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Study of the nutritional quality of the seeds of two varieties of beans (*Phaseolus vulgaris* L.) grown in Congo

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Abstract

Phaseolus vulgaris L. is a legume belonging to the order *Fabales* and the family *Fabaceae* and which is consumed throughout the world for its seeds and pods.

The seeds of this species provide a source of dietary protein available in many parts of the world; this is why they are considered "poor man's meat" because of their high protein content and low cost, compared to animal proteins.

The present study relating to the study of the nutritional value of bean seeds cultivated in Bouenza (Boko-Songho) and in the Plateaux (in Lékana) is a contribution to responding to the need for characterization of these varieties. Thus the general objective of this study which is to establish the nutritional value of these two local varieties "Kikata blanc" and "Lekana" is to use the standards established by the AOAC to achieve this objective, namely: the oven to determine the water content, the Soxhlet method to extract the fat, the Kjedahl method to determine the total protein content, the muffle furnace to know the ash rate, we deduced the carbohydrate content and finally we used spectrometry to identify the minerals in the ashes. We obtained the following results: Contents: Water: 7.65% ±0.07; Lipids: 0.81% ±0.02; Proteins: 10.6% ±0.14; Carbohydrates: 76.68% ±0.06; Ashes: 76.68% ±0.06; Energy value: 356.41 Kcal/100g. Among the minerals sought, we obtained: Phosphorus: 0.50% ±0.007; Magnesium: 0.14% ±0.007; Calcium: 0.04% ±0.006; Iron: 0.01% ±0.00. The results of this study allowed us to see that these two plants have good nutritional values.

In order to complete this study, it is appropriate to look for other minerals in the ashes from these seeds; to deepen the studies to know the factors responsible for the differences in the biochemical composition observed between the two varieties.

Keywords: Bean; Bouenza; Lékana; Congo; Biochemical Composition.

1. Introduction

Phaseolus vulgaris L. is a legume that is consumed worldwide for its seeds and pods [1]. It belongs to the *Fabaceae* family whose morphological characteristics are known. It has a root system with several lateral and adventitious branches [2].

Phaseolus vulgaris L is one of the dietary protein sources available in many parts of the world; It is therefore considered "poor man's meat" because of its high protein content (20-30%) and low cost compared to animal proteins. *Phaseolus vulgaris* is also rich in iron and fiber [3]. It represents a valuable supplement to the cereal-based diet [4]. On a global

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scale, beans are ranked 10th among the most consumed foods of plant origin (the first three being occupied by potatoes, cassava and tomatoes), and first among legumes consumed as vegetables dry (excluding soya), ahead of peas, chickpeas and beans [5].

Common bean is produced in subtropical and tropical regions, most often by smallholder farmers, and is a major staple crop in both developing and developed countries.

The largest producers by annual average during the period 2013 to 2017 are India (5.8 Mt), Myanmar (4.9 Mt), Brazil (3.0 Mt), United States (1.3 Mt) and Mexico (1.2 Mt) [6].

For the African region, from 2015 to 2020 the top ten (10) dry bean producing countries ranked in order are: Tanzania, Uganda, Kenya, Ethiopia, Rwanda, Burundi, Cameroon, Angola, Mozambique and the Democratic Republic of Congo.

In central and eastern Africa, beans partly cover the daily protein and energy needs of populations.

In Congo, beans are consumed in most departments as a complementary food; the seeds consumed include both locally produced and imported varieties. In the departments of Niari, Bouenza, and Pool, this plant is cited among the main crops which contribute to the dietary energy intake of households alongside cassava, peanuts and corn [7]. These three departments in the South (Niari, Bouenza and Pool) and the Plateaux department in the Center (Lékana District) are the main centers of bean production in Congo.

The present study relating to the study of the nutritional value of bean seeds cultivated in Bouenza (Boko-Songho) and in the Plateaux (in Lékana) is a contribution to responding to the need for characterization of these varieties. Thus the general objective of this study is to establish the nutritional value of these two local varieties “Kikata blanc” and “Lekana”.

