

Full Length Research Paper

Evaluation of proximate composition and microbial contamination in artisanal and manufactured bambara nut condiment

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The proximate composition and microbiological load of bambara nut (*Vigna subterranea L (Verd)*) condiment: inoculated and un-inoculated samples were evaluated. The proximate composition revealed variations between unfermented and fermented seeds of bambara nut with lipid having a value of 1.67 and 13.33%; protein value 6.53 and 11.44%; then carbohydrate was 77.8 and 49.46% respectively. The organisms associated with fermented Bambara nut condiment were *Staphylococcus saprophyticus* and *streptococcus species* in un-inoculated sample and *Bacillus subtilis* and *B licheniformis* in inoculated sample. This suggests that 'bambara nut condiment' is a good and cheap source of protein for the lower class, who cannot afford other expensive sources of proteins.

Keywords: Bambara nut, condiment, inoculated, un-inoculated and proximate composition.

INTRODUCTION

Vigna subterranean (L.) verde also known by its common name "bambara nut", its name is derived from the name of a mali tribe called "Bambara" (Murevanhema and jideani, 2013). Legume seeds are the most important sources of macronutrients, such as protein, carbohydrates, lipid and dietary fiber, in the diet of many populations, especially in developing countries. Legumes have a major role to play to fight against malnutrition. One of these legumes is the Bambara groundnut. Although it represents a common food staple in semi-arid area of Africa, the Bambara groundnut remains one of the crops less investigated (Bamshiye and Adegbola, 2011) but one with a great nutritional potential. A condiment is a substance applied to

food in the form of a source, powder, spread or anything similar, to enhance or improve the flavor. Fermentation is one of the oldest methods of food preservation known to man. In Nigeria and most African countries, condiments such as fermented locust bean (Iru), fermented melon seed (Ogiri), fermented bambara seed (Daddawa), fermented cotton seed (Ogiri) and fermented pigeon pea were widely used to season food. The production of condiment is largely on a traditional small-scale, household basis under highly variable conditions contact with appropriate microorganisms at the ambient temperature of the topics. The completion of fermentation is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation (Omafuvbe *et al.*, 2000). Condiments are also known to contribute to the calorie and protein intake and

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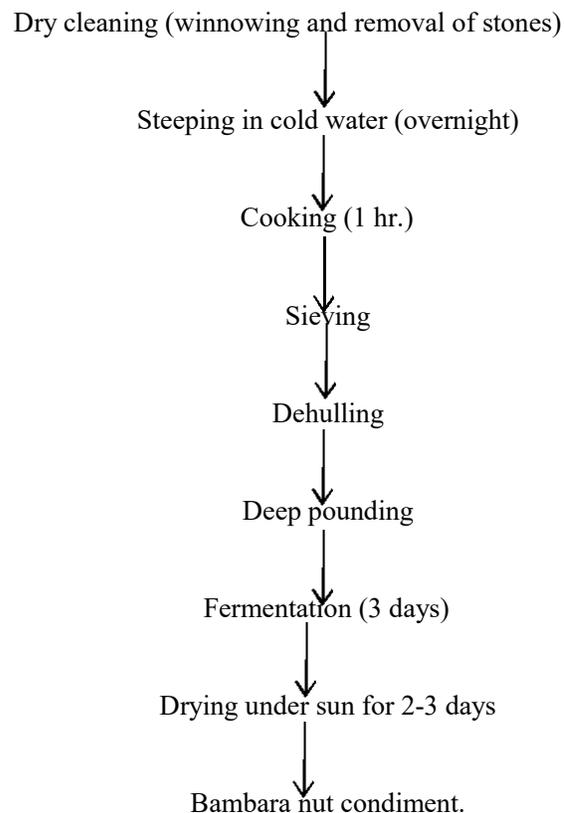


Figure 1. Flow chart for the local production of Daddawa (Odunfa, 1981)

are generously added to soups as low-cost meat substitute by low- income families in parts of Nigeria.

MATERIALS AND METHOD

Sample collection and processing

One kilo gram (1kg) of *Vigna subterranean* seeds were purchased from a local market in Sokoto, Sokoto State, Nigeria. The sample was taken to the Usman Danfodio University Sokoto (UDUS), Department of Biology, Botany unit, herbarium laboratory which was identified and authenticated and given the vouchers number as (ANS. 0301) and was then aseptically transferred to the microbiology laboratory of the Sokoto State University, for further analysis.

