Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.73, 2018

Some Nutritional Characteristics of a Local Landrace of Tepary Bean Seed from the Republic of Benin

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Abstract
The study was led to investigate the proximate nutritional value of white tepary bean from Benin, Africa. The proximate nutritional composition of Tepary bean seed such as crude protein, fat, and carbohydrate were determined by the use of standard methods of analysis, and minerals such as iron, manganese, calcium and magnesium were also determined by atomic absorption spectrometry. The result shows that white tepary bean contains 25.69% protein, 0.96% fat and 73.35% total carbohydrates, Iron (Fe) 0.6mg/100g; Manganese (Mn) 2.3 mg/100g; Magnesium (Mg) 51.8 mg/100g and Calcium (Ca) 48 mg/100g.
Keywords: Proximate, minerals, fatty acids, white tepary bean

1. Introduction

Tepary bean (*Phaseolus acutifolius* L. Gray) constitutes one of the most popular and widely cultivated species among the species of the genus *Phaseolus*. They are among the most important grain legumes for direct food use (Broughton *et al*. 2003). *Phaseolus* beans are grown all over the world, but principally in the south and central America, Africa, India, and China. It has a rich, nutty taste and it is an excellent source of fiber, carbohydrates, and protein. Although tepary bean is low in amino acids tryptophan, methionine, and cysteine, it has similar energy, protein, fat and carbohydrate levels as other commonly grown beans in Mesoamerica (e.g. black and red varieties of *Phaseolus vulgaris*). However, the presence of anti-nutritional factors in tepary bean such as trypsin inhibitors reduce the bioavailability of active trypsin needed for protein, and lectins digestion, which cause red blood cells to agglutinate. Therefore, due to the levels of anti-nutritional factors in tepary bean, it is considered toxic in a raw form and must be thoroughly processed before usage by human and animals by simple heating or chemical treatment. Its annual production is around 24 million tons (Popelka *et al*. 2004). The search for alternative sources of inexpensive protein has led to the cultivation of more Fabaceous crops because of their advantages in worldwide distribution and their high protein content (Obasi, 1993). Unfortunately, this attention on the Fabaceous crops has not been extended to all legumes, therefore, resulting in the underutilization and neglect of others leguminous species. One of such legumes is the tepary bean (*Phaseolus acutifolius* L. Gray). Despite its numerous potential benefits, it has been neglected by both government and researchers and suffers a risk of being extinct as it is now cultivated by mostly elderly farmers. It has not been utilized for milk production but has rich properties as soybean (*Glycine max*). Tepary bean is consumed as legume for about thousand years ago by native Americans (Nabhan and Felger. 1978), Uganda and Malawi (Mogotsi. 2006). Throughout the world, rainfed agriculture is threatened by frequent problem of drought. Moreover, there is a potential impact on the global economy and trade owing to the fact that 84% of world's cultivated lands are under severe impacts caused by global warming and drought, and associated consequences felt in developing countries by farmers "subsistence" or " small holder " (Morton. 2007). According to (Rao. 2001; Beebe *et al.,* 2008), drought touches over 60% of dry bean production worldwide. In 2009, a group of researchers established a molecular selection program in a conceptual framework for drought phenotyping which emphasized the importance of identifying plant traits and mechanisms that promote greater adaptation to drought stress (Salekdeh *et al*., 2009). A special interest is shown in some parts of the world for plants with the capacity to develop in semi-arid and arid areas with regard to desertification. Despite the studies and promotion of a number of species, many other plants such as tepary bean have been less exploited and used in their native regions because they are considered as less important and nutritive compared to other crops.

The objective of this paper was to show a proximate nutritional composition of the white tepary bean from Benin, Africa as it has not been done before.

2. Materials and Methods

2.1 Sample collection: One variety of white tepary bean were purchased from a farmer in Bohicon city, Benin.

2.2 Preparation of sample: Samples were washed with running water and secondly with distilled water and placed on sterilized paper towels to remove excess water and hasten drying. The dry bean samples were then grinded using sterile mortar and pestle, and the fine powder obtained was used for the proximate analysis. The flour was placed into a plastic jar and stored at 4ºC with 40% relative humidity until needed.