2. Material and methods

2.1. Plant material

The plant material used for this study consists of the seeds of two varieties of bean: the “Kikata blanc” variety grown in the Bouenza department (Boko-Songho) and the “Lekana” variety grown in the Plateaux department (Lékana).

Figure 1 Seeds of the “White Kikata” bean

Figure 2 Seeds of the “Lekana” bean

2.2. Methods

2.2.1. Sample analysis

Preparation of samples for analysis

The seeds used as samples were dried, crushed and wrapped in aluminum foil before carrying out the analyses. We thus constituted two groups of samples: one group for the seeds of the "Kikata Blanc" variety (KB) and another for the seeds of the "Lékana" variety (LKN).

Determination of water content

To determine the water content, 30 g of fresh samples were weighed then placed in an oven set at a temperature of 70°C until a constant dry mass was obtained.

Knowing the fresh mass (mf) and the dry mass (ms) of the samples, the water content was determined by the following formula: % Water = (mf-ms)/mf×100

With :

mf = Mass of fresh samples and ms = Mass of dried samples

Determination of lipid content

The lipid content was determined using a Soxhlet type extractor. In the WHATMAN cartridge, 30g of ground dried bean seeds were introduced into filter paper. This cartridge was placed in a Soxlhet extractor then connected to the refrigerant. 200 ml of N-hexane were placed in a 250 ml flask previously weighed (Mo), and on this flask, the Soxhlet extractor, topped with a condenser was positioned and the whole was placed on a heating flask. After several continuous siphonings, a mixture of oil plus solvent was obtained. The oil extracted from each sample was separated from the solvent using a rotary evaporator. After cooling, the flask containing oil was weighed (M1). The lipid content was determined by the following formula:

% lipids = (M1-M0)/M x 100

M0: mass of the empty balloon; M1: mass of the balloon with oil; M: mass of the weighed sample

Determination of protein content

The protein content of the samples was determined by the Kjedahl method. In a matra, 0.5 g of the crushed seeds of each sample was then added 10 ml of sulfuric acid, a tip of catalyst and a few glass beads. After cold digestion for approximately 48 minutes, the mixture then undergoes hot digestion for 2 hours at a temperature of 415°C. After cooling, 20 ml of distilled water and 30ml of sodium hydroxide (0.1N) were added until a brown colored solution was obtained. The ammonia is then distilled in an excess of soda then recovered in boric acid in the presence of a colored indicator (methyl red) up to a volume of 150 ml. The nitrogen titration was carried out with N/20 sulfuric acid until the indicator changed from green to pink.

The total nitrogen content was determined by the following formula: $\%N = (V H_2SO_4 \times 0.07)/\text{weighing}$

To determine the protein content, multiply the % N by 6.25 (conversion coefficient).

Determination of ash content

Determining the ash content makes it possible to evaluate the level of minerals contained in the sample. This operation consisted of incinerating the sample at a temperature of 550°C in a muffle furnace until a constant mass was obtained.

The ash content was calculated by the following formula:

Ash content (% MS) = (M2-M0)/(M1-M0)×100

With: M0: mass in grams of the empty crucible; M1: mass in grams of the incineration crucible loaded with sample; M2: mass in grams of the incineration crucible loaded with ashes.

Mineral dosage

The determination of minerals is carried out on the ashes obtained after incineration of the ground material.

- Phosphorus dosage

To carry out the phosphorus dosage, two solutions were previously made up as follows:

Solution A: In a 100 ml flask, 50 ml of distilled water and 10ml of sulfuric acid were put. After cooling, 0.6 g of ammonium molybdate and 0.014 g of potassium antimonyl tartrate were added and then the mixture was supplemented with distilled water to the mark;

Solution B: in a 100 ml flask, 2 g of ascorbic acid plus 50 ml of water distilled were mixed with 5 ml of concentrated hydrochloric acid. Distilled water is then added up to the mark.

The two solutions (A and B) were mixed in a 250 ml beaker to make a range. The prepared quantity was used to determine phosphorus.

