EFFECTS OF FERMENTATION ON THE NUTRITIONAL STATUS OF *CRESCENTIA CUJETE* L. SEED AND ITS POTENTIALITY AS AQUA FEEDSTUFF

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ABSTRACT

The effects of fermentation on the proximate and anti-nutrient composition of Crescentia cujete seeds were investigated. Two methods of fermentation were employed; the traditional fermentation (TF) and gut-filtrate treated fermentation (GFTF). Proximate and anti-nutrient contents were determined using standard procedures. Anti-nutrients tested for were alkaloids, phytate, tannins, saponins and flavonoids. All proximate contents varied significantly (p<0.05), TF fermented C. cujete seeds had the highest protein (18.73 %) and crude fibre (31.23 %) contents. Fermentation significantly increased all proximate compositions except for nitrogen free extracts. A 26.15 % decrease in Nitrogen free extract was effected by TF while, a 30.26 % decrease was effected by GFTF. Saponin and flavonoid was increased appreciably by TF (47.61 and 95.44 %, respectively) and GFTF (82.63 and 92.07 %, respectively). Fermentation effected appreciable reduction in alkaloid and phytate contents; 21.56 and 75.00 %, respectively for TF and 55.88 and 67.85 % respectively for GFTF. Fermenting C. cujete seeds is advocated for owing to its ability to significantly enhance (p<0.05) crude protein by 79.75 and 72.74 % for TF and GFTF respectively and lipid by 7.82 and 17.41 % for TF and GFTF respectively. Crescentia cujete seeds had high contents of protein, carbohydrate and lipids which suggest that they can serve as a good source of crude protein and energy for livestock and animal production, not forgetting the seed's medicinal potentials due to their high composition of phytochemicals.

Keywords: Anti-nutrients, *Crescentia cujete*, Fermentation, Proximate composition

INTRODUCTION

Calabash tree, gourd tree or *Crescentia cujete* tree belongs to the family of Binoniacea. Small flat seeds are embedded in the pulp (Arbonnier, 2004). The calabash tree is widely distributed in the Caribbean region, Mexico, Northern and Southern American and later introduced to tropical Africa from Senegal to Cameroon then to other parts of Africa (Arbonnier, 2004) and widely grown in the northern states of Nigeria. Most parts of the tree have been put to use: with the wood used as handles of tools, ribs in boat building and cattle yokes; the gourd for cups, containers and musical instruments; and the fruits have medicinal application.

Nutrition is the most expensive component in the intensive agriculture industry, where it represents over 50 % of operating costs. The high cost of commercial animal feed is one of the major limiting factors in the growth and development of agricultural sector in Nigeria. Production of high quality feed has been stated as one of the persistent bottle necks holding back great rapid expansion of aquaculture in Nigeria (Udo and Dickson, 2017). There is need to focus on using alternative, readily available and less competitive plant protein sources to complement the already available plant protein resources.

Fermentation has been identified as one of the less expensive means of increasing the

protein quality of feedstuff. The use of microorganisms to convert carbohydrates, lignocelluloses and other industrial wastes into foodstuffs rich in protein is possible due to the following inherent nature of microorganisms: ability to multiply rapidly; their amenability to modification genetically for growth on a particular substrate under particular cultural conditions; they have high protein content varying from 3.5 – 60 %; they have growth versatility in both slurry and on solids; and their nutritional values are as good as other conventional foods rich in protein (Ubalua and Ezeronye, 2008).

The phyto-constituents (phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins) found to be distributed in plants are antibiotic principles of plants, but these compounds were not well-established owing to lack of knowledge and techniques as reported by Hafiza *et al*. (2002). In view of the overall nutrient and chemical composition, *C. cujete* seeds may be adopted as an inexpensive alternative protein source to alleviate protein malnutrition among traditional people living in developing countries. The aim of this study was therefore to assess the effect of two methods of fermentation on selected chemical constituents of *C. cujete*.

