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Nutritionnel Assessment of *Cajanus Cajan* Seeds from Côte D'ivoire

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Original Research Article

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Abstract: The aim of this study was to investigate the physicochemical properties of *Cajanus cajan* cultivated in Côte d'Ivoire. Some anti-nutritional factors (trypsin activity) and α-amylase of *Bacillus subtillus* digestibility were evaluated for the seeds flours. The proximate composition revealed that pigeon pea had highest moisture (12.40 %), dry matter (87.60 %), ash (4.50 %), crude starch (39%), crude cellulous (11.9%) and crude protein (25.60%) respectively. While, carbohydrate and fat contents was found in values of 56.10 ± 0,1% and 1.8 ± 0.1 %, respectively. *Cajanus cajan* seed also contains several minerals. The appreciable value of potassium was 1.8mg/kg, while the levels of phosphor and sodium in the legumes seeds tested were found to be 0. 6± 0. 0 and 0.03±0,02 mg/kg respectively with a total energy value average 2818 ± 3 kcal per 100 g of flour. Anti-nutritional factor such as trypsin activity was found to decrease significantly in the whole seed at 55%. A significant improvement was observed in vitro digestibility of starch provide from whole seeds. Digestibility with alpha amylase represents 82% for whole flour and 88 % for flour made with seeds without film. Thus, the study indicated that pigeon pea flours would have great potential in various food applications. Therefore, the judicious use of seeds could be a source of additional nutrients in the diet of vulnerable population.

Keywords: Pigeon pea, physicochemical properties, trypsin activity, digestibility.

INTRODUCTION

The importance of grain legumes in the world is high due to their significance in human and animal nutrition. Legumes are the edible seeds of leguminous plant. They provide a significant amount of food in developing countries. They, along with cereals, roots and vegetables constitute the staple foods that are consumed [1].

Among legumes, Pigeon pea (*Cajanus cajan* L.), a perennial legume which belongs to *Fabaceae's* family, one of the most common tropical and subtropical legumes cultivated for its edible seeds**.** It is predominantly grown and consumed in India and some regions of Africa [2, 3]. Other common names are red gram, Congo pea, Gungo pea, and no-eye pea, arhar, tur dal which derived from the botanical terms Leguminosae [4].

Amongst these, pigeon pea or red gram (*Cajanus cajan* (L.) Millspaugh) is an important food legume that can be grown under rain fed conditions with least inputs [5].

It is observed that pigeon pea is economically and nutritionally an important legume and a major source of protein for the poor communities of many tropical and subtropical regions of the world [6]. Besides its nutritional value, pigeon pea also possesses various medicinal properties due to presence of a number of polyphenols and flavonoids. It is an integral part of traditional folk medicine in India, China, and some other nations [7].Literature on this aspect show that the extracts or components of Pigeon pea is known to prevent and cure human ailments like bronchitis, coughs, pneumonia, respiratory infections, pain, dysentery, menstrual disorders, curing sores, wounds, abdominal tumors, and diabetes in traditional folk medicine [8, 9].

The aim of the present study therefore is to determine some physical and chemical properties of seeds flours of *Cajanus cajan* cultivated in Côte d'Ivoire.

MATERIALS AND METHODS

Materials

Pigeon pea (Cajanus cajan L.) Taro) seeds used for this work were carried out from Abobo in Abidjan, portion of Côte d'Ivoire (West Africa). After collection, pods were immediately transported to the Laboratory of Nutrition and Food Safety (Nangui Abrogoua University, Abidjan, Côte d'Ivoire) and once in the laboratory, these pods are opened to release the grains that will be immediately weighed next, then, they were dried at 45°C during 72 h. Dried seeds were ground using the blender to obtain the crude flours. All chemicals and reagents used were of analytical grade and purchased from Sigma Chemical Company (USA).

Proximate composition

The moisture content was determined by drying in an oven at 105 °C during 24 h to constant weight. Nitrogen was determined by the Kjeldahl method reported by AOAC [10] and crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25. The crude fat content was determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent. The total ash content was determined by incinerating in a furnace at 550 °C. The total sugars content was determined as described by by Dubois *et al*.[11].Cellulose content was determined according to the gravimetric method of Van Soest [12] The carbohydrate content was determined by deference by deducting the mean values of other parameters that were determined from 100. Therefore % carbohydrate = 100- (% moisture + % crude protein + % crude fat + crude fiber + % ash) [13,14].

