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Full Length Research Paper

Nutritional and microbiological characterization of pulp powder of locust bean (*Parkia biglobosa* Benth.) used as a supplement in infant feeding in Northern Benin

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The microbiological and nutritional characterization of locust bean pulp powder (*Parkia biglobosa*) was investigated. Bacteria and fungi were isolated from this product. The bacteria isolated were essentially fecal coliforms. The fungal isolates were *Aspergillus niger, Aspergillus ochraceus* and *Penicillium digitatum*. The mean total plate count of samples was 2.8 × 10^3 cfu/g, while the mean coliform total count was lower than 10 cfu/g and the mean fungal count was 1.9 × 10^3 cfu/g. The respective mean moisture content and total acidity in locust bean pulp powder were 24.16 ± 2.45 and 2.10 ± 0.95%. Nutritional analysis showed that locust bean pulp powder has interesting nutritional potential. Carbohydrate content (6.28 ± 0.67%), protein content (4.129 ± 0.328%), carotenoid content (0.154 ± 0.03%) and the presence of minerals such as calcium (0.166 ± 0.005%), sodium (0.228 ± 0.006%), potassium (1.60 ± 0.071%) and magnesium (0.144 ± 0.002%) allowed its application as supplement in infant feeding in rural areas. Anti-nutritional factors such as oxalate and phytate were detected in samples, and values were lower than established toxic level. Finally, more attention should be made to its microbial quality in order to preserve children's health.

Key words: *Parkia biglobosa*, microbiological and nutritional characterization, food safety, Benin.

INTRODUCTION

According to the World Health Organization, malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (Tierney et al., 2010). The term protein-energy malnutrition (PEM) applied to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor (Tierney et al., 2010). The most common form of malnutrition in Africa is protein energy deficiency affecting over 100 million people, especially 30 to 50 million children under 5 years of age (Jildeh et al., 2010). Some legumes such as soybean, bean, and peanut, are important sources of protein and can therefore help to increase the protein intake of the diet of population. However, the low-income group, especially in rural areas, sometimes cannot afford these protein foods. *Parkia biglobosa* is a legume forest tree crop belonging to the family Mimosaceae which provides to West African population, a range of products used in food and traditional medicines. The main interest is the use of its fermented seeds, known for their very pronounced flavor, as condiment in Africa and designated by various names *Afitin*, *Iru* or *Sonru* in Benin, *Soumbala* in Burkina Faso, Mali and Niger and *Iru* in Southwest Nigeria. The fermented seed contains about 30 to 40% protein, 10 to 15% carbohydrates, 15 to 20% fat and 4% minerals (Codjia et al., 2003). In the northern regions of Benin, the pulp is collected, stored and later sold in the market in powder form. It is usually applied by these populations as a supplement in infant feeding and is added directly in infant porridges. Because of the vulnerability of child health, the poor conditions and duration of storage of the

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pulp, the precarious conditions of hygiene in the market, the direct use of this powder pulp in infant feeding and the risk of reoccurring of childhood diseases epidemiological, microbiological and nutritional quality of pulp powder of locust bean must be evaluated to ensure the health of consumers who are mostly children and infants.

MATERIALS AND METHODS

Collection of samples

Samples (pulps of *P. biglobosa*) were purchased from local markets (the major sales depot of *P. biglobosa*) in Ouenou, Tamarou, Gbegouru, Bori, and Teme, all in N'Dali, (north of Benin) and labeled A, B, C, D and E respectively. The samples were purchased from four different points in each market and were mixed together to give each composite sample which was used for the analysis. Fresh fruits were also harvested after maturation at N'Dali and taken to the laboratory where they were dried at laboratory temperature (25°C). Husks were manually removed. The floury pulp were grated, passed through a 25 mm sieve and kept in airtight container for laboratory analysis.

Determination of physicochemical parameters

Moisture content of samples was determined by desiccation using the method of De Knegt and Brink (1998). A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined. In order to obtain the pH of the samples, 5 g of each sample was weighed and suspended in 10 ml of distilled water. The pH was determined with a digital pH-meter (HANNA HI 98129). Acidity of samples, expressed as citric acid content per unit of volume, was determined by titration with 0.01 mol/L of sodium hydroxide solution, using phenolphthalein as indicator (AOAC, 1990).

