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Biodeterioration of Thermally Treated Jack Beans (*Canavalia ensiformis,* L) Cotyledons

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Abstract

Dehulled cotyledons of Jack beans (*Canavalia ensiformis*, L) were thermally treated by roasting and pressure cooking, allowed to deteriorate for seven days and the microorganisms involved isolated and identified.. This was after five days of natural solid state fermentation, using the natural micro flora of the air and banana leave used in wrapping. The physiochemical compositions of the deteriorated cotyledons were monitored. There was a significant increase in the microbial count for the first three days of deterioration followed by gradual decrease. A total of eighteen bacteria were isolated however *Bacillus subtilis, Leuconostoc lactic, Leuconostoc meseneroides, Listeria denitrificans, Listeria murrayii* and *Xanthomorias fragariae* were dominant in all the samples. A total of eight fungal isolates were involved which were all dominant except *Geniculosporium serpens* found in pressure cooked ground sample. All treated samples had higher protein content than the control but the carbohydrate content was significantly low when compared with the control; however there was a significant decrease in the antinutrient content of the treated samples when compared to the control.

Keywords: Biodegradation; Cotyledon; Ant-nutrition; Solid-substrate; Microorganisms.

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1. Introduction

Jack bean (*Canavalia ensiformis*, L) is an under-utilized novel legume with high crude protein content and amino acid profile making it a potential substitute for other protein sources for human consumption and production of fish meal and animal feeds. Animals require a supply of protein and energy to be in good health, and this promotes competition between men and animals over the limited source that is available. Jack bean (*Canavalia ensiformis*, L) has been tested and found to have a considerable adaptability to acidic soils; hence offers potential has a good protein source [1]. This legume contains 30% crude protein (CP) and 53% starch. The seed contains heat sensitive physiological factors (trypsin and subtilisn inhibitors and haemagglutin), toxic due to the presence of canavanine, a non-protein amino acid [2], and consequently the usefulness of Jack bean

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as a potential protein source is limited. Because of its anti-nutritional factor, the general use of this legume for animal feed is best limited to not more than 5% of the diet. Therefore, several methods adopted to detoxify the beans, such as, soaking, pressure cooking, heating and roasting, ensiling or autoclaving the bean to reduce the inhibitors has been partially effective [3].

Biodeterioration can be defined as the breakdown of food or any undesirable change in the property of a material caused by the vital activities of microorganisms, either directly or indirectly by products of their metabolism [4]. Biodeterioration may lead to loss of nutritional value, organoleptic and color changes, and most importantly, safety may become compromised. It refers to the chemical changes in organic substances caused by the action of specific enzymes produced by microorganisms, such as, moulds, bacteria, and yeasts which usually result in the breakdown of complex organic substances into simpler ones. A few species of moulds (*Aspergillus oryzae* in soy sauce manufacture) have been used in the production of fermented food. *Aspergillus* species compete with *Penicillium* and *Fusarium* species for dominance in foods and food plants while Aspergilla generally grow at higher temperatures or lower water activities than Penicillia [5].

Several efforts and researches have been carried out on possible ways of eliminating the toxicity of Jack beans [6] but up till this moment there has been none in biodegradation of the major anti-nutrients of *Canavalia* beans. Knowing that, biodeterioration is the microbial mediated breakdown of processed food and can probably reduce harmful organic matters to harmless end product. Therefore, we carried out this work to study the effect of biodegradation process on the toxic components of jack beans. Hence, there may be the need for the isolation and detection of microorganisms that will go beyond the safe fermentation period in degrading the antinutrients in jack bean.

2. Materials and Methods

2.1. *Source of sample*

Two kilograms of dry, healthy, clean, mature pods of Jack bean were obtained from the Federal University of Technology, Akure and Oke-Ako, Ekiti. The healthy clean seeds were stored in sterile, transparent polythene bag in the refrigerator before used.