Calories were obtained by the summation of multiplied mean values for protein, fat and carbohydrate by their respective Atwater factors, 4, 9 and 4 [15].

Mineral analysis

The minerals, such as potassium and sodium were analyzed after nitric acid digestion using an atomic absorption spectrophotometer (Model No. 560, Elmer Corp, Norwalk/ United States). Phosphorus was estimated colorimetrically (UV-visible spectrophotometer, Model DR 2800/United States). All results for mineral composition are recorded on the basis of edible portion of uncooked sample as mg/100 g dry weight.

Trypsin inhibitor activity

Trypsin inhibition was performed by modifying the method originally described by Erlanger *et al*. [16] and the results were expressed as milligram of inhibited trypsin/gram of extract protein. *Cajanus cajan* flour was stirred with 15mL buffer (0.1M of Tris-HCL, pH8.2, containing 20 mM of CaCl2) at room temperature for 3h. After, the extract was centrifuged at 35.600 for 30 min. Then, 100 µl of the extract suspension, 50 µl of trypsin solution (0.1% in 1mM HCl) and 450 µl of buffer (0,1M of Tris-HCl, pH 8.2, containing 20mM of CaCl2) were mixed in tube and incubated at room temperature for 5 min. Aliquot of 500 µl of this reaction was added into another tube containing 500 µl of buffer (0.1M Tris-HCl, pH8.2 containing 20 mM CaCl2) and 500 µl of D,L-BApNA solution (200 µl of stock solution, prepared with 130.47mg D,L-BApNA in 5 mL dimethyl sulfoxide, diluted with 10 mL buffer). After 5 min, the reaction was terminated by adding 300 uL of acetic acid (60%), mixed thoroughly, and absorbance was measured at 410nm against reagent blank (enzyme and extract were substituted by the buffer).

The measurement of extract crude protein was carried out according to the methodology outlined by Smith *et al*. [17] using bovine serum albumin (0.2%) as standard. Reagents A (1% of bicinchoninic acid, 0.16% of potassium tartrate. 1.8% of sodium carbonate, 0.4% of sodium hydroxide and 95% of sodium bicarbonate, pH 11.3) and B (1% of copper sulfate) were mixed in ratio 50:1 and to 1ml of this solution 50uL of extract suspension was added. After 30 min at 37°C, the reaction was cooled for 20 min and the absorbance was measured at 562 nm. The trypsin inhibitor activity was calculated as follows:

Milligram of inhibited trypsin/gram of extract protein-

$$\frac{(AXB)}{C\ X\ 1000\ XP}$$

Where A is the difference between the enzyme control and sample absorbance. B the simple dilution factor, C the trypsin factor (0.09), [18] and P is the extract protein concentration in g/ml. The obtained values were converted into percentage (g/ 100 ml).

Digestibility

The in vitro digestibility of flour was carry out using an α-amylase purified from *Bacillus subtilus* according to the method of Kouame *et al.* [19]

Amylase activity was measured at 37°C for 20 min in 0.6 ml of 100 mM acetate buffer (pH 5.0) containing 1% (w/v) of soluble starch (Sigma) and 0.58 U of amylase solutions. The reaction was halted by addition of 2. 4-dinitro-salicilic reagent before estimating the reducing sugars released by the Bernfeld method [20].

This enzyme assay was performed as described below [19]. Briefly, purified alpha-amylase extract (50 µl) was incubated with 0.5 % solution starch (80 µl) on 100 mM phosphate buffer pH 7.0 (170 µl) at 37°C for 30 min. The amount of reducing sugars produced was determined by dinitrosalicylic acid (DNS) method with maltose as the standard.

Statistical procedure

All determinations were carried out in triplicate for each nutrient analysis. For all analyses, the mean and standard deviation for each nutrient analyzed were calculated and reported.

RESULTS AND DISCUSSION

Physico-chemical Characteristics of *Cajanus cajan* Seeds

The physico-chimical analysis values of the seeds of *Cajanus canjan* from Côte d'Ivoire studied are summarized in Table 1. The moisture content was found to be 12.4%. This value is higher and significally different to those reported by pele G *et al*.[21]for Nigeria *Cajanus cajan seeds* (7-10%) in sample A,B,C that represent soaking for 24 h (sample A); sun drying and milling (sample B); soaking for 12 h, de-hulling, sun drying and milling (sample C) . However, it is lower than value of sample D (13.65%) reported in the same study, but similar to those reported by Zar *et al*. [22]with Mung bean flour (12.72%)**.** Furthermore, high moisture content is associated with increase of microbial activities which accelerates spoilage**;** this is an indication that *Cajanus cajan* seeds are highly perishable.