MATERIALS AND METHODS

Sample Preparation: The leaves and seeds of *C. cujete* were taken to the Herbarium in the Department of Botany, Ahmadu Bello University, Zaria, Nigeria, for identification. The seeds were air-dried, divided into three portions and put into separate polyethene bags. Two of the portions were fermented. Two methods of fermentation were employed; the traditional fermentation (TF) and gut-filtrate treated fermentation (GFTF). For the TF 10 kg of the seed was ground into granules and wet with water (1 litre of water to 10 kg seed) and bagged in duplicate packs of 5 kg inside white cellophane paper. They were then packed inside two different black polythene bags, tightly tied to exclude air for five days at room temperature to facilitate anaerobic fermentation. After which the fermented seeds were sun-dried for three days before milling (Apata *et al*., 1999). The GFTF followed a similar treatment as the TF except that freshly obtained cow gut-filtrate was used to wet the seeds rather than water. The three portions were transported to the Food Science Laboratory, Institute of Agricultural Research, Zaria, Nigeria, for analyses.

Proximate Analysis of Seeds: Proximate analysis of *C. cujete* seed was determined in triplicates using recommended methods of the Association of Official Analytical Chemist (AOAC, 2000). Samples were analysed for crude protein by the micro-Kjeldahl method of determining nitrogen and calculated as N x 6.25. Moisture content was determined by drying to a constant weight, crude lipid was determined as extracted ether using petroleum spirit or N-hexane within the soxhlet apparatus, crude fibre and ash contents were determined by incinerating dry samples in a Muffle furnace set at 550 °C. Carbohydrate was calculated as nitrogen free extracts by difference [100 – (% crude protein + % lipid + % moisture + % crude fibre)] (AOAC, 2000).

Determination of Anti-nutrients in the Seeds: Alkaloid, saponin, tannin and phytate were determined using recommended methods of the Association of Official Analytical Chemist (AOAC, 2000).

Determination of Alkaloids: Two grams of the sample was weighed into a 250 ml beaker and 100 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was then filtered and filtrate was concentrated on a water bath to one quarter of the original volume. The entire/whole solution was allowed to settle. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed to determine the alkaloid content as described by Harbone (1973).

Determination of Phytate Content: Phytate was determined using the method described by Wheeler and Ferrel (1971). Four gram of the sample was soaked in 100 ml of 2 % HCl for 3 hours and then filtered. 25 ml filtrate was

dispensed into a conical flask and 5 ml of 0.3 ml ammonium thiocyanate solution was added as indicator. Thereafter, 53.5 ml distilled water was added to the mixture to give it a proper acidity and this was titrated with standard iron III chloride solution, which contains about 0.00195 g (1.95 g) of iron per millimeter until a brownish colour persisted for 5 minutes. Phytate content was calculated by; titre value x 0.00195 x 1.19 x 100.

Determination of Tannins: Two grams of the sample was poured into a beaker containing 50 ml of distilled water and heated to 60 °C. Then it was filtered and the residue was discarded. 10 ml of 4 % copper acetate solution was added to the hot filtrate and boiled for another 10 minutes. The precipitate was filtered and the filtrate was discarded. The residue was dried using a filter paper and dried sample was then scraped from filter paper into a pre-weighed crucible. The weight was recorded as W. The crucible containing the sample was incinerated in a muffle furnace at 550 °C, then cooled in a desiccator and then reweighed as W1. The difference between the weight of sample before ashing and the ashed residue after incineration represents the tannin content as described by Joslyn (1970).

Determination of Saponin Content: Saponin content was determined using the AOAC (1990). Saponin extract was done using acetone and methanol. Crude lipid content of samples was extracted with acetone while methanol was used to extract saponin. Two grams in triplicate were folded in filter paper and put in thimble and extracted by influxing in a Soxhlet extractor. Extraction was done with acetone in a 250 cm^3 capacity round bottomed flask containing 100 cm^3 methanol, fitted to the extractor and extraction sustained for another 3 hours. The weight of flask was taken before and after extraction to note the change in weight. Methanol was recovered by distillation after the second extraction and the flasks oven-dried and allowed to cool at room temperature and weighed. The saponin content was calculated using the formula: Saponin (mg/100 g) = A – B x 100 / Sm, where A= mass of flask and extract, B = mass of empty flask and Sm = sample mass.