Nutritional analysis

The carbohydrate was determined according to phenol sulfuric acid method (Agbo and Ronald, 1996; Ezoua et al., 1999). A standard curve was obtained using the following concentration of sucrose in (mg/ml) 2.5 2.0, 1.25, 1.0, 0.5 g of each sample with 9 ml of distillated water was measured into test-tube. 2 ml of phenol solution (1%) and 1 ml of concentrated H_2SO_4 solution were added. This was shaken for 15 min and boiled for 30 min. It was then allowed to cool. The absorbance was then read off a spectrophotometer (spectrum lab 22) at 700 nm. The sugar concentration was then obtained by extrapolation from the standard curve. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction as described by Pearson (1976). Carotenoids content was determined according to the method described by AOAC (1995). Ash was determined according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990). Fiber content was determined by the ISO method (ISO, 1981). 2 g of finely ground defatted sample were weighed and boiled with sulfuric acid solution (0.255 mol/L) for half an hour followed by separation and washing of the insoluble residue. The residue was then boiled with a sodium hydroxide (0.313 mol/L) solution followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace at 600°C and the loss in mass was determined. Minerals were analyzed by the method reported by Oshodi (1992). Minerals were analyzed by dry-ashing 1 g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Flame photometer was used to determine sodium and potassium contents of the samples, while calcium and magnesium were determined using atomic absorption spectrophotometer (Perkin Elmer, Model 403).

Anti-nutritional factors analysis

Total oxalate was determined as described by Day and Underwood (1986). 1 g of sample was weighed into 100 ml conical flask. 75 ml H_2SO_4 (3 mol/L) was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 ml of the filtrate was then taken and titrated while hot against 0.05 mol/L of $KMnO_4$ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 ml of 0.05 mol/L of $KMnO_4$ as equivalent to 2.2 mg oxalate (Ihekoronye and Ngoddy, 1985; Chinma and Igyor, 2007). Phytate was determined using the method of Reddy and Love (1999). 4 g of each sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content (Okon and Akpanyung, 2005).

Microbiological analysis

To 25 g of each sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total Coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the Faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using EMB (Eosine Methylene blue). Tryptone Sulfite Neomicin Agar was used for Anaerobic Sulfito-Reducer (ASR) count and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution (Singh et al., 1991). The isolation of fungi from samples was performed using dilution plating method. 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further 10-fold serial dilutions up to 10^{-4} were made. One mililitre of each dilution was separately placed in Petri dishes, over which 10 to 15 ml of Potato Dextrose Agar with 60 µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days (Rampersad et al., 1999). The identification of the bacterial isolates was based on cultural, morphological, and biochemical characteristics following standard methods (Buchanan and Gibbons, 1974) while that of fungi was also based on cultural and morphological characteristics using standard taxonomic schemes (Singh et al., 1991; Bryce, 1992). Microbiological parameters were evaluated periodically during 16 days.

Statistical analyses

The data generated from these studies were analyzed using

Table 1. Physicochemical parameters of locust bean pulp powder from markets.

Sample	Moisture (%)	pH	Acidity (%)
A	23.54	4.2	1.68
B	22.58	4.3	1.34
C	26.61	3.1	3.06
D	22.09	4.7	1.42
E	25.98	3.2	3.02
Mean	24.16 ± 2.45	3.9 ± 0.8	2.10 ± 0.95

Table 2. Nutritional content of locust bean pulp powder from markets.

Sample	Carbohydrate (%)	Protein (%)	Carotenoid (%)	Ash (%)	Fiber (%)
A	6.89	4.457	0.187	7.85	22.84
B	6.95	4.228	0.173	7.62	22.56
C	5.83	3.892	0.137	7.54	21.34
D	6.41	4.238	0.145	7.83	22.57
E	5.34	3.832	0.128	7.34	21.82
Mean	6.28 ± 0.67	4.129 ± 0.328	0.154 ± 0.033	7.637 ± 0.214	22.226 ± 0.614

Table 3. Antinutritional factors content of locust bean pulp powder from markets.