2.2. *Preparation of samples*

Healthy clean seeds were soaked in boiled distilled water for 2 hours and dehulled by pressing the swollen seed between fingers. The cotyledons were rinsed subsequently in three changes of sterile water and were divided into two equal parts. Half of which was dried in drying cabinet in the laboratory at 70^{o}C for eight hours. The dried cotyledons were blended to fine powder of about 0.8 mm screen mesh using the stainless Marlex. The powder was packed in sterile polythene and labeled. Both the cotyledons and the ground powders were further divided into two equal parts, with half of each pressure cooked and the other half surface sterilized with 1% sodium hypochlorite; before fermenting. The

samples were grouped into 4 main categories: Pressure cooked cotyledon, Non-pressure cooked cotyledon, Roasted-ground cotyledon pressure cooked, Roasted-ground cotyledon non-pressure cooked.

Solid state technique of fermentation was employed for five days, using spontaneous inoculums. Two hundred and fifty grams (250g) of cotyledons were aseptically packed in calabash lined with banana leaves and covered with lid. The covered calabash was then wrapped with jute bag and kept in laboratory cupboard for five days at ambient temperature.

2.3. *Physicochemical analysis*

The pH and temperature of the fermenting/deteriorating substrate was taken twice a day with 12 hourly intervals using a potable pH meter and thermometer. Starting from the fifth day when deterioration is due to set in.

2.4. *Isolation and characterization of microorganisms*

Isolation of microorganisms (Bacteria and Fungi) was done by making serial dilution of 1g of deteriorating samples at every 24 hour interval.

2.4.1. *Fungal cultures*

About 0.5 milliliter of 10^{-2} and 10^{-6} dilutions were transferred into sterile plates in duplicate using pour plate method as recorded by Harrigan [7]. Malt Extract Agar (MEA) was used for the culturing and incubated at 30 ^{o}C for 72 hours for (10^{-2} diluents) and for 24 hours (10^{-6} diluents). The colonies formed were counted and recorded.

2.4.2. *Bacterial cultures*

About 0.5 milliliter from 10^{-5} dilution was transferred in duplicate using pour plate method with Nutrient Plate Count Agar being used for culturing. The plates were incubated at 37 ^{o}C for 24 hours and the colonies formed were counted and recorded. Representative of each colony based on their cultural and morphological features were picked and sub-cultured on fresh Nutrient agar plates for bacteria and Malt extract agar plates for fungi, until pure culture is obtained according to Food and Agriculture Organization [8] and characterized using standard procedures [7].

2.5. *Proximate analysis*

All the samples were dried in the oven at 55 ^{o}C, ground, sieved and put in labeled bottles and kept in refrigerator pending analysis. The following parameters were determined for the various flour samples; moisture content, crude protein, ash, crude fiber, fat and carbohydrate contents (by difference) using the standard AOAC [9] method. The protein was determined from estimates of total nitrogen using a conversion factor of 6.25 according to Etheridge *et al*. [10].

2.6. *Anti-nutritional content determination*

10.0 ml of 70% aqueous acetone was used to extract tannin from 0.2g of finely ground sample and supernatant stored in ice using the procedure of Makker and Goodchild [11]. Oxalate was determined by titrating hot filtrate against 0.1N $KMnO_4$ solution by the procedure of Day and Underwood [12]. For the determination of phytic acid content, the Wheeler and Ferrel [13] method was used in which the phytic acid present was extracted with 3% TCA by shaking. And the supernatant was precipitated as ferric phytate by adding excess ferric chloride.

2.7. *Statistical analysis of data*

Quantitative data are presented as means plus standard error. Statistical evaluation of data was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test [14].

3. Results and Discussion

A total of sixteen different bacteria species (Table 1) were isolated from the four different samples of both the ground and whole cotyledons. These are *Actinobacillus equilli, Bacillus pasteus, B. pumilus, Enterobacter spp, Lactobacillus spp, Leuconostoc lactic, L. cremoris, L. mesenteroides, Listeria denitrificans, L. murrayii, Micrococcus luteus, Proteus vulgaris, Streptococcus thermophillus, Xanthomonas compesuns, X. fragariae* and *Zymonomas spp.* Table 2 shows that eight different fungi (moulds) were involved in the deterioration of the fermented samples, which includes *Aspergillus flavus, A. fumigates, A. niger, Geotrichum albicum, Geruculosporium serpens, Gibellula suffuta, Penicillium chrysogenum,*and *Rhizopus stolonifer*. Most of the bacteria and fungi species isolated were those that have both endogenous and exogenous enzymes that are able to utilize the chemical composition of most leguminous plants. The result of more than one microorganisms working together in sequence is in consonant with the findings of Hutton [15] and Kay [16].

