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Effect of Spontaneous Fermentation on the Chemical Composition of Thermally Treated Jack Beans (*Canavalia ensiformis* L.)

¹R.A.O. Gabriel and ²F.C. Akharaiyi

¹Microbiology Department, Adekunle Ajasin University,
P.M.B. 001, Akungba Akoko, Ondo State, Nigeria

²Microbiology Department, Federal University of Technology,
P.M.B. 704 Akure, Ondo State, Nigeria

Abstract: To enhance the nutritional quality of jack beans (*Canavalia ensiformis*) in an expensive model, natural fermentation was employed on thermally treated jack beans. Two treatment were employed, boiled (100°C for 2 h), ground pressure-cooked (120°C for 30 min) and raw cotyledon unfermented (control). These were contained in separate gourd containers overlaid with clean banana leaves, covered and wrapped in jute bags. They were left for four days under solid substrate fermentation. Microbiological, chemical and mineral analyses were assessed on the samples. *Bacillus licheniformis*, *Bacillus subtilis* and *Streptococcus thermophilus* dominated with 100% each frequency of occurrence among the nine bacteria isolates while *Rhizopus nigricans* and *Aspergillus niger* were dominant with 75% each frequency of occurrence among the five fungal isolates. Evaluation of antinutritional contents of fermented samples showed remarkable reductions in the tannin of the boiled fermented (0.10%) and ground pressure cooked fermented (0.07%), phytate and cyanide contents in that order was 893.83, 235.53 mg/100 g; 1.02, 7.07 mg kg⁻¹ contents in comparison with the unfermented sample 0.22 mg/100 g, 1246.33 mg/100 g and 41.63 mg kg⁻¹ (tannin, phytate and cyanide) contents, respectively. The protein contents of the boiled fermented (28.15%), carbohydrate in ground pressure cooked-fermented was 28.85 and 27.12% in the unfermented. Carbohydrate in that order was 48.35, 45.90 and 42.25% contents. Calcium in the boiled-fermented sample was 420 ppm, 246 ppm in ground pressure cooked-fermented and 105 ppm in the raw unfermented. Iron was 4.0, 16.0 and 9.5 ppm, respectively for boiled-fermented, ground pressure cooked-fermented and raw unfermented. Sodium, in that order was 413.0, 416.5 and 329 ppm. Zinc was 280.3, 119.7 and 166.3 ppm, respectively for boiled-fermented, ground pressure-cooked fermented and raw unfermented, respectively.

Key words: Effect, solid substrate, thermal treatment, fermentation, jack bean, biochemical composition

INTRODUCTION

The increasing demand for source of protein in developing countries coupled with the relatively high cost of imported proteins, has led to a search for alternatives, particularly legumes indigenous to the tropics such as jack beans (*Canavalia ensiformis*). Legumes are foods that are rich in protein. They occur for up to 80% of dietary protein and may be the only source of protein for some groups. Quite often, they are inedible in their raw unfermented or uncooked state. Their cooked forms are eaten as meals and are commonly used in fermented forms as condiments to enhance the flavours of food (Oniofiok *et al.*, 1996; Dakwa *et al.*, 2005; Omafube and Oyedapo, 2000). Although fermented food

Corresponding Author: R.A.O. Gabriel, Microbiology Department, Adekunle Ajasin University, P.M.B. 001, Akungba Akoko, Ondo State, Nigeria

condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitudes in terms of consumer tastes and preferences for such foods (Achi, 2005). The matured dry seeds of jack beans are used as foodstuff, but are not popular because of their unattractive flavour, texture and the fact that they require soaking and boiling in salt water for several hours before they can be utilized. The dried seeds are sometimes used as feeds for livestock, but are not very palatable and can cause outbreak of poisoning, unless cooked or limited to less than 30% of the total feed for animals (Berrie, 1977). The seeds of all *Canavalia* species are known to contain toxic substances and this has restricted the use of this bean as food and feed in their raw form. The toxicity of jack beans has been attributed to the presence of protein globulins, concanavalin A and B (D' Mello *et al.*, 1998); two toxic amino acid canavanine and canaline (Bressani *et al.*, 1987). In addition, a hydrocyanic acid contents of 0.0108% and saponnins have been reported in jack beans among other antinutritional factors such as tannins and phylate (Pulseglove, 1974). A lot of work has been done on the thermal and chemical detoxification of jack beans (D'Mello and Walker, 1991; Ologhobo *et al.*, 1993) but no much work on the use of fermentation techniques has been done. Since heat treatment alone is not enough to render jack beans inhibition negative (D'Mello and Walker, 1998) and chemicals are expensive and in most cases are not readily available in some rural and urban areas of developing countries like Nigeria. This study therefore examines the use of fermentation in further detoxification of thermally treated jack beans to either be used as animal feed or for human consumption.