Local Production of Bambara nut condiment (daddawa)

Preparation of raw material

The process was done according to (Odunfa, 1981).The raw seeds were pre-processed before the real production

steps. The pre-processing consists of a selection by manually sorting. The seeds were winnowed to eliminate stones and other impurities and repeatedly washed with water (2 to 3 times). The water cleaning step is in fact a sorting by gravity in the sense that immature seeds and spoiled seeds as well as other light impurities were float while heavy impurities (stones, sand) were deposited as sediment.

Fermentation

The rapped pounded seeds were kept in an container to ferment naturally (spontaneously) for three days. During the fermentation the microorganisms used the nutritional component of the seeds converting them in to product that contribute to the chemicals composition and taste of the condiments. At the end of the fermentation, the ammonia-like flavor condiment was dried in an open sun and will be repeatedly turned to form balls and enable a good drying for 2 to 3 days according to the intensity of sunshine.

Laboratory Production of Bambara Nut Condiment

The method of Fadahunsi and Olubunmi (2009) was adopted. 200g of *Vigna subterranea* seeds were weighed

Table 1: Proximate compositions of unfermented seeds of bambara nut locally and laboratory produced condiment

Proximate Components (%)	Crude protein	Nitrogen	Moisture	Ash	Lipid	Fibre	Carbohydrate
Raw sample	6.533	1.05	8.17	9.83	1.67	1.67	77.80
Inoculated sample	11.31	1.81	18.50	6.00	11.33	0.67	55.51
Un-inoculated sample	11.58	1.85	27.67	1.67	15.33	0.50	43.42
Mean of fermented samples	11.44	1.83	23.08	2.16	13.33	0.58	49.46

Table 2: Biochemical Characteristics of bacteria isolated from Bambara nut condiment

Samples	GM	SH	SF	CT	CG	MR	VP	IN	UR	NaCl	CR	HM	GL	SC	LC	HS	P.Isolates
Ino	+(rd)	+	+	+	-	-	+	-	-	+	+	-	+	+	-	-	<i>B.subtilis</i>
Ino	+(rd)	+	+	+	-	-	+	-	-	+	+	-	+	+	+	-	<i>B.Licheniformis</i>
Un-ino	+(c)	+	-	+	-	-	-	-	+	+	+	-	+	+	+	-	<i>S.Saprophyticus</i>
Un-ino	+(ch)	+	-	-	-	+	-	-	+	+	+	+	+	+	-	-	<i>Streptococcus spp</i>

key terms:

Ino: inoculated sample + (positive) p. isolate (probable isolate)

unino: un-inoculated sample - (negative), rd: rod

GS: Gram reaction. SH: Starch hydrolysis. SF: Spore forming.

CT: Catalase. CG: Coagulase.

MR: Methyl red.

VP: Vorges proskauer.

IN: Indole.

UR: Urea. NaCl: NaCl(6.5%) growth. CR: Citrate. HM: Hemolysis. GL: Glucose. SC: Sucrose. LC:

Lactose. HS: H₂S production

using electric weighing balance. The seeds were washed and stepped in 500ml of distilled water for 18h, the seeds were then transferred in to pressure cooker containing 400ml of distilled water, which were boiled with hot plate for about 90 min until soft, this were sieved and allow to cool for 15 min, dehulled (removing of the seeds coat) and transferred to a sterile mortar followed by mashing in to pulp, the mashed nut were sterilized with autoclave, after sterilization the mashed nuts were aseptically inoculated with the starter cultures, wrapped in a polyethylene bag and kept in a air tight container, incubated at 37⁰C for 72h.

Proximate Analysis

Samples were analyzed in triplicate for proximate composition in accordance with the Official Methods of the Association of Official Analytical Chemists (AOAC, 2000). Ash was determined by incinerating two grams (2 g) each of ground unfermented and fermented seeds of bambara nut at 550 °C in lenton furnaces (England) over night. Fiber was determined by drying two gram (2 g) each of ground unfermented and fermented seeds of bambara nut over night at 105⁰C in the oven (Gallenhamp Oven BS) and incinerated at 550⁰C for 90 minutes in lenton furnaces (England). Moisture Content was determined by drying two gram (2 g) each of ground unfermented and fermented seeds of bambara nut over night at 105⁰C in the oven (Gallenhamp Oven BS). Crude lipid was determined using

saturated method. Two grams (2 g) of ground unfermented and fermented seeds of bambara nut were weighed into 50 ml conical flask and N-hexane was added and allowed to stand at room temperature overnight. It was drained into an empty flask, earlier weighed and designated W1. It was placed in an oven to allow the N-hexane to evaporate in the oven (Gallenhamp Oven BS). Protein (Nitrogen percent × 6.25 (% N × 6.25)) was determined by the Micro-kjeldahl Method. Soluble carbohydrate is not determined directly but obtained as a difference between the sum of ash, protein, crude lipid and crude fiber.