Table 1. Bacteria isolated from thermally treated biodeteriorated Jackbeans cotyledon.

Pressure cooked cotyledons	Not pressure cooked cotyledons	Pressure cooked ground cotyledons	Not pressure cooked ground cotyledon
Listeria denitrificans	*Actinobacillus equilli*	*Bacillus pumilus*	*Listeria denitrificans*
Leuconostoc lactic	*Xanthomonas fragariae*	*Xanthomonas campesuns*	*Leuconostoc lactic*
Bacillus pumilus	*Bacillus pumilus*	*Leuconostoc mesenteroides*	*Bacillus pasteus*
Bacillus pasteus	*Proteus vulgaris*	*Actinobacillus equilli*	*Leuconostoc mesenteroides*
Lactobacillus spp	*Enterobacter spp*	*Streptococcus thermophillus*	*Micrococcus luteus*
Leuconostoc cremoris	*Listeria murrayii*	*Zymonomas spp*	*Listeria murrayii*

Table 2. Fungi isolated from thermally treated biodeteriorated jack beans cotyledon.

Pressure cooked cotyledons	Not pressure cooked cotyledons	Pressure cooked ground cotyledons	Not pressure cooked ground cotyledon
Aspergillus flavus	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
Aspergillus niger	*Aspergillus niger*	*Aspergillus niger*	*Aspergillus niger*
Aspergillus fumigatus	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigatus*
Rhizopus stolonifer	*Rhizopus stolonifer*	*Rhizopus stolonifer*	*Rhizopus stolonifer*
Penicillium chrysogenum	*Penicillium chrysogenum*	*Gibellula suffuta*	*Gibellula suffuta*
Gibellula suffuta	*Gibellula suffuta*	*Geotrichum albium*	*Penicillium chrysogenum*
Geotrichum albium	*Geotrichum albium*	*Geruculosporium serpens*	*Geotrichum albium*

Of the sixteen bacteria species isolated from the different samples, only six [*Bacillus pumilus* (100%), *Leuconostoc lactic* (50%), *L. mesenteroides* (50%), *Listeria denitrificans* (50%), *L. murrayii* (50%) and *Xanthomonas fragariae* (50%)] have high frequency of occurrence. The most frequent of the six is *Bacillus pumilus* with hundred percent occurence (Table 3). It was isolated from all the samples, which is an indication that, it possesses endogenous enzymes that are capable of utilizing the component of the substrate. This occurrence establishes the fact that this organism initiates the deterioration and grows until a by-product produce inhibits its further growth and activity [16].

Table 3. The frequency of occurrence of bacteria isolated from thermally treated biodeteriorated Jackbean cotyledon.

Organisms / Sample	*Actinobacillus spp*	*Actinobacillus equilli*	*B. pasteus*	*B. pumilis*	*Enterobacter spp*	*L. delbrueockii*	*Leuconostoc cremoris*	*Leuconostoc lactic*	*L. mesenteroides*	*Listeria denitrificans*	*Listeria murrayii*	*Micrococcus luteus*	*Proteus vulgaris*	*S. thermophillus*	*X. campesuns*	*X. fragariae*	*Zygomonas spp*
Pressure cooked cotyledons	O	O	X	X	O	X	X	X	O	O	O	O	O	O	O	X	O
Not pressure cooked cotyledon	X	O	O	X	X	O	O	X	O	X	X	O	X	O	O	O	O
Pressure cooked ground cotyledons	O	X	O	X	O	O	O	O	X	O	O	O	O	X	X	X	X
Not pressure cooked ground cotyledon	O	O	O	X	O	O	O	X	X	X	X	X	O	O	O	O	O
Percentage occurrence	25	25	25	100	25	25	25	50	50	50	50	25	25	25	25	50	25

Legend: O – Absent; X – Present.

