using glass filter, with 1g celite, and the filtrate is washed with 78% ethanol, 95% ethanol and acetone in turn. After overnight, the residue in the glass filter is weighed for the insoluble fiber. The filtrate collected is added 95% ethanol and distilled water. For extract of soluble fiber, the solution is filtered using a glass filter with celite and the filtrate is washed with 15ml of 78% ethanol, 95% ethanol and acetone, in turn. After overnight, the residue in the glass filter is weighed for the insoluble fiber.

### Amino acids

Amino acids are determined using a HPLC using appropriate column according to the method.<sup>21</sup>

50g of powder in hexane (100mL) is delipided using soxhlet dispositive. The free amino acids content in delipidate powdered material is extracted in distiller water (100mL) using soxhlet dispositive during 8hours. The free amino acids content in distiller water are ready for HPLC analysis. The total protein is extracted from delipidate powder used to extract free amino acids with 1250 $\mu$ L of 2% SDS in 0,05M sodium phosphate buffer PH 6.9. The extracts will be centrifuged at 10500rpm for 10min. Fifty microlitres of the supernatant is hydrolyzed using 100 $\mu$ L 6N HCl in an oven at 110°C for 24h. After hydrolysis, 50 $\mu$ L of CaCO<sub>3</sub> is added to neutralize the reaction. The whole is then centrifuged at 10,500rpm for 10min and 10 $\mu$ L of supernatant can be use for HPCL analysis. A standard solution containing 1.25 $\mu$ mol/mL of each amino acid in 0.1N hydrochloric acid will created.

Chromatography is carried out at a constant temperature of 30°C using a gradient elution as follows. Eluant A is an aqueous buffer prepared by adding 0.5mL/L TEA to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluant B is acetonitrile-water (60:40 [v/v]).

The detection limit of each amino acid is calculated in accordance with American Chemical Society guidelines.

### Fatty acids and phytostérols

#### Fatty acids

The oil extracted from powdered whole seeds in a Soxhlet extractor with hexane is analysed by the standard AOAC methods.<sup>19</sup> Crude oil is analyzed as methyl esters to determine the fat acids composition. Fatty acid methyl esters (FAME) were obtained through a two steps method with sodium methoxyde and HCl as catalysts, and then analyzed by capillary column gas chromatography using appropriate column. The identification of the peaks is made by comparison of retention times of methyl esters obtained and analyzed in the same conditions of known oils as olive, and sunflower.

#### Phytostérols

The unsaponifiable and sterolic fractions are obtained using standard IUPAC method.<sup>22</sup> After isolation from the thin layer

chromatography plate, the sterol fraction prepared according to the standard EN ISO6799, and is further analyzed by gas chromatography with a FISON GC 8000 unit equipped with a FID detector. Sterols are quantified by internal standard method using cholesterol. They are identified by comparison to the data obtained by running authentic sterol standard.

### Mineral composition and trace elements

The main minerals found in bean are Na; K; Ca; Mg; Fe; Mn and Zn.<sup>23</sup>

Mineral contents of powder sample are determined by atomic absorption spectrometry/flame photometry according to the methods.<sup>24</sup>

For wet digestion of sample, 1g of the powdered sample is taken in digesting glass tube. 12ml of HNO<sub>3</sub> is added to the food samples and mixture is kept for overnight at room temperature. Then 4ml perchloric acid (HClO<sub>4</sub>) is added to this mixture and is kept in for the fumes block for digestion. The temperature was increased gradually, starting from 50°C and increasing up to 250-300°C. The digestion completed in about 70-85min as indicated by the appearance of white fumes. The mixture is left to cool down and the contents of the tubes are transferred to 100ml volumetric flasks and the volumes of the contents are made to 100ml with distilled water. The wet digested solution is transferred to plastic bottles labeled accurately. Put the sample in many tube to centrifuge it at 3000rpm to 10min. Use supernatants for mineral determination.

### Determination of Iron (Fe), Zinc (Zn), Calcium (Ca), Manganese (Mn) and Magnesium (Mg) by Atomic Absorption Spectrometry

The digested sample was analyzed for mineral contents by Atomic Absorption Spectrophotometer. Different electrode lamps were used for each mineral. The equipment is run for standard solutions of each mineral before and during determination to check that it is working properly.