Determination of Flavonoids: Flavonoids level was determined by gravimetric method as described by Allen (1974). 10 g of sample was extracted with 100 ml of 80 % aqueous methanol at room temperature. It was filtered and the residue was transferred into a crucible for drying. This was dried at a constant weight in an oven. The percentage flavonoid was calculated as follows: % Flavonoids = Weight of dried residue / Weight of original sample x 100.

Data Analysis: Data generated were statistically analyzed using One-way ANOVA adopted to test for significant difference ($p<0.05$) in the nutritional and anti-nutritional contents between the seeds of raw *C. cujete* seed, *C. cujete* seed fermented traditionally with water and *C. cujete* seed fermented with cow gut filtrate. The outcomes were presented as mean ± standard error of mean.

RESULTS AND DISCUSSION

Table 1 presents the proximate composition of *C. cujete* seeds. Fermentation significantly affected ($p<0.05$) the proximate composition of the seeds. The ash, protein, lipid and fibre contents of the seeds were increased significantly, while there was significant reduction ($p<0.05$) in moisture and nitrogen free extracts contents. *C. cujete* fermented with water had the highest protein and fibre contents (18.73 and 31.23 %), while raw *C. cujete* had the highest moisture (19.16 %) and nitrogen free extracts (49.86 %) content. A 26.15 % decrease in nitrogen free extract was effected by TF while, a 30.26 % decrease was effected by GFTF. The reduction in moisture contents of the fermented seeds could be attributed to the temperature and the duration of drying of the fermented samples, and this was contrary to the report of Ojokoh *et al*. (2015).

The increase in crude protein content recorded with fermentation could be due to the fermentation activities that led to increase in number of microorganisms (Ojokoh *et al*., 2014)

Table 1: Proximate composition of raw and fermented *Crescentia cujete* seeds

Crescentia cujete seed	Parameters (%)					
	Moisture	Ash	Protein	Lipid	Fibre	NFE
Raw	19.16 ± 0.08^{a}	3.59 ± 0.02^{c}	10.42 ± 0.23^{c}	17.00 ± 0.21^{b}	5.30 ± 0.03^{c}	49.86 ± 0.42^{a}
TF	17.27 ± 0.10^{c}	8.86 ± 0.03^{b}	18.73 ± 0.05^{a}	18.33 ± 0.24^{ab}	31.23 ± 0.09^{a}	36.82 ± 0.14^{b}
GFTF	17.78 ± 0.15^{b}	9.49 ± 0.05^{a}	18.00 ± 0.08^{b}	19.96 ± 0.78^{a}	23.17 ± 0.10^{b}	34.77 ± 0.84^{c}
P-value	0.00	0.00	0.00	0.02	0.00	0.00

Means with the same superscript along columns do not vary significantly (p>0.05), Note: TF – Traditional Fermentation, GFTF – Gut-filtrate Treated Fermentation

Table 2: Anti-nutrient composition of raw and fermented *Crescentia cujete* seeds

Crescentia cujete seed	Parameters (mg/g^{-1})				
	Alkaloid	Phytate	Tannin	Saponin	Flavonoid
Raw	2.04 ± 0.05^{a}	0.28 ± 0.01^{a}	2.95 ± 0.03^{b}	17.85 ± 0.30^{c}	45.68 ± 0.46^{b}
TF	1.60 ± 0.03^{b}	0.07 ± 0.00^{b}	3.30 ± 0.05^{a}	26.35 ± 0.02^{b}	89.28 ± 0.28^{a}
GFTF	0.90 ± 0.01^{c}	0.09 ± 0.00^{b}	2.50 ± 0.02^{c}	32.60 ± 0.70^{a}	87.74 ± 0.49^{a}
P-value	0.00	0.00	0.001	0.00	0.00