Leuconostoc lactic and *L. mesenteroides* were more predominant with the roasted ground samples. This is an indication that the initial heat treatment of the substrate provides a better platform for the *Leuconostoc* species to utilize the substrate. *Listeria denitrificans* and *L. murrayii* were isolated from the unpressured cooked samples of both ground and whole cotyledons, while *Xanthomonas fragariae* was dominant only in the two pressure cooked samples. This corroborates the fact that during the initial growth period of the most frequent organism, other organisms also develops to take over proliferation [16].

All the fungi on Table 4, shows a high frequency of occurrence of 75 to 100 %. *Aspergillus species* dominated all the four samples, *Geotrichum albicum, Gibellula suffuta* and *Rhizopus stolonifer* were also isolated from all the samples. The dominancy of the *Aspergillus species* over others was confirmed by the fact that they are common components of the aerosol, which agrees with the findings of Kanaani *et al.* [17]. This is an indication that the fungi played a crucial role in the deterioration of the fermented substrate, because appropriate nutrients were available for these molds to germinate, which was what Samson and his group [18] detected. These organisms have been found to be responsible for the fermentation of most legumes and consequently remain during post fermentation. This is also in agreement with Eka [19], who reported the role of Aspergillus *spp, Botrytis cenerae, Rhizoctonia solani, Trichoderma vinde, Chaetomium globosum* and *Myrothecium verrncaria* in the deterioration of carbohydrate in food or plant materials. For *Aspergillus* and *Rhizopus species*, the process of degradation is the means of obtaining nutrients made available in jack beans, which is in line with the work of Pitt and Hocking [20].

Table 4. The frequency of occurrence of fungi isolated from thermally treated biodeteriorated jackbean cotyledon.

Samples \ Organism	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*G. serpens*	*Geotrichum albium*	*Gibellula suffuta*	*P. chrysogenum*	*Rhizopus stolonifer*
Pressure cooked cotyledons	X	X	X	O	X	X	X	X
Not pressure cooked cotyledon	X	X	X	O	X	X	X	X
Pressure cooked ground cotyledons	X	X	X	X	X	X	O	X
Not pressure cooked ground cotyledon	X	X	X	O	X	X	X	X
Percentage occurrence	100	100	100	25	100	100	75	100

Legend: O – Absent; X – Present

The pH of the deteriorating whole cotyledon samples has an initial rise for the first 60 hours of deterioration from 6.2 to 7.2 and later started to fall as compared to the zero hour between 6.2 and 5.6. The pH of the two ground samples were found to have a steady decline from 6.8 to 5.6 when compared with the zero hour (Fig. 1). This could be as a result of alcohol production which occurred due to the enzymatic activities of the microorganisms [15, 21].

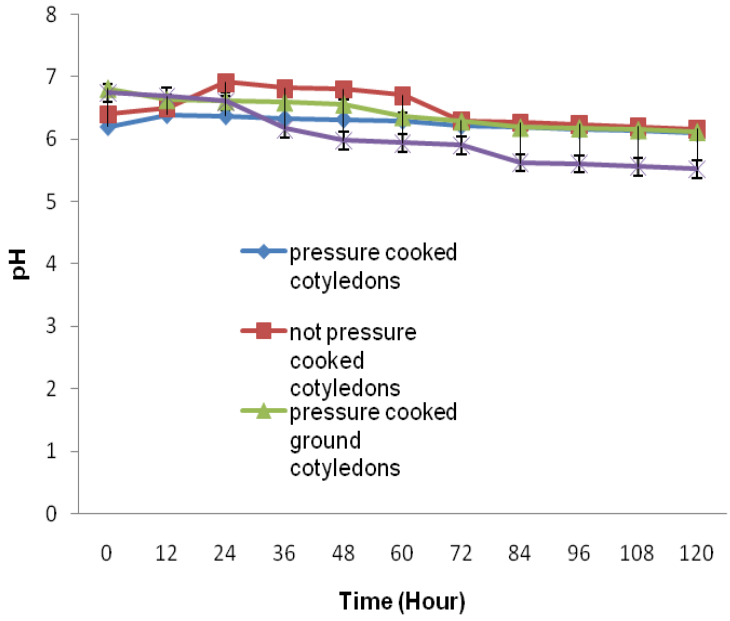

Fig. 1. pH of thermally treated jack bean cotyledons during biodeterioration.