The dilution factor for all minerals except Mg is 100. For determination of Mg, further dilution of the original solution was done by using 0.5ml original solution and enough distilled water is added to it to make the volume up to 100ml. Also for the determination of Ca, 1.0ml lithium oxide solution is added to the original solution to unmask Ca from Mg. The concentrations of minerals recorded in terms of "ppm" are converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$MW = \frac{\text{absorbency} \times \text{dry wt.} \times D}{\text{Wt. of sample} \times 1000} \left( \text{mg / g} \right)$$

### Determination of Sodium (Na) and Potassium (K) by flame photometer

Na and K analysis of the sample are done by the method of flame photometry. The same wet digested food sample solutions as used in Atomic Absorption Spectrometry are used for the determination of Na and K. Standard solutions of 20, 40, 60, 80 and 100 milliequivalent/L are used both for Na and K. The calculations for the total mineral intake involve the same procedure as given in Atomic Absorption Spectrometry.

## Vitamins

### Fat soluble vitamins

#### \*vitamin E ( $\alpha$ -tocopherol)

It is particularly about vitamin E ( $\alpha$ -tocopherol) and  $\beta$ -carotene.

Tocopherol in the oil sample is determined by HPLC using appropriate column. 10mg of the crude oil is dissolved in 10ml of hexane. An aliquot of this solution is injected on to column the mobile phase is hexane/isopropanol (99/1v/v) at a flow rate of 1ml/min. The tocopherol is identified by comparison of retention times with authentic standard.

#### \* $\beta$ -carotene

The value of  $\beta$ -carotene concentration is obtained by HPLC analysis.<sup>25</sup> In order to avoid possible degradation; the  $\beta$ -carotene is extracted directly in oil sample with solvent without saponification. Aliquots (2g) of oil were extracted 5min with acetone: hexane (4:6). After the extraction, the solvent is evaporated to dryness under a stream of nitrogen and the residue is reconstituted with 1ml of eluent solution and is colled in a screw-cap vial for HPLC analysis. Determination of  $\beta$ -carotene, consisted in the treatment of sample extraction with 1000 $\mu$ l aqueous potassium hydroxide solution (60% w/v) for 15min in a 45°C water bath. Following saponification, each sample is extracted with 1000 $\mu$ l hexane. The sample is vortexed for 3min, centrifuged at 1500 $\times$ g for 5min, and the organic hexane layer is decanted into an evaporating tube. This procedure is repeated and the second hexane extract is combined with that from the first. The hexane is then evaporated to dryness with a stream of nitrogen gas. The remaining residue is reconstituted with 1ml of eluent solution. Aliquots of 20 $\mu$ l are used for HPLC analysis using appropriate column.

### Water soluble vitamins

Common beans are an excellent source of the water-soluble vitamins thiamin, riboflavin, niacin and folate (also known as folacin and folic acid). But the main water soluble vitamin in bean is folic acid.<sup>13</sup> Analytical technique used for determination of folate content is HPLC-method.<sup>26</sup>

Standard stock solution of folic acid is prepared by dissolving 25.0mg of folic acid in 50.0ml of water. 1ml of the standard stock solution of folic acid is diluted to 50ml with 15 % methanol solution.

Twenty mg of sample powder are weighed and transferred into a 50ml volumetric flask and 15% of methanol solution is added. The mixture is sonicated (15min) and diluted to the mark with the same solvent. 1ml of this solution is transferred into a 10ml volumetric flask, diluted to the mark with the same solvent and filtered through a 0.2 $\mu$ m Millipore filter.

Prior to injection into the chromatographic system, all analytical solutions are degassed by sonication. All the prepared sample solutions are first chromatographed to ensure that interfering peaks are not present. 10 $\mu$ l and 100 $\mu$ l aliquots of the standard solutions and sample solutions are injected.

### Anti-nutrients factors

Tannins; phytate, proteases inhibitors; lectins or hemagglutinins.

## Tannins

Total tannins are determined colorimetrically as described.<sup>19</sup>

In flask of 100ml, one introduces: 5ml of distilled water; 1ml of acetone powder extract; 5ml of reagent of Folin Ciocalteu; 10ml of the solution saturated with  $\text{CO}_3\text{Na}_2$  (this saturated solution will be prepared starting from 43.75g of sodium carbonate dissolved in 100ml of hot water (70 with 80°C) after cooling the solution is filtered then adjusted to 125ml). After mechanical agitation, the preparation rests during 30 minutes. The measurement of the optical density is made to 760nm. A range standard of tannic acid is prepared under the same conditions of which concentrations go from 0 to 0.1g/l. Quantity of tannins are calculated using the equation of the calibration curve.