MATERIALS AND METHODS

Source of Sample

Matured dried pods of jackbeans (*Canavalia ensiformis*) were harvested from an ornamental garden in Akure, Ondo State, Nigeria, in 2006.

Preparation of Samples

Clean and healthy *Canavalia ensiformis* seeds were soaked in warm water for 24 h to aid dehull of the seeds. The dehulled cotyledons were washed in clean water, drained and spread out on wire mesh to air dry. 900 g of the air-dried cotyledons were weighed and divided equally into three parts for different treatments. The first treatment involves boiling the cotyledons in a steamy pot at 100°C for 2 h. The second treatment was grinding the dried cotyledons, mixed with 500 mL distilled water in a stainless dish and pressure cooked at 120°C for 30 min. The third set up served as control experiment in which the cotyledons were left in their raw form unfermented but as well contained in the gourd containers as the treatments. These treatments were packed in separate gourd containers overlaid with clean banana leaves, covered and finally wrapped in jute bags. The experiment in its solid state was left for four days to ferment.

Microbiological Analysis

The fermented samples were aseptically mixed with sterile spatula and 10 g each was weighed in a sterile beaker and mixed with 10 mL sterile distilled water. 1 mL was then diluted serially and 1 mL each was pour plated on nutrient agar plates in triplicates and incubated at 37°C for 24 h (bacteria growth) and on acidified malt extract agar incubated at 28±1°C for 72 h (fungal growth). This was done for isolation of micro organisms. Representatives of the various bacterial organisms were purified by streaking on freshly prepared nutrient agar and transfer of fungal mycelia on freshly prepared acidified malt extract agar. They were incubated appropriately and single isolates of bacteria were identified according to tests and descriptions given in Collins' and Lyne (1984) and in Bergey's manual of systematic bacteriology (Holt *et al.*, 1994; Sneath *et al.*, 1986) while purified fungi isolates were identified according to the criteria of Rhode and Hartman (1980).

Chemical Analysis

The proximate compositions of ash, fat, crude fibre, moisture, carbohydrate and protein contents of the fermented samples were evaluated with the standard methods of AOAC (1990).

Tannin Determination

Tannin was determined according to the method of Makker *et al.* (1993) which is based on the ability of tannin-like compounds to reduce phosphorus tungsto-molybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity is measured at 700 nm and it is proportional to the amount of tannin present.

Phytate Determination

The methods of Young and Coreavese (1940) was used for phytate determination. Four grams each of ground pressure cooked-fermented, mashed or boiled-fermented were soaked in 100 mL 2% HCl for 3 h and filtered against a standard FeCl₃ solution containing about 0.00195 g Fe mL⁻¹ until a brownish yellow colour persisted for 5 min.

Mineral Analysis

The mineral analysis was carried out according to published methods (Perkin-Elmer, 1988). In general, the chemical analysis result was calculated as proportional to dry weight.

Cyanide Determination

Cyanide content was determined using alkaline pirates methods as described by Balagopalan *et al.* (1988).

Statistical Methods

The whole experiment was replicated three times. All the data obtained were analysed using the analysis of variance to determine variances and Duncan's multiple range tests to separate the means. (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the proximate analysis (Table 1) shows that the average protein content of ground-pressure cooked fermented Jack beans was higher (28.55%) and least in the raw unfermented sample (27.12%). The heat treatment employed however, helped in softening the samples thus making more available the protein contents of the Jack beans. The higher protein contents (%) of the thermally treated fermented samples over the raw unfermented sample may be due to the interplay of the microbial activities involved in the fermentation since the samples were more liable to fermentation strategy. In accordance with (Whitaker, 1981; Rombouts and Nouts, 1995), it could also be due to the larger surface interaction with the microbial enzymes and the number and types of microbial species involved in the fermentation (Table 4). The average moisture contents of the thermally treated sample were also higher than the raw unfermented sample. The ground-cooked sample (14.35%) was higher in moisture contents than the boiled fermented (13.35%) (Table 1). This could be as a result of the involvement of more bacteria species than fungi species in the samples fermentation (Table 4). These findings are in agreement with the previous results of (Eka, 1980; Sanmi and Ogbonna, 1990). The fat content of the fermented sample was between 4.15-5.45% while in the unfermented, it was 5.75%. The reduced fat content values recovered from the thermally treated fermented sample may be due to the duration of the thermal process of the samples coupled with the microbial activities. The microbial activities during fermentation could as well resulted to the peculiar aroma, texture, taste and colour of