Sample	Oxalate (%)	Phytate (%)
A	11.17	3.19
B	15.10	4.12
C	14.18	2.13
D	17.19	4.75
E	9.17	2.34
Mean	13.36 ± 3.83	3.30 ± 1.45

Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (Alder and Roessler, 1977; Ogbeibu, 2005).

RESULTS

The results of physicochemical characterization of different samples of locust bean pulp powder from markets (Table 1) showed that the moisture content of different samples ranged from 22.09 to 26.61%, with an average of 24.16%. The pH was between 3.1 and 4.7 with a mean acidity of 2.36%. The locust bean pulp powders are rich in nutrients (Table 2) such as carbohydrates (6.28%), proteins (4.29%), carotenoids (0.154%), ash (7.63%) and fiber (22.23%). The analysis of anti-nutritional factors revealed the presence of oxalate (9.17 to 15.10%) and phytate (2.13 to 4.75%) (Table 3). All samples analyzed were rich in minerals such as calcium, magnesium, potassium and sodium, with a higher content of potassium (1.5 to 1.7%) (Figure 1). The result of proximate composition of fresh pulp of locust bean is as shown in Table 4. The moisture content and acidity were respectively 11.02 ± 0.41 and 2.10 ± 0.95%. Ash, protein and carbohydrate content were 8.05 ± 0.22, 9.61 ± 0.43 and 47.63 ± 0.27%, respectively. Fat content was very low and fiber was 23.68 ± 0.14%. All samples analyzed were also rich in minerals such as calcium, magnesium, potassium and sodium, with a higher content of potassium (1.8 ± 0.32%) (Figure 1). The analysis of anti-nutritional factors also revealed the presence of oxalate (12.74 ± 1.74%) and phytate (3.21 ± 0.96%) (Table 3). The total flora count of samples from markets ranged from 6×10^1 to 9×10^3. The enumeration of total coliforms and fecal coliforms was less than 10 cfu/g with an absence of spores of anaerobic sulfite reducers (ASR). Fungal flora was high (3×10^2 to 7×10^3 cfu/g) with the presence of fungi such as *Aspergillus niger*, *Aspergillus ochraceus* and *Penicillium digitatum* (Table 8). These bacteria and fungi were also able to growth in the pulp stored at room temperature (Table 7). However, the microbial contamination of fresh pulp was very low with the absence of pathogens (Table 6).

DISCUSSION

The results obtained from microbial analysis of locust bean pulp powder from markets show that they were contaminated with microorganisms of public health concern. The high total bacterial and coliform count may be a consequence of the low level of hygiene maintained during the processing and sale of locust bean pulp