2.3 Protein determination

1g of the flour was mixed with 4 mL of hexane for 12 hours in a Soxhlet apparatus for solidification, freeze dried and stored at -18 ºC for further use. The Crude protein content (N * 6.25) in the sample was determined according to the micro-Kjeldahl method (AACC, 1983). 0.20g of the sample was placed into a digestion tube and 15mL of H_2SO_4 was added. The contents of the tube were gently swirled until they were thoroughly mixed, followed by the addition of Kjeldahl catalyst (5g) and heating for 2 hours to obtain a clear liquid. The pre-cooled content was transferred and diluted with distilled water in 100mL volumetric flask. An aliquot (10mL) from a digest (10mL of 2% boric acid and 4 drops of mixed indicator) was transferred into a distillation flask attached to the distillation apparatus 10mL NaOH was then added, followed by the distillation of Nitrogen into the distillation flask till it reached the 150mL mark. Distilled water was used to wash the condenser tip and titrated the distillate against 0.025 N H_2SO_4 until a pink endpoint was reached. The precedure was performed in triplicates. Total crude protein was obtained by multiplying the % N by 6.25.

2.4 Lipid extraction

The fine powdered tepary bean samples (1g) were hydrated with 10mL of distill water and left for 1h under magnetic stirring at room temperature due to the minimum amount of distill water required to wet the flour. The solvent combination of chloroform/methanol ($CHCl_3$/ MeOH, 2/1, 86 mL) was added to the sample and extracted for 3 min using a homogenizer (Agilent 7890B-GC). A separating funnel was used to collect filtered solution and the solid residue washed with 22 mL of the extractive solvent that had been collected in the separating funnel where the organic phase was washed with 21.4 mL / 0.73 (w: v) NaCl aqueous solution. Afterwards, the organic phase was siphoned, dried over anhydrous sodium sulfate, filtered, and dried under reduced pressure with a rotary evaporator. The liquid extract was weighed and trans esterified. Each extraction was performed in triplicate.

Sixty milligrams (60mg) of tepary bean sample powder were put into the test tube, added 4 ml isooctane and 200 mL potassium hydroxide solution, then shacked violently for 30 seconds until it was clear. 1g of the sodium bisulfate was placed into a test tube and shake violently for 30 seconds until the liquid was clear. 0.5 mL of the liquid was put in a small brown bottle and analyzed with a gas chromatograph.

The GC analysis was performed on an Agilent DB-Wax Column (30 m * 0.25 mm * 0.25 mm i.d., 0.20 µm film thickness) fitted with flame ionization detector (FID) and SP2380 (Supelco, Bellefonte, PA, USA). He at 1 mL/min was used as the carrier gas and the initial oven temperature held at 180 ºC for 1.5 min. This was then increased to 210 ºC at 2 ºC/min, subsequently to 220 ºC at 5 ºC for 1.5min. The injector and detector temperatures were configured at 250 and 300 ºC, respectively with a split ratio (10:1). Identified FAME peaks were compared to the retention times of reference standards FAME determinations were performed in triplicate with mean values.

2.5 Minerals determination

The samples were prepared and analyzed by atomic absorption spectrometry instrument Agilent 200AA using the flame method. The standard curve was prepared at different concentration depending on the mineral to check (Fe; Mn; Mg; and Ca). 0.5g of the samples was weighed and accurate to 0.001g in which was added 5 ml nitric acid then placed in a microwave digestion for 30 min. The samples were then cooled to room temperature and water to a volume of 25 mL before being tested.

In accordance with the instructions prescribed by the manufacturer, the instrument (section of fuel, oxidizing gases, burner fuel and wavelength) has been set up. A specific wavelength was chosen for each Fe, Mn, Mg and Ca (Fe248.3nm, Mn279.5nm, Mg285.2nm and Ca422.7nm) minerals, respectively. For each metal, the sample and standard solution was aspirated into the flame and the absorbance of the ions was recorded Fe 0.5,1,2,4,6 (mg / L); Mn 1,2,4,6,8 (mg / L); Mg 1,2,4,6,8 (mg / L) and Ca 1,2,4,6,8 (mg / L). The graph of absorbance versus concentration gives a linear graph. Using the absorbance values, the concentration of each metal in a sample was calculated based on the slope of the graph. The exact concentration of the element of interest was calculated by applying the following formula.

Concentration mg/L = absorbance of sample # Slope obtained from the graph

2.6 Carbohydrates determination

The colorimetric analysis was the one used in this experiment for the determination of the total carbohydrates. At different volumes (1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 8 ml, 10 ml, 13 ml and 20 ml) the glucose standards of the stock solution aliquots were pipetted and transferred to nine beakers. of 30 ml. To obtain a final volume of 20 ml, a quantity of distilled water was added. 2 ml of each solution was transferred to 10 test tubes. 10 ml of sulfuric acid (H_2SO_4) at 96% concentration and 2 ml of phenol were pipetted and added to each of the 10 tubes. For 10 minutes, a light orange color developed. Cuvettes having a path length of 1 cm were used to transfer all the solutions and using a UV spectrophotometer (Shimadzu Mini-1240 UV-VIS model) the absorbances were

measured at 485 nm. The measurements were taken three times in a row to determine the average. The same method was used for the reference solution, with the difference that the glucose solution (2 ml) was replaced by distilled water.