the end product (Wang and Hesseltine, 1982; Fudiyansyah *et al.*, 1995). The average ash contents of the fermented samples was between 2.15-2.75% while the raw unfermented sample was 3.15%. Average crude fibre of the fermented samples was between 3.25-3.32% and was 3.75% in the raw unfermented. (Table 1) All these resulted to the effects of microbial enzymes involved in the fermentation process (Beuchat, 1984; Rombouts and Nout, 1995). These observations may affect the carbohydrate content which increases in all the fermented samples due to the reduction in fibre content and increase in both reducing sugars and total soluble sugars in accordance with (Aykroyd and Doughty, 1982; Odetokun, 2000).

The mineral (Ca, Fe, Mg Zn k and Na) contents of the samples is shown in Table 2. Calcium was higher in the thermal treated samples (246.0-420.0 ppm) than the raw unfermented (105.0 ppm). Sodium iron, potassium, magnesium and zinc were also found higher in the thermally treated samples than the raw unfermented sample (Table 2). However, the values of the mineral contents obtained from the Jack beans indicates that thermally treated fermented Jack beans may serve as a good source of Ca, Fe, Na, Mg, Zn and K and may as well satisfy the nutritional need for consumers because their values as obtained is reasonably high and could be acceptable when compared to FAO (1968).

The antinutrient contents (tannin, phytate and cyanide) is shown in Table 3. Tannin in the raw unfermented was (0.22 mg/100 g) which was quite higher than the thermally treated fermented samples (0.07-0.10 mg/100 g). This indicates that when jack beans is subjected to complex microbial fermentation, microbial enzymes interaction is increase which then influence the decrease in tannin content as observed in the fermented samples. Meanwhile, with the result obtained after fermenting the Jack beans, it could be considered safe, with regard to tannin poisoning since the content is far below the critical values of (0.70-0.90 mg/100 g) being poisonous as reported by (Aletor, 1993a). Phytate content in the Jack beans samples was also found to be reduced with thermal treatment and fermentation process. The raw unfermented samples had a phytate content as high as (1246.33 mg/100 g) compared to the thermally treated fermented samples (235.53-893.83 mg/100 g). The heat treatment though helped softening the samples, might not be the major cause of phytate

Table 1: Proximate composition of fermented jack beans (% dry weight)

Treatment of sample	Ash	Crude fibre	Crude protein	Fat	Carbohydrate	Moisture
Boiled fermented	2.75±0.20c	3.25±0.02c	28.15±0.20a	415.00±5.00e	48.35±0.20e	13.35±6.00b
Ground pressure-cooked fermented	2.15±0.20a	3.32±0.15ab	28.85±0.20e	5.35±4.30be	45.90±0.20c	14.35±2.70d
Raw unfermented (control)	3.15±0.20e	3.75±0.02c	27.12±0.20e	5.75±4.30e	42.25±0.20a	13.05±2.70a

Values are Means±Standard Error for three samples; Mean followed by the same letter(s) within the group are not significantly different at p<0.05 using Duncan's multiple range test

Table 2: Mineral Composition of spontaneous fermented jack beans (ppm) Dry weight

Treatment of sample	Calcium	Iron	Potassium	Magnesium	Sodium	Zinc
Boiled fermented	420.0±5.8d	4.0±0.3b	480.0±2.0a	71.3±0.7b	413.0±0.6c	280.3±0.6e
Ground pressure-cooked fermented	246.0±0.7c	16.0±0.6d	454.0±1.2a	150.0±1.2d	416.5±1.2c	119.7±0.6b
Raw unfermented (control)	105.0±1.2a	9.5±0.5±0.6c	480.0±1.2c	112.0±1.2c	329.0±1.2a	166.3±1.2d

Values are Means±Standard Error for three replicates; Mean followed by the same letter(s) within the group are not significantly different at p<0.05 using Duncan's multiple range test

Table 3: Some antinutrient contents of spontaneous fermentation of jack beans (Dry weight)