The dosage was carried out as follows: in a plastic pill bottle, we put 0.5 ml of sample to be dosed, 10 ml of distilled water and 3 ml of reagent. After mixing, the color was allowed to develop for 30 minutes after it had stabilized. We then measured the absorbance at 660 nm with a spectrophotometer. A calibration curve was drawn from the range points. From there, we deduced the concentration for each sample.

Iron dosage

The iron dosage was carried out using the Ortho-phenantroline method. Iron solution of 10mg/l concentration was used for the range.

In seven (07) pill bottles, 5 ml of the sample were added as well as 5 ml of phosphorus, 2 ml of 3% sodium citrate, 2 ml of sodium acetate and 2 ml of ortho-phenantroline at 0 .2%. 30 minutes after the stabilization of the coloring, the absorbance was measured at 490 nm with a UV spectrophotometer. A calibration curve was plotted from the range points and then we finally deduced the concentration for each sample.

- Calcium dosage

To determine the calcium, we took 20 ml of the sample, which we then introduced into a 250 ml Erlenmeyer flask to which we added 30 ml of distilled water, 1 ml of 1% KCN, 5ml of N triethanolamine hydrochloride. The mixture obtained was brought to a pH of 12.5, by adding a 2.5N sodium hydroxide solution. A pinch of calcon was also added. The dosage was finally carried out with an EDTA N/50 solution up to the bend to obtain the calcium concentration.

- Magnesium dosage

To measure the magnesium, we took 20 ml of the sample in a flask which we put in a 250 ml Erlenmeyer flask then we added 30 ml of distilled water. We then adjusted the pH of the solution to 10 with a buffer solution by adding a pinch of the NET. We then dosed the mixture with an EDTA N/50 solution until the indicator changed.

We made the blank and compared the results (turns). From this last volume we subtracted the volume of the blank then that of the calcium dosage alone and we carried out the calculation to obtain the magnesium concentration.

- Determination of total carbohydrate level (G)

Carbohydrate content (G) was estimated by the difference method. According to the method of Manzi (1999) cited by other authors [8]; it was calculated by subtracting from 100, the sum of humidity (H), fat (MG), proteins (P) and ash (C) contained in the sample.

- Determination of the crude fiber rate (FB)

After acid and then alkaline treatment, hydraulic oxidative degradation of cellulose and lignin occurs. The residue obtained after filtration is weighed, calcined, cooled and weighed again. Weight loss gives the crude fiber rate.

We introduced into a 500 ml flask, 1 g of the delipidated ground material and 100 ml of sulfuric acid at 0.255 N. The mixture obtained was heated to reflux for 30 minutes and filtered under vacuum through filter paper and washed with water distilled. The residue was returned to the flask and treated with 100 ml of 0.313 N sodium hydroxide. The mixture was heated for 30 minutes and filtered. The residue was washed three times with distilled water and transferred to a previously weighed crucible (P1) and then dried in an oven at 130°C for 2 hours then allowed to cool and weigh (P2). It was then calcined for 30 minutes in a muffle furnace then cooled followed by weighing (P3).

The fiber percentage was calculated using the following formula:

$$\%\mathbf{Raw\ fibers} = \frac{(\mathbf{P2} - \mathbf{P1}) - (\mathbf{P3} - \mathbf{P1})}{\mathrm{Me}} \text{ x } \mathbf{100}$$

P1: mass of crucible and sample; P2: crucible mass and dried sample

P3: mass of the crucible and the sample after incineration; Me: mass of the sample

Determination of Energy Value (EV)

The total energy value was calculated according to the method of Manzi (1999) cited by other authors [8].

It is determined using the formula below:

VE (kcal/100g) = (CHO x 4) + (CL x 9) + (CP x 4) with CHO = % of carbohydrates,

CL = % of lipids and CP = % of proteins.