Fig. 2 shows the temperature changes during the deterioration of the samples. Generally, it was observed that there were increases in temperature for all the samples. For the whole cotyledon samples, the increase was from 30 to 36^{o}C, while the ground samples were from 28 to 40^{o}C. The increase in temperature of all the samples implies that energy is given out during deterioration process hence the reaction is an exegetic one. Heat was also generated by the jute bags used in wrapping the samples. The heat generated from both ends helped to facilitate the deterioration rate by keeping the bacteria near their optimum growth temperature; hence the microbial enzymes were able to perform at their optimum [16]. In addition, Hocking *et a.* [5] detected that the dominant molds grow more rapidly at higher temperatures.

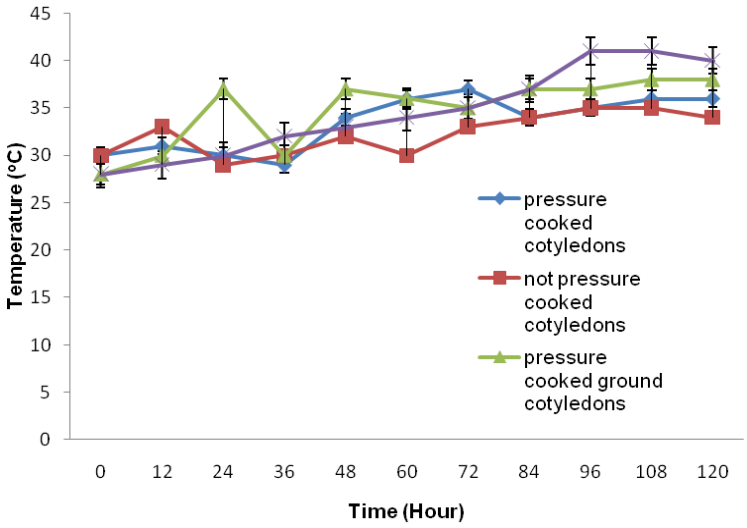

Fig. 2. Temperature of thermally treated jack bean cotyledon during bideterioration.

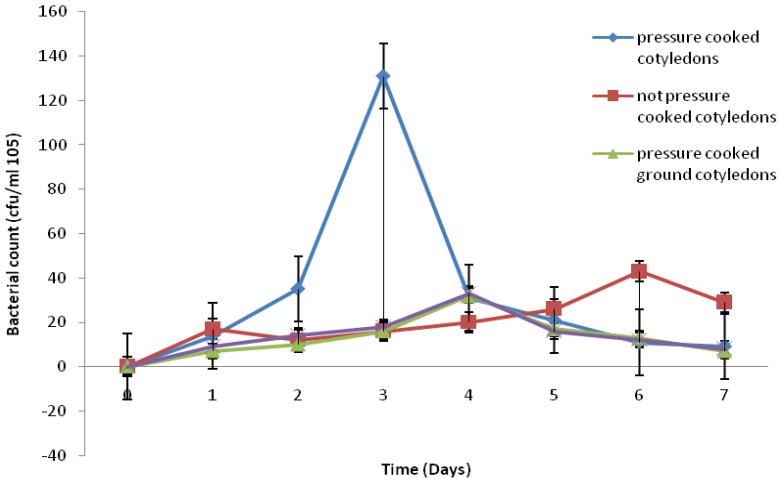

Fig. 3. Total bacterial count of thermally treated biodeteriorated jack bean cotyledons.