2.7 Energy calculation

The rate of energy that is expressed in kilocalorie (Kcal) was obtained by multiplying the values found in this experiment (crude protein, fat, and carbohydrate). Knowing the law of nutrition which states that: 1g of protein = 4 Kcal, 1g of carbohydrate = 4 Kcal and 1g of fat = 9 Kcal; The values obtained were multiplied by their energy index corresponding to their group. The calculation was done as follows;

Energy (Kcal/100g) = {[% C.P # 4] + [% total carbohydrate # 4] + [% C.F # 9]}

Where: C.P, Crude Protein, C.F, Crude Fat.

3. Results and Discussion

3.1 Proximate analysis and Minerals composition of white tepary

This study was performed to determine the nutritional value of white tepary bean from Benin Africa. The proximate composition and minerals determined using standard procedures are reported in table 1 and 2.

Table 1. Proximate composition of the white tepary bean

Nutrients	Proteins	Fat	Carbohydrates	Energy
Composition %	25.69	0.96	73.35	404.8Kcal/100g

Table 2. Minerals composition of white tepary bean

Minerals	Iron	Manganese	Magnesium	Calcium
Composition (mg/100g)	0.6	2.3	51.8	48

The result of specific minerals tested are shown in table 2. The results showed that, white tepary bean from Benin, Africa contains Iron (Fe) 0.6 mg/100g; Manganese (Mn) 2.3 mg/100g; Magnesium (Mg) 51.8 mg/100g and Calcium (Ca) 48 mg/100g. Since no previous report about the mineral composition of tepary exist in Africa, the current results were compared to previous analysis of the same bean found at a different location. The iron composition in tepary bean was lower 0.6mg/100g in comparison with other previous research Nabhan *et al*., (1985) observed a higher iron concentration (8.0) in tepary obtain from Arizona. This may be due to the difference in geographical area, method of analysis among others etc. Awal *et al*., (2000) noticed that the nutritional composition can be influenced by the method of analysis. The contrary was found for the manganese content (2.3 mg/g) of tepary bean obtained from Benin, Africa relative to that from Arizona 1.8 mg/g magnesium (51.8mg/g) and calcium (48mg/g) levels of tepary bean from Benin was somehow higher amongst all minerals checked but lower in comparison to the one from Arizona (Scheerens *et al*., 1983). Despite the fact that it showed fewer values, it still gives the health benefits associated with the consumption of beans such as those important for energy production, enzyme activity, heart and nervous system (magnesium), strengthen bones, teeth and promote muscle growth. The diverse range of the mineral contents could be an indication of the interdependence of the soil nutrients available to the growing plant and mineral levels present in the seed.

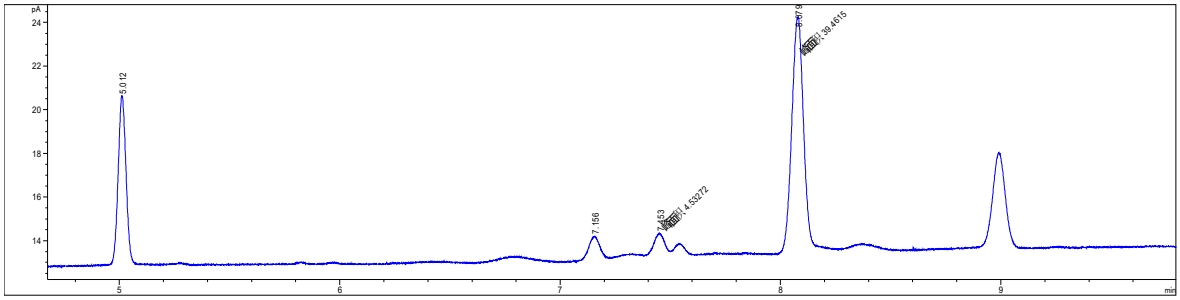
3.2 Fatty acids in white tepary bean

Lipids or fatty acids are substances soluble in organic solvents such as ether, chloroform or benzene, in this study, chloroform was used as solvent to extract and quantify the fatty acids, the result is reported in the table-3 and the chromatograph illustrated in fig. 1.