The protein content of *Cajanus canjan* seeds from Côte d'Ivoire (25.6%) is comparative to that of dry legumes seeds" (20-40%), [23] but still higher than lentils (22.7 %, [24]Mung bean full fat flour (22%)[22]) chick pea (18.3 %, [24]) and lower than the value of soybean (40.4%) reported by silva *et al*. [25]. This result is in agreement with the value obtained by peer researchers such as Pele and et *al*. [21] which obtain 25.03 % with pigeon pea from Nigeria soaking for 24 h.

Crude cellulose content of Cajanus cajan seeds (11.9%) were significantly higher than those of peanut kernel (1,11%, Musa and Serap, 2003) [26]; but smaller in Soya beans(22%) and Faba beans (18%) obtained by Helmut *et al.* [23]. The higher fibre contents may be advantageous since their consumption could enhance digestion and prevent constipation. High crude fibre in the vegetable according could also help in blood cholesterol attenuation, as well as blood glucose attenuation when consumed [27, 28].

The fat content obtain (1.8 %) is higher compared with those reported by Zar *et al*.[22]; in Mung bean flour; but similar to those presented by Ononogbu [29]; with *Cajanus cajan* seeds (1.79%) from Nigeria. Furthermore, Data showed that low fat would be advantageous to prevent hypertension and hypercholesterolemia and suggests that the consumption of *Cajanus cajan* seeds may protect against cardio-related chronic diseases such as diabetes and hypertension (Ogbunugafor *et al*. [30], [31].

Carbohydrates content in this study (56.1 %) were lower to those reported by Zar *et al*. [22]; in Mung bean seed (59.77%). Data show that Carbohydrates are the most important food energy provider among the macronutrients, accounting for between 40 and 80 percent of total energy intake [13]. The high caloric values obtained in this study could be explained to high proteins, lipids and total carbohydrate contents.

The ash content of the seeds obtained from the present study is low when compared with the value of 5.58 % reported in *Cajanus cajan* seed soaking for 24 h in Nigeria [21] but compares favorably with the value of 4.11 % found in soaking for 12 h, de-hulling, sun drying and millingseeds of the same studies.

The data in table 1 also reveal that there were great variations in the mineral content of *Cajanus cajan seeds*. The potassium content, 1.8 mg/100 g for *C. cajan* was much higher than phosphor (0.6) and sodium (0.03). Potassium is a very significant body mineral, important to both cellular and electrical functions. It is one of the main blood minerals, which carries an electrical charge (potential).It prevents stroke and aids in muscle contraction together with sodium [32, 33]. However, low sodium content might be one of the reasons it is employed in enthno-medicine for the treatment of hypertension [34]

Table-1: proximate composition of *Cajanus cajan* seeds (g/100 g dry weight)

parameters	*Cajanus cajan*
Dry matter	87,6 ±0.12
moisture	12,4±0.05
Crude protein	25,6± 0,5
Crude fat	1,8± 0,1
Total sugars	4,6± 0.7
cellulose	11,9 ± 0.04
carbohydrate	56,1 ± 0.1
ash	4,5 ±0.1
Starch	39,2 ± 1.4
potassium	1,8±0.01
phosphor	0,6±0.07
sodium	0,03±0.4
Energy (KCAL/KG)	2818±3,09

Trypsin inhibitor

Pigeon pea seeds also contain some anti-nutritional factors like other legumes (Figure 1). The trypsin inhibitor of the seed of *Cajanus cajan* was quite high in Whole wheat flours than that made with flours provided of seeds without film. In this study, the activity of trypsin inhibitors was evalued of 53 %. However**,** anti-nutritional factors can be eliminated or reduced by processing. Data showed that Heat or toasted treatment has been shown to reduce trypsin inhibitor activity by the fact that it sensitive to denaturation [37]. Trypsin inhibitors are Kunitz factor and Bowman-Birk factor [36] found in raw soybeans that inhibit protease enzymes in the digestive tract. According to Kamath and belavady [35], pigeon pea seeds also have some amounts of unavailable carbohydrates which reduce the protein digestibility and adversely affect the bio availability of certain vital nutrients. Result showed that there is significant decrease in trypsin content this could be due to sprouting of pigeon pea which reduced the effect of anti trypsic activity that could compromise the protease absorption.