3. Results

The results of the analyzes carried out are presented in the table below:

Table 1 Nutritional value of the beans studied

Var.			**KB**	**LKN**
Parameters analyzed and their contents	Water, organic matter and ashes (%)	Water	7.65% ±0.07	14.4% ±0.6
		Lipids	0.81% ±0.02	2.37% ±0.44
		Proteins	10.6% ±0.14	8.92% ±0.24
		Carbohydrates	76.68% ±0.06	69.59% ±0.34
		Fibers	10.6% ±0.14	7.6% ±0.14
		Ashes	4.26% ±0.02	4.72% ±0.09
	Mineral materials (%)	Fe	0.01% ±0.00	0.006%±0.00
		Ca	0.04% ±0.006	0.11% ±0.014
		Mg	0.14% ±0.007	0.33% ±0.00
		P	0.50% ±0.007	0.59% ±0.007
	V.E. (Kcal/100g)		356.41	335.37

Var. =Variety, V.E= Energy value

4. Discussion

4.1. Water content

From the point of view of the environment where the crops were grown, it is possible that the humidity levels recorded on these two varieties were influenced by the rainfall levels of each site. The department of Bouenza, growing area of

the KB variety, is part of the Niari Valley, a geographical area where the level of annual rainfall is around 800 to 1,800 mm (this is the area where it rains the most less in the Republic of Congo) with average temperatures of around 21 to 27° C [9]. The Koukouya Plateau, a growing area for the LKN variety, on the other hand, is part of the Batékés Plateaux where the annual rainfall reaches very high levels, around 1,600 to 2,500 mm (this is the area where it rains the most in Congo) and average temperatures are between 22 to 25°C [9]. It therefore rains more on the Koukouya Plateau (in Lékana) than in the Niari Valley (in Bouenza). These rainfall particularities could influence the water content of the seeds: the seeds from the area where it rains the most (seeds of the "Lekana" variety) have more water than those from the area where it rains the least (seeds from the variety "Kikata Blanc". There is also the richness in minerals of the soils of the different sites.

The water content of these seeds can also be influenced by the duration or conditions of their storage. It is obvious that the longer the time that elapsed between the harvest of these seeds and their use in our study, the lower their water content.

The high humidity of the seeds of the LKN variety would require more drying for their conservation. The seeds of the KB variety keep better than those of the LKN variety thanks to their low water content. The LKN variety would be more exposed to fungal diseases (rots) than the KB variety.

The results obtained in this study are in an order of magnitude similar to those obtained by certain authors [10], on 4 varieties of beans in Madagascar: 7.88%±0.02-12.87±0.03. However, humidity levels reported by other authors: 14%-15% [11]; 12.09%±0.16-14.47%±0.20 [12]; 11.72%±0.05 [13] are close to that expressed by the seeds of the LKN variety (14.4%±0.6) but higher than the value obtained on the KB variety (7.65%±0.07). The water content of the KB variety is very low compared to the results of several other works relating to the analysis of bean seeds, it is nevertheless close to 9.19%, a value obtained by certain authors [14], out of four different varieties of common bean in Burundi, slightly higher than 6.56%±0.60, value obtained by certain authors on a variety of red bean (small red Kidney bean) in Cameroon [15], but very high compared to 2.4%±0.23, value obtained by other authors in Nigeria on a variety of red kidney bean (Red Kidney bean) [16].

4.2. Lipid content

The results obtained show that the LKN variety has a higher fat content (2.37% ±0.44) than that of the KB variety (0.81% ±0.02). The expressed contents are still very low, this makes us think that these two varieties can be stored for long periods without risk of oxidation due to these low fat contents.

The lipid levels found in the two varieties are close to certain values reported in the literature: 0.33%-1.33% [17]; 1.24%±0.04-1.75%±0.02 [10]. However, the rates we obtained are much lower than 15.8%±0.10, a value obtained in another study [16]. The results obtained in this study are also lower (notably the content of the KB variety: 0.81% ±0.02) than the values ranging from 2.20% to 5.03% [18].