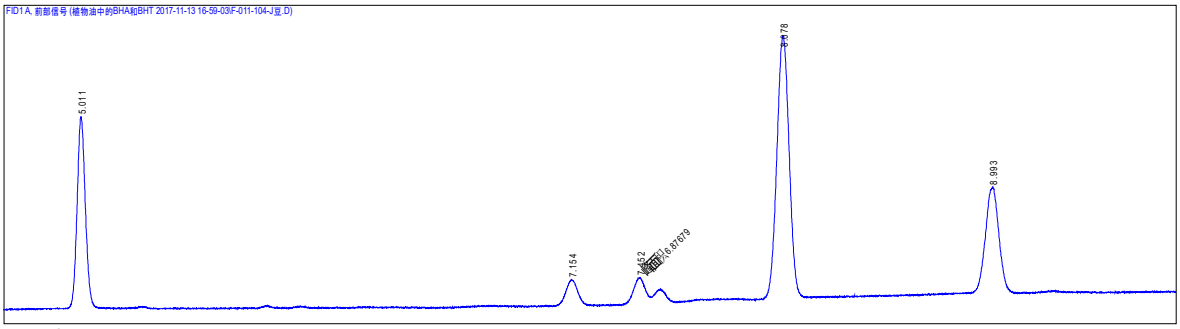
Table 3. Fatty acid composition of white tepary bean

Tepary bean	Methyl hexadecanoate	Methyl stearate	Methyl Oleate	Methyl Linoleate	Methyl Linolenate
RT/min	5	7.1	7.4	8	8.9
Peak area	25.6	4.5	6.5	47.2	20.6
g/100g	0.178	0.022	0.035	0.209	0.524

Test 1



Test 2



Test 3

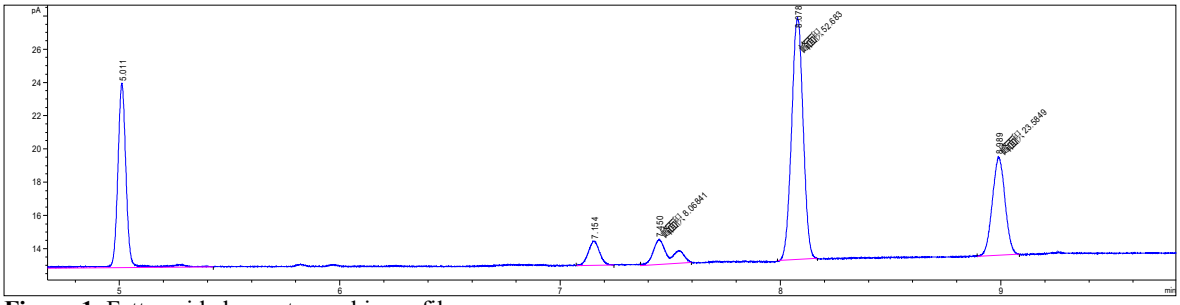


Figure 1. Fatty acid chromatographic profile

The lipid content in tepary bean ranged from 0.73 to 1.18% and composed mostly of polyunsaturated fat (Scheerens *et al*.,1983; Idouraine *et al*., 1993a). Seed fats usually contain low levels of saturated fatty acids, palmitic acid is the widest spread saturated fatty acid in vegetable oils in addition to the presence of small amounts of stearic acid. They contain palmitic, linoleic, oleic, and linoleic acids. Amongst the five fatty acids, 2 saturated acids (palmitic and stearic) and 3 unsaturated acids (oleic, linoleic and linolenic). The linolenic acid was found with the highest value (0.524g/100g) with a peak area of 20.6 and a retention time of 8.9 followed by linolenic acid (0.209g/100g) which showed 47.2 at the peak area and 8 as retention time. Oleic acid (0.035g/100g) was the lowest among unsaturated acid with a peak area 6.5 and 7.4 retention time. In the group of saturated acid, palmitic acid appeared to be higher (0.178g/100g), peak area 25.6 and retention time than stearic acid (0.022g/100g), peak area 4.5 and 7.1 retention time. This result confirmed the health benefit of with tepary bean in free fatty acids. Natural fats such as palmitic and stearic acids are the best fatty acids for mammalian nutrition (Hayes. 2002). Palmitic acid has an intermediate impact on lipoprotein profile and its consumption along with monounsaturated fatty acids or polyunsaturated fatty acids shows neutral effect. Foodstuffs processing P/S ratio below 0.45 is not advisable for human consumption as it leads to cardiac diseases (Department of Health, 1994).

4. Conclusion

Based on the current results, it can be said that white tepary bean from Africa is as much as nutritive than those found elsewhere with regards to the protein, fat, total carbohydrates, minerals, and calculated calories. The grain can provide substantial amounts of these recommended daily intake nutrients. Great attention has to be given to the nutritional properties of this undervalued crop of Africa which is under extinction.

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