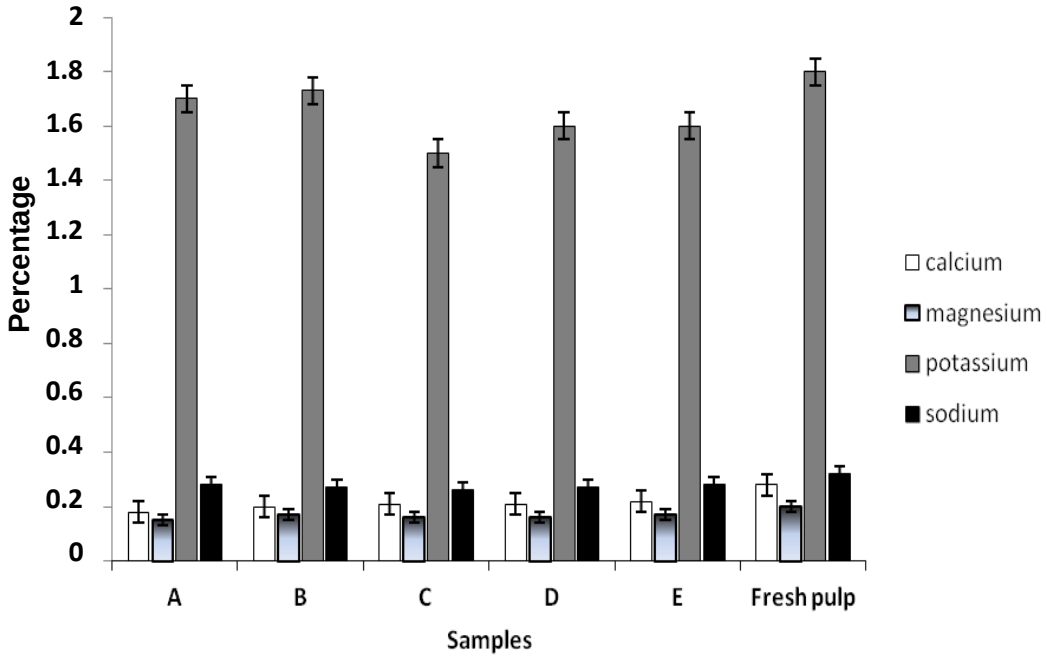

Figure 1. Minerals content of locust bean pulp.

Table 4. Proximate composition of fresh pulp of locust bean.

Item	Percentage composition						
	Moisture	Acidity	Ash	Protein	Fat	Carbohydrate	Fiber
Fresh pulp	11.02 ± 0.41	0.2 ± 0.06	8.05 ± 0.22	9.61 ± 0.43	0.01 ± 0.004	47.63 ±0.27	23.68 ± 0.14

Table 5. Anti-nutritional factors content of fresh pulp of locust bean.

Item	Percentage composition	
	Oxalate	Phytate
Fresh pulp	12.74 ± 1.74	3.21 ± 0.96

powder. During the sale, dirty hands are dipped into the powder for product selection by both hawkers and consumers. The exposure of products while they were displayed for sale can also serve as a source of contamination. The detection of coliforms may indicate possible faecal contamination. Being enteric bacteria, their presence indicate poor hygienic practice among handlers of products. Their presence in samples could pose a serious threat to food safety, due to the fact that locust bean pulp powder is a ready-to-eat food which is consumed without further processing. Great attention should therefore be given to the microbiological safety of these products. Similar results were found at the street foods which are mostly marketed under the same conditions (OMS, 2004). In Africa, several studies on street food showed that their hygienic quality is very poor and this is a clear risk on the health of consumers (OMS, 2004). These precarious hygiene conditions promote the risk of fecal contamination and therefore the reoccurrence risk of food borne illness. The contamination also varied according to sampling zones. It may be due to the environmental conditions in the markets or the level of hygiene maintained during the processing and sale. The fungal isolates: *A. niger, A. ochraceus* and *P. digitatium* species are known spore formers. It therefore means that they can easily contaminate locust bean pulp powders which are usually exposed in market. Their growth can result in the production and accumulation of mycotoxins. The result of proximate composition of pulps from markets indicates that the moisture content is quite high. This high moisture content would encourage microbial growth and so deterioration. However, the low level contamination of fresh pulp, compared to those from markets, confirmed that the important source of contamination was the low level of hygiene maintained during the processing and sale of locust bean pulp. The low microbial count is a reflection of the low fat and moisture contents of the fresh pulp which is an indication that the pulp can be stored in a tight container for a long time without spoilage (Gernah et al., 2007). This is in

Table 6. Microbial count of locust bean pulp (cfu/g).

Sample	Total bacterial count	Total coliforms count	Faecal coliforms count	A.S.R spores count	Mould and yeast count
Fresh pulp	03	00	00	00	00
A	7×10^2	00	00	00	5×10^2
B	6×10^2	04	03	00	3×10^2
C	4×10^3	04	02	00	2×10^3
D	6×10^1	03	01	00	4×10^1
E	9×10^3	07	04	00	7×10^3
European Union criteria (2005)	-	10	10	Absence/10 g	Absence/10 g
Conformity (%)	-	100	100	100	00

A.S.R: Anaerobic Sulfito-Reducer

Table 7. Microbial growth in locust bean pulp from markets stored at room temperature (cfu/g).