4.3. Protein content

The highest protein level was obtained on the KB variety (10.6% ±0.14). The LKN variety gave a fairly low rate of 8.92% ±0.24. These results show that the KB variety is slightly richer in protein than the LKN variety. The difference between these protein levels is very small (1.68%). However, the quantities of proteins obtained in this study are much lower than those reported in the literature: 15.3%±0.20 [16]; 17.08%-25.46% [19] and 22%-26% [25]. These latter values represent double or even triple those we obtained. This difference could be explained particularly by the genotype of the plant material used and the cultural care. These protein weaknesses could require improvement of these varieties by crossing with varieties richer in protein in order to obtain cultivars with higher protein levels.

4.4. Ash content

The ash contents of the two varieties are 4.72%±0.09% for the LKN variety and 4.26%±0.02 for the KB Variety. These two values are very close but the quantity of ash of the LKN Variety dominates that of the KB variety. Ash being a good indicator of the concentration of mineral elements in a sample of plant organ, the results obtained suggest that the quantity of minerals of the LKN variety could be greater than that of the KB variety.

The results of this study are close to values ranging from 3.57%±0.07 to 4.55%±0.07 [10], but also from 3.1%-4.2% [21] and 4, 4%±0.52 [16].

4.5. Mineral content

Four minerals were quantified in this study: Iron, Calcium, Magnesium and Phosphorus. Among these four minerals, phosphorus is the most abundant element in both varieties. The contents of these four elements in the two cultivars varied in the following order: phosphorus > magnesium > calcium > iron. The highest values for three of these elements (Phosphorus, Magnesium and Calcium) were recorded on the LKN variety, the highest content for Iron was recorded on the KB variety.

The contents recorded on the LKN variety are: 0.006%±0.00; 0.595±0.007; 0.11%±0.014 and 0.33%±0.00 respectively for Iron, Phosphorus, Calcium and Magnesium, and for the KB variety: 0.009%±0.00; 0.505%±0.007; 0.044%±0.006 and 0.145%±0.007 respectively for Iron, Phosphorus, Calcium and Magnesium. As these figures indicate, the LKN variety is richer in mineral matter than the KB variety.

The Calcium contents recorded in a study on six bean varieties in Mexico: 0.0696% ±6 - 0.173% ±40 [22], are on average higher than that obtained for the KB variety (0.044% ±0.006) but close of the value obtained on the LKN variety (0.11% ±0.014). The contents obtained in other studies in Burundi: 0.0552% [14] and Nigeria: 0.0549% [16], are higher than that found on the KB variety (0.044% ±0.006) but lower than that obtained on the LKN variety (0.11% ±0.014).

The iron content of around 0.0115% [16] is close to that obtained on the KB variety (0.01%) but higher than that obtained on the LKN variety (0.006%); those reported in another study (0.0048% ±1.6 - 0.00613% ±2.5) are similar to the result recorded on the LKN variety (0.006%) but lower than the rate found on the KB variety (0.01%). The rate of 0.00763% obtained by certain authors [20] is lower than that found on the KB variety: 0.01% but close to that found on the LKN variety: 0.006%.

The magnesium concentrations of the samples which are respectively 0.145% ±0.007 for KB and 0.33% for LKN), are close to those obtained by other authors whose values obtained oscillate between 0.112% ±0 and 0.218% ±10 [22] and are less than 0.8209% [16] but greater than 0.0382% [20].

The quantities of phosphorus found in this study (0.505%±0.007 for KB and 0.595%±0.007 for LKN) are higher than those obtained by other authors on the same product: 0.0316%±5-0.0515%± 2 [22], 0.456% [20], and 0.003% [16].

4.6. Total carbohydrate content

Whatever the variety, the carbohydrate level is very high (76.68% ±0.062 for the KB variety and 69.59% ±0.34 for the LKN variety). This confirms the trend observed in other work on beans: 73.59% [13]; 56%-60% [11]. However, it is fair to say that the level of total carbohydrates is higher in the LKN variety (76.68% ±0.062) than in the KB variety (69.59% ±0.34). Such an observation suggests that the seeds of the LKN variety could have a better energy value than that of the seeds of the KB variety, especially if their quantities of lipids also express high values. However, the value expressed by each of the two varieties is greater than 69.59% ±0.34; this allows us to say that these beans are good sources of carbohydrates and that they can be used in the production of infant flour.