It is shown in Fig. 3 that the total viable bacterial counts increased initially and later began to drop. For the whole cotyledon samples the pattern of increase in number of the total viable bacterial counts was determined by the thermal treatment, hence they showed

different peaks. The peaks for the pressure cooked cotyledons samples were on the third day, with 134 x 10^5 cfu/ml being the highest peak, while that of the non-pressure cooked was on the sixth day. The rate of bacteria proliferation of the pressure cooked whole cotyledon was higher and faster than the non-pressure cooked. This may be due to the fact that pressure cooking has softened the cotyledon hence easy accessibility of the nutrients by the bacteria which was in line with the finding of Gabriel and Akharaiyi [22]. While for the ground cotyledon, the rate of proliferation of the non-pressure cooked was almost at par with that of the pressure cooked higher, this may be due to the fact that grinding makes the nutrients accessible while the cooking makes for more availability [22].

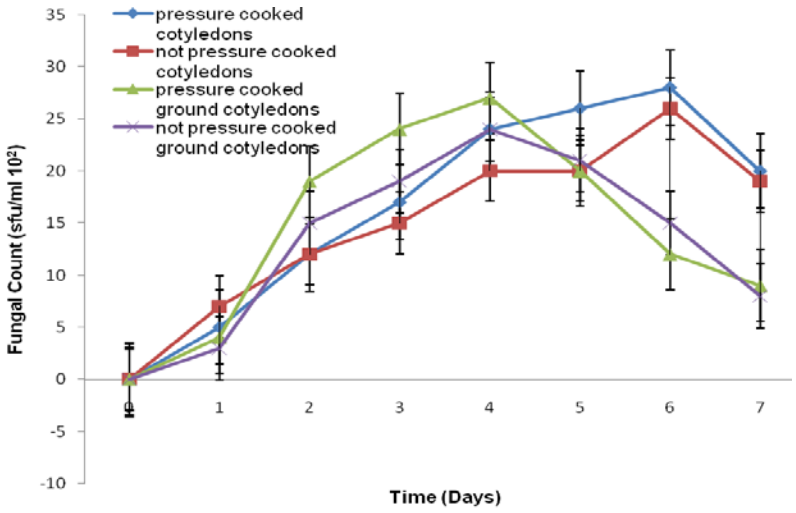

Fig. 4. Total fungal count of thermally treated biodeteriorated jack bean cotyledons.

With Fig. 4, the total viable fungal counts shows similar pattern of initial increase and later drop as shown in Fig. 3. The two ground cotyledon sample's chart has the same pattern irrespective of their thermal treatments, and the samples both had their highest peaks at the fourth day. Their state of matter and the available surface area were also determinant factors in the pattern of their curves. There was a difference in the rate of proliferation; the fungal proliferation of the ground cotyledons was faster than the whole cotyledons, while in both cases the pressure cooked was higher than the non-pressure cooked. The growth peaks for the ground cotyledon samples whether pressure cooked or not, was on the fourth day (23 x 10^2 sfu/ml and 28 x 10^2 sfu/ml for non-pressure cooked and pressure cooked respectively). While the growth peaks for the whole cotyledon samples was on the sixth day and the non-pressure cooked (26 x 10^2 sfu/ml) was lower

than that of the pressure cooked (29 x 10^2 sfu/ml). All these are also due to the fact that the nutrients were both accessible and available for the molds to penetrate and absorb**.**

Table 5. Proximate composition of thermally treated biodeteriorated jackbean cotyledon (sem).