RESULTS AND DISCUSSION

The proximate composition of unfermented and fermented Bambara nut was presented in Table 1. Bambara nut was allowed to ferment naturally by indigenous microorganisms (normal flora) present in the nuts for about 3 days in un-inoculated sample. While in the inoculated sample the fermentation was carried out by starter cultures (inoculated isolate). Pure cultures of the four isolates were obtained and identified by employing several biochemical tests. The identities were *staphylococcus saprophyticus*, and *streptococcus* spp in un-inoculated sample. While in inoculated sample were *Bacillus subtilis*, and *B. licheniformis*. Their biochemical characteristics are showed in Table 2 and their mean count in Table 3.

Table 3: Result of mean bacterial count during bambara nut condiment production.

Samples	Mean bacterial count (CFU/g)
Un-ino	3.1×10^6
Ino	3.8×10^6

Key terms:

Ino: inoculated sample

un-ino: un-inoculated sample

The proximate composition of unfermented locally fermented and laboratory- based fermented seeds of Bambara nut (Table 1) shows variation. Protein content increased from 6.53-11.44% within 0-72h fermentation period. This increase may be due to the structural proteins that are integral part of the microbial cells (Tortoral *et al.*, 2002). Also the increased in growth and proliferation of the starter cultures and the normal flora may account for the increasing trend in crude protein (Oboh, 2006). Recently, many workers have reported different proximate composition values for the unfermented and fermented seeds of Bambara nut. Yagoub *et al.* (2004) reported that cooking followed by fermentation resulted in deviation of nutrients from the raw seed. He also reported a total protein of (29.79%), true protein (28.44%), non protein nitrogen (1.35%) and water soluble protein (6.81%) for raw karkade seeds and that they were changed during 'furundu' preparation to varied extents. The changes in nitrogenous constituents have been reported by workers to be a result of leaching out effect during working (Saikia *et al.*, 1999). Earlier studies have shown that moisture content increased in fermented seeds probably due to the cooking period (Parkouda *et al.*, 2008). however, there is increase in lipids content in fermented samples from (1.67%-13.33%) from 0-72hours fermentation. which is in line with finding of (Yagoub *et al.*, 2004; Bengaly *et al.*, 2006; Parkouda *et al.*, 2008), reported an increase of lipid content during the fermentation of African locust beans for soumbala production, this is due to a selective utilization of carbohydrate by the micro flora during the fermentation. Anhwange *et al.* (2006). The fiber content of the fermented Bambara nut condiment decreased from 1.67% - 0.58% within 0 – 72 hours fermentation period. This agreed with the result of Oboh (2006) that fermented foods such as legume has lower fibre content. Fibers are known to help in lowering of serum cholesterol, control blood sugar and increase in stool bulk which may prevent colon cancer and other digestive disorder (NSRL, 2002). The finding of this work showed the decrease in ash content during the fermentation which is contrast with the reports of Sefa-Dedeh *et al.* (2000) on cowpea fortification of traditional foods. The reduction was observed between 0-72hours of fermentation period with values ranging from 77.8% - 49.46%. The reduction in carbohydrate content could be

attributed to the possible bioconversion of the substrate by enzymes from starter cultures and normal flora other substrates especially protein and fats, in addition to the portion used as carbon and energy sources by the microorganisms.

CONCLUSION

The fermentation of Bambara nut for the production of 'dawadawa' is a less laborious and time consuming. 'Dawadawa' production is a process where lipolysis, proteolysis as well as degradation of carbohydrates seem to be equally important. Bambara nut is a good and cheap source of protein for human nutrition. 'Dawadawa' could be considered as an affordable fish or meat substitute particularly for low income earners in developing countries such as Nigeria. The microbial examination highlighted the need of using starter culture more specifically *Bacillus* species in bambara nut fermentation as well as other leguminous seeds fermentation as it has greater advantage of improving the fermentation process, quality of the product and also increasing the nutritional content specifically amino acids (protein) content of the product.

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