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Nutritional Composition of *Canavalia ensiformis* (L.) (Jack Beans) as Affected by the use of Mould Starter Cultures for Fermentation

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ABSTRACT

In order to investigate the role of the individual mould strains in the improvement of the nutritional status of jack beans during natural fermentation. The part played by individual mould strains was investigated using each as a starter culture inoculum, using pressure-cooking as the mode of thermal treatment. One hundred gramme of ground, 40 min, pressure cooked samples were inoculated with the single starter cultures under aseptic condition using 1 mL of each inoculum and the samples were homogenized using a sterile scapula after which fermentation was allowed to take place under controlled environment of temperature of 30±3°C for 14 days. Physical sensory changes, fermentation parameters, proximate and antinutritional composition were determined during fermentation. The total titratable acidity of the fermenting substrates varied between 0.07% for *N. crassa* fermented substrate to 0.26% for *R. oryzae* fermented substrate. While there was a general decrease in value for the pH of the fermenting substrates from 6.6 to 5.9. Proximate analysis revealed that the protein content of substrates increased from 26.21 g 100 g^{-1} in the control to 33.51 g 100 g^{-1} with *N. crassa* as the inoculum, while the fat content decreased from 11.95 g 100 g^{-1} in the control to 1.95 g 100 g^{-1} in the substrate fermented with *A. niger*, respectively. The mineral composition showed an increase in magnesium, sodium, potassium and iron compared with the control (30.07, 18.51, 23.51 and 0.00 μg g^{-1}, respectively). The antinutrient composition revealed that both the canavanine and phytate content of the fermented substrates decreased significantly from 0.79 mg g^{-1} (control) to 0.53 mg g^{-1} (*A. niger* fermented substrate) and 5865.60 mg 100 mg^{-1} (control) to 1352.60 mg 100 g^{-1} (*A. niger* fermented substrate), respectively. Therefore, the use of single starter culture mould fermentation can be used to improve the nutritional quality of *Canavalia ensiformis* L.

Key words: Solid substrate, cultures, biochemical value, fermentation, jack beans

INTRODUCTION

In view of the fact that, supplementation of animal protein is expensive and not easily affordable for mono-gastric animals, legumes provides a viable economical alternative in developing countries (Famurewa and Raji, 2005). Legumes have long shelf life and provide more proteins, abundant carbohydrates, high fibre, low fat and possess high concentration of polyunsaturated fatty acids. *Canavalia ensiformis* (commonly known as jack bean) is, considered to be the one with the highest potential as an economic crop of all the *Canavalia* genus, in view of it's excellent

agronomic characteristics (Udedibie and Nwaiwu, 1988) requiring no staking if planted on open land. Apart from jack beans ability to establish relatively with ease many of it's unusual growth attributes include, outstanding capability of well nodulated mycorrhizal colonized for continuous growth and harsh climatic conditions. To fulfill the growing demands of plant-based proteins for humans and livestock need, research on the possibilities of employing underutilized legumes as inexpensive and elegant source of protein than the conventional sources has to be pursued. Some underutilized wild legumes adapted to adverse conditions have been explored for their nutritional advantages (Bhagya *et al*., 2006; Sridhar and Seena, 2006); but they possess some anti-nutritional factors which make their utilization very difficult, hence the need for their detoxification. Many means of detoxification has been explored but found to be expensive for the poor, fermentation as an affordable means of detoxification is being considered in this work. From the work of Gabriel *et al*. (2004), it was evidence that fermentation was effective in the detoxification of *Canavalia ensiformis* with solid state fermentation being more effective than the liquid state one. In this same study effect of different thermal treatment was considered along side with the different fermentation technique. Gabriel and Akharaiyi (2007) looked at the effect of spontaneous fermentation on the chemical composition of thermally treated Jack beans and found it to improved greatly with both bacteria and fungi playing a great role. Gabriel *et al*. (2009), considered the effect of single bacterial starter cultures on the nutritional value of *Canavalia* ensiformis using solid state fermentation. Since, mould is used in the fermentation of soy beans in the production of *Tempeh,* this study was designed to utilized the individual ability of starter mould cultures to ferment jack bean seeds. The moulds used as starter culture inoculum were intrinsic organisms with the exception of *Rhizopus oryzea* which were isolated from local rice (that is, ones isolated from the natural spontaneous fermentation of *Canavalia ensiformis).*

MATERIALS AND METHODS

Preparation of samples: The jack bean seeds used for this study were obtained in May, 2007 from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The clean healthy seeds were soaked with boiled distilled water for 3 h to remove the seed coat and rinsed in changes of distilled water. The dehulled seeds were dried at 70°C for 36 h in the drying cabinet and ground with the Marlex portable stainless grinder. The materials were stored in sterile transparent polythene bags, tightened and kept at -20°C for further usage.