Samples	Moisture (mg/100g)	Ash (mg/100g)	Fat (mg/100g)	Protein (mg/100g)	Fibre (mg/100g)	Carbohydrate (mg/100g)
Pressure cooked cotyledons	10.43 ± 0.37^{b}	4.54 ± 0.01^{b}	5.31 ± 0.14^{e}	38.21 ± 0.55^{e}	2.43 ± 0.02^{a}	38.84 ± 0.51^{a}
Not pressure cooked cotyledons	12.06 ± 0.11^{c}	5.03 ± 0.01^{d}	0.77 ± 0.11^{a}	32.71 ± 0.02^{c}	4.80 ± 0.03^{d}	44.65 ± 0.01^{b}
Pressure cooked ground cotyledons	10.70 ± 0.31^{b}	4.41 ± 0.05^{a}	2.80 ± 0.01^{d}	30.24 ± 0.01^{b}	3.00 ± 0.01^{b}	48.85 ± 0.12^{d}
Not pressure cooked ground cotyledons	9.37 ± 0.35^{a}	6.00 ± 0.02^{e}	1.22 ± 0.14^{b}	34.63 ± 0.01^{d}	2.44 ± 0.25^{a}	46.36 ± 2.29^{c}
Raw cotyledons	13.27 ± 0.01^{d}	4.60 ± 0.01^{c}	2.10 ± 0.02^{c}	24.36 ± 0.01^{a}	3.10 ± 0.01^{c}	52.38 ± 0.02^{e}

Key: DM = Dry matter. SEM = Standard error of the mean. a, b = Values in the same column with different superscripts differ significantly ($p < 0.05$).

The proximate composition of the deteriorating Jack beans cotyledons when compared with the raw cotyledons which act as the control is shown in Table 5. There was a considerable decrease in the moisture content of the deteriorating beans as the days progresses, when the raw cotyledon (13.27 ± 0.01 mg/100g) was compared with the non-pressure cooked ground cotyledon sample (9.37 ± 0.35 mg/100g) being the lowest. This was due to the fact that the moisture content of the substrate were being used up by the proliferating organisms as explained by both the groups of Kanaani [17] and Hockings [5]. The ash content of the pressure cooked samples showed significant decrease when compared with the control since the pressure cooking helps in making the nutrients accessible, the mineral content might have been used up by the microbial enzymes. The thermal treatment affected the crude fat content of the pressure cooked samples whether it was ground or not, they both showed significant increase in comparison with the raw sample (2.10 ± 0.02 mg/100g), while the non-pressure cooked ones showed a significant decrease. The accessibility of the nutrients in the pressure cooked samples enables the numerous microbial enzymes to turn the complex polymers in the substrate to lipids, as detected by the Baker's group [23]. For all the degraded samples there were significant increases in crude protein content when compared with the control. Apart from the fact that the microbial biomass will add to the crude protein content of the substrate, it is also possible that some of the complex unavailable polypeptide been broken down by the

microbial enzyme as reported by Gabriel's group [24]. With the exception of the non-pressure cooked whole cotyledons, there was a significant decrease in the crude fiber content when compared with the control. The carbohydrate content showed a significant decrease when compared to the control (Table 5). The findings of Pandit and coworkers corroborate this work, in which they reported that frequent involvement of *Neurospora intermedia* in the deterioration of roasted or cooked food products may be as a result of possession of powerful enzymes useful in bioconversion operations [25].

Table 6. Antinutritional content of biodeteriorated Jackbean cotyledons (sem).

Sample	Phytate (mg/100g)	Oxalate (mg/100g)	Tannin (mg/g)
Pressure cooked cotyledons	11.90 ± 0.25^{b}	3.87 ± 0.02^{c}	0.75 ± 0.04^{c}
Not pressure cooked cotyledons	21.84 ± 0.34^{d}	4.95 ± 0.02^{d}	0.98 ± 0.02^{d}
Pressure cooked ground cotyledons	9.07 ± 0.36^{a}	2.12 ± 0.04^{a}	0.60 ± 0.01^{a}
Not pressure cooked ground cotyledons	16.89 ± 0.34^{c}	2.79 ± 0.15^{b}	0.68 ± 0.02^{b}
Raw cotyledons	26.78 ± 0.34^{e}	5.67 ± 0.02^{e}	1.29 ± 0.01^{e}

Key: DM = Dry matter. SEM = Standard error of the mean.
a, b = Values in the same column with different superscripts differ significantly ($p < 0.05$).

4. Conclusion

In conclusion, combination of treatments and well-monitored fermentation process would greatly reduced if not eliminate the anti-nutrients present in jack beans and consequently bring about new products as protein sources.

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