The values of total carbohydrates obtained on our samples are close to those obtained by certain authors in Brazil (69.89% to 72.47%) [12]. The results are, however, superior to those reported by other authors on eight (08) improved varieties of dry beans in Ethiopia and who had obtained values ranging from 62.05% to 65.08% [23]; from 56% to 60% [11] and from 49.0%±0.50 [16].

4.7. Dietary fiber content:

The dietary fiber rate is equal to 10.6%±0.14 for the KB variety and 7.6%±0.14 for the LKN variety. The value expressed by the KB variety is higher than that of the LKN variety. The averages of the two varieties are higher than those found by other authors on red kidney beans and who obtained a value of 3.6%±0.50 [16], the value of 3.0% ±0.02 3, on pinto bean [24], the values ranging from 1.0% ± 0.1 to 1.7% ± 0.0, on six different varieties of dry bean [22]. However, these averages are lower than those reported by other authors on peanuts (30.77%) and soya (15.61%) [25]. Considering our results, we can say that the two varieties that we studied have dietary fiber contents which could give them a certain laxative power. The IOM (Institute of Medicine Food and Nutrition Board) recommends the consumption of foods rich in fiber, in fact fiber could reduce the risks linked to cardiovascular diseases and cancer [25].

4.8. Energetic value

The energy values measured are very high for the two types of bean, with: 356.41Kcal/100g or 1491.57 KJ/100g for the Bouenza variety and 335.37Kcal/100g or 1403.52 KJ/100g for the variety plates. These figures indicate that the KB

variety grown in Bouenza has a higher energy value than that of the LKN variety which originates from Lékana, this opinion agrees with the values obtained when determining their quantities of carbohydrates (the carbohydrate content of the KB variety is superior to that of the LKN variety).

The energy values of the varieties concerned by our work are higher than those obtained by certain authors in Ethiopia on 8 different bean cultivars (1320.01-1375.74 kJ/100g of dry matter.) [28], but lower than those obtained by other authors: 1678.4 kJ/100g of dry matter [20] and 1851.4 kJ/100g of dry matter [24].

5. Conclusion

The present study was carried out with the aim of evaluating the nutritional values and antioxidant potential of two varieties of bean: "Kikata blanc" (KB) cultivated in Bouenza, Niari and Pool and "Lékana" (LKN) cultivated in the Plateaux department. These two varieties are among the most marketed and consumed in our country, the results of this study allowed us to note that these two plants have good nutritional values.

The highest levels of humidity and ash (14.4% ± 0.6 and 4.72% ± 0.09 respectively) were recorded on seeds of the "Lékana" variety. Regarding minerals, except for iron, the "Lékana" variety was found to be richer than that of Bouenza with the following values: 0.11%±0.014; 0.33% and 0.595%±0.007 respectively for calcium, magnesium and phosphorus. On the other hand, Bouenza seeds have an iron content (0.009%) higher than that of Plateaux seeds.

Among the macronutrients, carbohydrates, proteins and fibers are in higher proportions (76.68±0.062; 10.6% ±0.14 and 10.6%±0.14 respectively) in Bouenza seeds. Lipids, on the other hand, are more abundant in Plateaux seeds (2.37%±0.44). The energy value of the Bouenza variety is clearly in line with its high carbohydrate, fiber and protein contents, it turned out to be the highest (356.41Kcal/100g).

Several factors could explain the differences observed in the results obtained: environmental factors, genetic factors, factors relating to agricultural work for example.

In summary, this study generated information regarding the nutritional importance of these bean varieties commonly consumed in the Republic of Congo. The results of this study can help promote the use of these varieties in various fields: food, selection and varietal improvement in plant production.

In order to complete this study, it would be interesting to:

- Look for other minerals in the ashes from these seeds;
- Deepen the studies to know the factors responsible for the differences in the biochemical composition observed between the two varieties.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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