Treatment of sample	Tannin (mg/100g)	Cyanide (mg kg ⁻¹)	Phytate (mg/100g)
Boiled fermented	0.10±0.6c	1.02±5.8a	893.83±4.8d
Ground pressure-cooked fermented	0.07±0.6b	7.07±1.2c	235.53±3.6a
Raw unfermented (control)	0.22±0.6e	41.63±0.6e	1246.33±6.5e

Each value represents the mean of three determination; Mean followed by the same letter(s) within the group are not significantly different at p<0.05 using Duncan's multiple range test

Table 4: Occurrence of isolated microorganisms

Organisms	Bf	Gp-cf	Ru	Frequency of occurrence (%)
<i>S. aureus</i>	1	1	1	75
<i>Peptostreptococcus</i> sp.	0	1	-	25
<i>Micrococcus lactis</i>	0	2	-	50
<i>Bacillus licheniformis</i>	2	2	-	100
<i>Lactobacillus</i> sp.	1	2	-	75
<i>Streptococcus thermophilus</i>	2	2	-	100
<i>Bacillus subtilis</i>	2	2	-	100
<i>Leuconostoc mesenteroides</i>	2	2	-	50
<i>Bacillus cereus</i>	1	1	1	75
<i>Mucor mucedo</i>	1	1	0	50
<i>Rhizopus nigricans</i>	1	2	0	75
<i>Aspergillus niger</i>	1	2	0	75
<i>Saccharomyces cerevisiae</i>	1	1	0	50
<i>Girellura suffulta</i>	1	0	0	25

Bf = Boiled fermented; Gp-cf = Ground pressure cooked fermented; Ru = Raw unfermented

reduction in the Jack beans but the presence of hydrolytic enzyme (Phytase) present in the microorganisms especially fungi species involved in the fermentation (Akindahunsi *et al.*, 1999). A reduction level was also seen in the cyanide content when the raw unfermented (41.63 mg kg⁻¹) was compared to the thermally treated fermented samples with between (1.02-7.07 mg kg⁻¹) cyanide content. This may be due to the combined effect of the hydrolytic activities of the microbial enzymes as reported by (Akindahunsi *et al.*, 1999) and the thermal treatments of boiling, cooking and drying processes employed which also could ensure the removal of cyanogenic glucosides in accordance with the findings of (Aletor and Fetuga, 1988). The cyanide level of the fermented jack beans samples were far below the detrimental level of 30 mg kg⁻¹ as reported by many researchers (Aletor, 1993b), thereby making the samples considered safe with regard to cyanide poisoning. The microflora of the fermented Jack beans samples were dominated mainly by *Bacillus licheniformis*, *Bacillus subtilis* and *Streptococcus thermophilus* while the microfauna of the fermented Jack beans samples were dominated with *Aspergillus niger* and *Rhizopus nigricans* (Table 4). Hence, this type of fermentation involves a complex microbial process, this may result in the variations in quality of food products prepared from Jack beans. In addition, the types of fermentation process employed will influence the quality of the final product. However, the population and composition of the microbiota as well as the reduction of cyanogenic glucoside is similar to the reports in earlier studies by (Eka, 1980; Rombouts and Nout, 1995; Okafor *et al.*, 1984; Odetokun, 2000).

The fermentation of Jack beans was found to be mediated by a diverse microflora and eventually dominated by heat loving bacteria. This pattern of microbial succession is a general feature of fermenting plant materials (Deaschel *et al.*, 1987; Achi and Akubo, 2000; Obile *et al.*, 2004; Achi and Akomas, 2006). Hence complex microorganisms are involved in the fermentation, strong starter cultures may reduce fermentation time and will further minimize the risk of incidental microflora that might cause spoilage or undesirability in end products.

CONCLUSIONS

Heat treating *Canavalia ensiformis* aids fermentation by microorganisms. Also heat treatment at 120°C for 30 min suits a better form of softening *Canavalia ensiformis* than boiling at 100°C for 2 h. This does not indicate that *Canavalia ensiformis* can not be utilized when boiled using steam pot but the time of boiling can be extended to 4 h to make the sample well cooked. At this research stage, it could also be concluded that spontaneous fermentation, a very cheap process of utilizing *Canavalia ensiformis*, would efficiently increase the nutritive value of *Canavalia ensiformis* and reduce the antinutrients (tannin, phytate and cyanide) to safety level, thereby making the nutrient

bioavailable for human and animal utilization as a source of protein for nourishment most especially in the underdeveloped and developing countries where *Canavalia ensiformis* are wasted away and used as ornamental plant.

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