## Phytic acid

Phytic acid is determined according to the method.<sup>27</sup>

Phytic acid is extracted from 3g powder sample with 50ml of 3% TCA by shaking at room temperature followed by high speed centrifugation. The phytic acid in supernatant is precipitated as ferric phytate by adding excess ferric chloride and centrifuged. The ferric phytate is converted to ferric hydroxide with a few ml of water and 3ml of 1.5N NaOH, and then the iron content present in the sample is estimated. The phytate phosphorus is calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio. The phytic acid is estimated by multiplying the phytate phosphorus by the factor 3.55 based on the empirical formula  $\text{C}_6\text{P}_6\text{O}_{24}\text{H}_{18}$ .

## Proteases inhibitor: trypsin inhibitor activity case

Trypsin inhibitor activity was determined according to the method<sup>28</sup> using benzoyl-DL-arginine-P-nitroanilide hydrochloride as the substrate.

A 4g of defatted sample is treated with 40ml of 0.05M sodium phosphate buffer, pH 7.5 and 40ml of distilled water. The sample is shaken for 3h and centrifuged at 700g for 30min at 15°C. Supernatant is diluted in order to obtain inhibition between 40 and 60% of enzyme activity. Incubation mixture consisted of 0.5ml trypsin solution (5mg/ml), 2ml 2% (w/v) Bapna, 1.0ml sodium phosphate buffer (pH 7.5, 0.1M), 0.4ml HCl (0.001M) and sample extract (0.1ml). Total volume of incubation mixture was maintained at 4.0ml. Incubation is carried out in a water bath at 37°C for 20min after which 6.0ml of 5% TCA (trichloroacetic acid) solution is added to stop the reaction. Blank sample is treated similarly through the entire determination. Absorbance (A) is read at 410nm wavelength in a spectrophotometer. Results are expressed as trypsin inhibitor units (TIU). One TIU is defined as an increase of 0.01 in absorbance units under conditions of assay. Trypsin inhibitory activity is defined as the number of TIU.

## Hemagglutinin

Hemagglutinin activity is estimated according to the method.<sup>29</sup>

The powder (5g) is mixed with 0.15M NaCl (1:8, w/v) for 48h at 4°C, and filtered through 80-mesh grid. Subsequently, the filtrate is centrifuged at 9168g for 30 minutes, and the supernatant is fractionally precipitated with ammonium sulfate at 10%-100% saturation, respectively. The four pellets are combined, dissolved in a minimal volume of water, and dialyzed against distilled water at 4°C. Bradford's method is used for protein quantification, using bovine serum albumin (BSA) as the standard.

## Conclusion

As shown in this study, nutritional properties and nutrients chemical analysis of common beans seed (*Phaseolus vulgaris* L.). Common beans are often a main source of protein, dietary fiber and minerals in diet, occupying a very important worldwide place in human alimentation, offering benefits for human health. Therefore, separate methods are required for estimating nutrients composition.

## Acknowledgments

None.

## Conflict of interest

Author declares no conflict of interest.