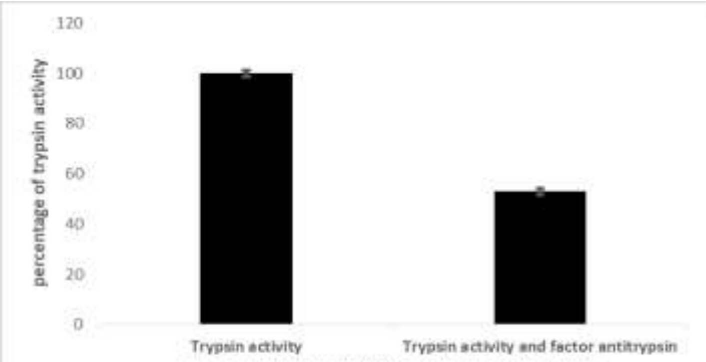
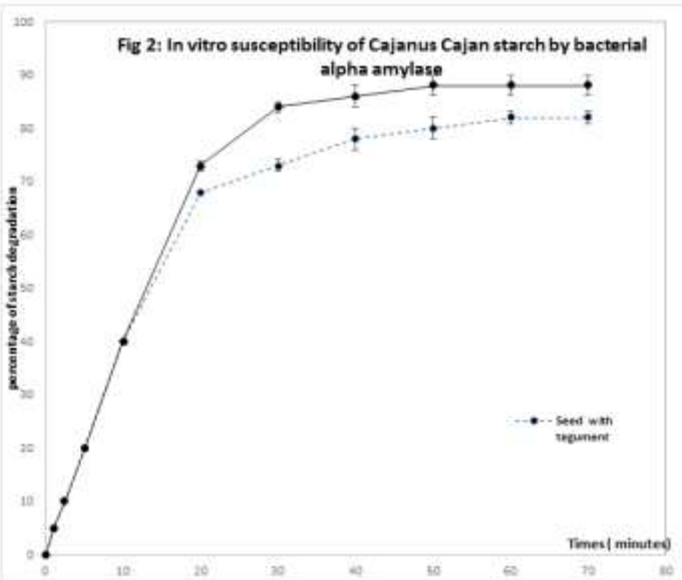


Fig 1: **TRYPSIN ACTIVITY OF CAJANUS CAJAN SEEDS**

Digestibility

In vitro digestibility of flour is significantly affected by the present or not of the film in the seed. A-amylase in vitro digestibility of flours is increased in flours made with seeds without film. Digestibility of flour made of seeds without film is higher than that of flour made with film (Figure 2). Values of alpha amylase indicate the degradation of starch at 82% for whole flour but 88 % for flour made with seeds without film. This difference in digestibility is explained by existence of cellulosic structure which is a constraint for enzyme activity. Indeed, cellulose represents insoluble fiber which includes hemicellulose, pectin and lignin. They are indigestible polymers which are resistant to hydrolysis by enzymes in the alimentary tract [38]. The cellulosic wall constitutes a protective envelope for starch; it acts as a real physical barrier preventing the entry of the enzyme to the starch enclosed in the cells of the parenchyma. Dietary fiber is important in aiding absorption of water from the digestive track. It also has health benefits such as lowering of blood pressure and serum cholesterol, protection against cardiovascular diseases, diabetes, obesity and colon cancer [39].

Fig 2: In vitro susceptibility of Cajanus Cajan starch by bacterial alpha amylase



CONCLUSION

Results of this study reveal that Ivorian pigeon pea seeds contained appreciable physicochemical characteristics as proteins, carbohydrates, cellulose, fat and mineral elements which are important in human health. Protein content of pigeon pea was significantly higher while the fat content was significantly lowed.

The anti-nutritional factors such as trypsin activity were found to decrease significantly in the whole seed flour at 55%. The presence of cellulose constitutes a protective envelope which constitue physical barrier for starch total degradation. Then the degradation of starch by alpha amylase was evalued at 82% for whole seeds and 88% with seeds without cells

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