Means with the same superscript along columns do not vary significantly (p>0.05), Note: TF – Traditional Fermentation, GFTF – Gut-filtrate Treated Fermentation

and proteolytic activities of enzymes produced by microorganisms during fermentation (Amankwah *et al*., 2009). Soluble low molecular weight peptides and amino acids that contribute to flavour are produced through the enzymatic breakdown of proteins (Ouoba *et al*., 2003) as a result of fermentation. Bashir and Suleiman (2018) also reported significant increase of protein (10.17 %) and lipid (46.62 %) content of fermented *Tamarindus indica*. Fermentation significantly ($p<0.05$) reduced the soluble carbohydrate content. The reduction in carbohydrate content with fermentation could be attributed to utilization of fermentable sugars by lactic acid bacteria for growth and other metabolic activities (Ojokoh *et al*., 2013). The values for crude fibre for this study are contrary to the reports of Igbabul *et al*. (2014) and Ojokoh *et al*. (2015) for pearl millet and Acha.

The anti-nutrient composition of *C. cujete* seeds indicated that all anti-nutrient composition varied significantly ($p<0.05$). The raw sample had the highest alkaloid (2.04 mg/g^{-1}) and phytate (0.28 mg/g^{-1}) contents, while TF had the highest tannin (3.30 mg/g^{-1}) and flavonoid (89.28 mg/g^{-1}) contents (Table 2). Saponin and flavonoid increased appreciably in TF seeds (47.61 and 95.44 % respectively) and GFTF seeds (82.63 and 92.07 % respectively). Fermentation caused appreciable reduction in alkaloid and phytate contents (21.56 and 75 %) respectively in TF seeds and 55.88 and 67.85 % respectively in GFTF seeds.

Bashir and Suleiman (2018) suggested that low level of alkaloid in fermented samples could be due to the extended hours spent during soaking of the seed sample to remove seed coat and fermentation process. Kumar *et al*. (2012) reported that alkaloids are removed from seed materials by aqueous extraction and treatment. Steinkraus (2002) also reported that many potential toxins such as trypsin inhibitor, phytate and cyanogens are reduced or destroyed during soaking and hydration that raw substrates undergo in various fermentation processes.

The reduction in phytate content may be attributed to the activity of the endogenous phytase enzyme from the raw ingredient and inherent microorganisms which are capable of hydrolyzing the phytic acid in the fermented food preparations into inositol and orthophosphate (Sandberg and Andlid, 2002).

Saponins increase the digestibility of carbohydrate rich foods by detergent-like activity that reduces viscosity, preventing the normal obstructing action of such foods in the intestine. Simultaneous consumption of saponin and tannin results in the loss of individual toxicity because the formation of tannin-saponin complexes inactivates the separate biological activity of both tannins and saponins (Kumar *et*

al., 2012). The presence of flavonoids in *C. cujete* also indicates that the seeds have biological functions such as being anti-oxidant and anti-tumor (Okwu, 2004). The seeds also possess anti-inflammatory properties (Lipkin and Newmark, 1999), and hence they can be used in the treatment of wounds, haemorrhoids, liver inflammation, rheumatism and pains (Olaniyi *et al*., 2018).

Conclusions: *Crescentia cujete* seeds had high contents of protein, carbohydrate and lipids which suggest that they can serve as a good source of crude protein and energy for aqua feed production, not forgetting the seed's medicinal potentials due to their high composition of phytochemicals. Fermenting *C. cujete* seeds and its inclusion in fish feed formulations is advocated for owing to its ability to significantly enhance ($p<0.05$) crude protein by 79.75 and 72.74 % for TF and GFTF respectively, and lipid by 7.82 and 17.41 % for TF and GFTF respectively.

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