## References

- Rosalinda T Laguna, Virginia S Claudio. *Nutrition and Diet Therapy Reference Dictionary*. New York: Chapman & Hall; 1995. 69 p.
- Willett WC. Diet and health: what should we eat? *Science*. 1994;264(5158):532–537.
- Lennernas M, Fjellstrom C, Becker W, et al. Influences on food choice perceived to be important by nationally representative samples of adults in the European Union. *Eur J Clin Nutr*. 1997;51:S8–S15.
- Debouck DG. Systematics and morphology. Common beans; research for crop improvement. Wallingford, UK: CAB Publishing; 1991. p. 55–118.
- Voyses OL, Fernandez FC. *Bean Improvement by introduction and selection*. International Center for Tropical Agriculture, Colombia; 1986. 32 p.
- Juhi M, Rattan DS, Vikas SJ, et al. Assessment of phenolic components and antioxidative activities of *Phaseolus vulgaris* L. *International Journal of Integrative Biology*. 2010;9(1):26–30.
- Mitchell DC, Lawrence FR, Hartman TJ, et al. Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *J Am Diet Assoc*. 2009;109(5):909–913.
- Pujolà M, Farreras A, Casañas F. Protein and Starch Content of Raw, Soaked and cooked beans (*Phaseolus vulgaris* L.). *Food Chemistry*. 2007;102(4):1034–1041.
- Nowacki E. Heat-stable and anti-nutritional factors in leguminous plants. *Advances in Legume Science*. UK: Royal Botanic Gardens, Kew, Richmond, Surrey; 1980. p. 171–177.
- Liener IE. Implications of anti-nutritional components in soybean foods. *Crit Rev Food Sci Nutr*. 1994;34(1):31–67.
- United States Department of Agriculture: Health and Human Services. *Dietary Guidelines for Americans*. 7th ed. Washington, DC: U.S. Government Printing Office; 2010. 154 p.
- United States Department of Agriculture: Center for Nutrition Policy and Promotion. *Food Guide Pyramid: A Guide to Daily Food Choices*. Avenue Building 005, Room 107, BARC-West Beltsville, Maryland; 2005. 16 p.
- United States Department of Agriculture: Branded Food Products Database. Composition of Foods Legumes and legume products; raw, processed, prepared. Agriculture Handbook; 2016. p. 8–16.
- Akerberg AK, Liljeberg HG, Granfeldt YE, et al. An *in vitro* method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fiber. *J Nutr*. 1998;128(3):651–660.

15. Landa-Habana L. Effect of cooking procedures and storage on starch bioavailability in common beans (*Phaseolus vulgaris* L.). *Plant Foods Hum Nutr.* 2004;59(4):133–136.
16. Lindorff K, Rogen P, Poci E, et al. Protein folding and the Organization of the Protein Topology Universe. *Trends Biochem Sci.* 2005;30(1):13–19.
17. Sotelo A, Sousa H, Sánchez M. Comparative study of the chemical composition of wild and cultivated beans (*Phaseolus vulgaris* L.). *Plant Foods for Human Nutrition.* 1995;47(2):93–100.
18. United States Department of Agriculture: National Nutrient Database for Standard Reference, Release 26. *Composition of Foods Raw, Processed, Prepared.* Beltsville, Maryland 20705; 2013. 136 p.
19. AOAC. *Official methods of analysis.* 15th ed. Washington, DC, USA; 1990. 12 p.
20. AOAC. Official methods of analysis of the AOAC. *Total, soluble and insoluble dietary fiber in foods.* Enzymatic-gravimetric method, MES-TRIS buffer. 16th ed. Arlington; 1995. 4 p.
21. Gonzalez Castro MJ, Lopez Hernández J, Simal Lozano J, et al. Determination of Amino Acids in Green Beans by Derivatization with Phenylisothiocyanate and High-Performance Liquid Chromatography with Ultraviolet Detection. *Journal of Chromatographic Science.* 1997;35(4):181–185.
22. IUPAC. Oils and fats. *Standard Methods for the Analysis of Oilseeds, Fats and Derivatives.* 7th ed. International Union of Pure and Applied Chemistry, Blackwell Scientific Publications, Oxford; 1987.
23. Tako E, Glahn RP, Laparra JM, et al. Iron and Zinc: Bioavailabilities to Pigs from Red and White Beans (*Phaseolus vulgaris* L.) are Similar. *J Agric Food Chem.* 2009;57(8):3134–3140.
24. AOAC. *Official methods of analysis of the association of official's analytical chemists.* 17th ed. Association of official analytical chemists, Arlington, Virginia; 2003.
25. Furtado J, Siles X, Campos H. Carotenoid concentrations in vegetables and fruits common to the Costa Rican diet. *Int J Food Sci Nutr.* 2004;55(2):101–113.
26. AOAC. *Total Foliates in Cerals and Cereal foods.* In: 10th ed. Gaithersburg; 2004.
27. Wheeler EI, Ferrel RE. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry.* 1971;48:312–316.
28. Kakade ML, Simons N, Liener IE. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soy bean samples. *Cereal Chemistry.* 1969;46:518–528.
29. Liener IE, Hill EG. The effect of heat treatment on the nutritive value and hemagglutinating activity of soy bean oil. *J Nutr.* 1953;49(4):609–620.