Days	Total bacterial count	Total coliforms count	Faecal coliforms count	A.S.R spores count	Mould and yeast count
1	7.0×10^2	04	03	00	3×10^2
4	1.0×10^3	04	03	00	7×10^2
8	1.5×10^3	06	05	00	9×10^2
12	2.7×10^3	08	05	00	1.2×10^3
16	3.2×10^3	12	09	00	6.0×10^3
European Union criteria (2005)	-	10	10	Absence/10 g	Absence/10 g

Table 8. Prevalence of fungi isolated from locust bean pulp samples from markets.

Fungal isolated	Prevalence (%)				
	A	B	C	D	E
Aspergillus niger	43.5	40.2	42.4	39.6	44.6
Aspergillus ochraceus	28.3	23.4	29.3	31	27.3
Penicillium digitatum	25	29	22	24	23
Other	3.2	7.4	6.3	5.4	5.1

accordance with the report of Owoyale et al. (1987), Omojola et al. (2011) and Compaoré et al. (2011).

The high nutritional potential of locust bean pulp such as its proteins, carbohydrates, carotenoids (provitamin A) and its mineral contents (Tables 2 and 4, Figure 1), justified its uses as supplement in infant feeding in northern Benin. Minerals are important in human nutrition. It is well known that enzymatic activities as well as electrolyte balance of the blood fluid are related to adequacy of Na, K and Mg. Potassium is very important in maintaining the body fluid volume and osmotic equilibrium. Metal deficiency syndrome like rickets and calcification of bones is caused by calcium deficiency. Several studies on nutrition in developing countries have shown that adequate nutrient intake (daily calories, daily protein, daily fat, minerals and vitamins) is an essential ingredient for improved well-being, economic growth and development, since a healthy body enhances the capacity to learn which in turn determines productivity and economic growth (Flores, 2001; Smith and Haddad, 2001; Diao et al., 2007). According to Musgrove (1993) and Benson (2008), adult productivity depends to a considerable extent on the contribution of health and nutrition during early childhood. From birth to age 4 months, all the nutritional needs of children are fully covered in milk. But between 4 and 6 months breast milk is not sufficient to cover the needs for energy and protein of the child. This is the period during which nutrients necessary for child growth must supplement the breast milk slurry (Claeson et al., 2001). Quantitative protein requirements are about 20 g per day between 6 months and 3 years. Ideally, the amino acid composition of these

complementary proteins should be identical to that of breast milk that is containing the same proportion of the nine essential amino acids (Hedley et al., 2004). Fortunately, it is possible to reconstruct a protein mixture composition meeting the needs of the child by mixing cereal flour with legume flour. Amino acids absent in cereal proteins are then supplemented by the amino acids present in legumes. Among the 12 vitamins essential for the child, some are particularly important, such as vitamin A. It is involved in vision, and especially protects the conjunctiva of the eye and cornea against infection. Its deficiency is a real public health problem. Vitamin A is present in foods in the form of provitamin A (carotenoids) which is present in the vegetables (Shi, 2000; Newborn-Cook et al., 2002) and fruits such as pulp of locust bean (Tables 2 and 4). This is in agreement with that obtained by Compaoré et al. (2011) in pulp of locust bean commonly used in food fortification in Burkina faso. However, according to Ladeji (2004), oxalate can bind to calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as kidney, causing kidney stones (Oke, 1969). However, the values obtained for locust bean pulp powder were below the established toxic level. According to Oke (1969), a phytate diet of 1 to 6% over a long period decreases the bioavailability of mineral elements in monogastric animals. Phytic acid can bind to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing the bioavailability of the element for absorption (Erdman, 1979). Phytic acid also has a negative effect on amino acid digestibility (Makkar and Becker, 1998). However, values obtained from these locust bean pulp powder samples are lower than established toxic level.

Conclusion

This survey underlined the nutritional potentiality of locust bean pulp powder (*P. biglobosa*) used as supplement in infant feeding in northern Benin. However, due to the fact that it is used for infant diet, more attention (in the storage and selling methods) should be paid to its microbial quality in order to preserve children health.

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