Preparation of starter cultures: Cultures of *Aspergillus niger* strain MF4803, *Neurospora crassa* strain MF4806, *Penicillium italicum* strain MF4807 and *Rhizopus oryzae* strain MF4801, previously isolated from natural fermentation were used. The fungal isolates, were cultivated by streaking on malt extract agar plates and incubated at 30°C for 72 h. One colony was picked and transferred to a tube containing 10 mL malt extract broth and incubated at 30°C for 48 h. 0.1 mL of this culture was used to inoculate10 mL malt extract broth and incubated at 30°C for 36 h. This culture was centrifuged (3000 rpm, 10 min), the pellet was washed in 10 mL sterile peptone physiological salt solution (1 g peptone, 8.5 g NaCl in 1000 mL distilled water, pH 7.2), centrifuged again and redistributed in peptone physiological salt solution. This procedure achieved an inoculum containing 10^7-10^8 cfu mL^{-1}, as viable count in both malt extract agar.

Preparation of samples: A 100 grams of ground jack beans each, were pressure cooked for 40 min before inoculation. Samples were inoculated with the single starter cultures under aseptic

condition using 1 mL of each inoculum and the samples were homogenized using a sterile scapula after which fermentation was allowed to take place under controlled environment of temperature for 14 days. This was done by placing the covered transparent plastic vessel used for pressure cooking in the water bath at optimal temperature of 30±3°C. Control was set-up by pressure cooking one hundred gramme oven dried, ground jack beans for 40 min and transferred aseptically into dry sterile 1-litre beaker and covered with non-absorbent cotton wool and aluminium foil for 14 days (Njoku *et al.*, 1990).

Physico-chemical analysis: At every 48 h interval for the whole 14 days samples were taken during fermentation and analyzed for, titratable acidity and pH (AOAC, 2000).

pH determination: The pH of the samples was determined according to the method of AOAC (2000). 10 g of sample was mixed in 100 mL of CO_2-free distilled water. The mixture was allowed to stand for 15 min, shaken at 5 min interval and filtered with Whatman No. 14 filter paper. The pH of the filtrate was measured using a pH meter (Model HM-305, Tokyo, Japan).

Total titratable acidity (TTA): Ten mililiter aliquots (triplicates) were pipetted and titrated against O.1 M NaOH to phenolphthalein end-point and the acidity was calculated as g lactic acid/100.

Sensory evaluation: Sensory characteristics of the fermented were assessed by 10 trained members of the Department of Microbiology of the Fededral University of Technology, Akure, Nigeria. Physical states of the substrates were assessed for their changes in color, odour (aroma), sliminess, texture and overall acceptability during the fermentation period (Steinkraus, 1997). The panelists were instructed to sip water before and after assessing each product. The judges recorded sensory characteristics of each sample using 8 - point hedonic scale as described by Ihekoronye and Ngoddy (1985), where:

8 : Like extremely
7 : Like very much
6 : Like moderately
5 : Like slightly
4 : Dislike slightly
3 : Dislike moderately
2 : Dislike very much
1 : Dislike extremely

Each treatment was evaluated three times by each panelist.

Proximate analysis: After fermentation, all the substrates were dried in the oven at 50°C, ground, sieved and kept at -20°C pending analysis. The following proximate parameters were determined; moisture content, crude protein, ash content, crude fibre, fat and carbohydrate (was estimated by difference) (AOAC, 2000).

Mineral composition: Preparation of aqueous solution of substrates for Atomic Absorption Spectrophotometer (AAS) analysis which was used for calcium, iron, magnesium, potassium, sodium and zinc; were carried out and 2 g of the seeds samples were ashed. Fifteen mililiter of 20% v/v of nitric acid solution was added to the crucible to break up the ash. This was boiled and filtered into a 100 mL volumetric flask and then diluted to 100 mL with glass distilled deionised water (AOAC, 2000).

Anti-nutritional factors: Tannin content was determined by the method of Makkar *et al.* (1993). Phytate was determined using the modified procedure of Young and Greaves according to Oboh (2006). Canavanine content was determined by HPLC using method for gradient condition (Acamovic and D'Mello, 1990). Oxalates were determined by standard method (AOAC, 2000).

Statistical analysis: Data were analyzed according to the analysis of variance (ANOVA) procedures (Gomez and Gomez, 1984).

RESULTS

There was a significant increase in the colour change of all the substrates fermented with moulds when compared with the control. The substrate fermented with *A. niger* (3.0) had the highest scores for colour change followed by the substrates fermented with both *N. crassa* and *P. italicum* (2.8) The substrates fermented with *A. niger, N. crassa* and *P. italicum* (2.8) had the highest score for odour when compared with the control (1.0). There was a general significant change in both sliminess and texture by all the mould fermented substrates of 1.8 and 1.3, respectively (Table 1). There was a significant increase in the total titratable acidity of the substrates fermented with *A. niger* and *R. oryzae,* while the substrates fermented with *P. italicum* and *N. crassa* had a significant decrease. The highest total titratable acidity value was 0.26% for *R. oryzae* fermented substrate and the lowest value was 0.07% for *N. crassa* fermented substrate (Fig. 1). The pH value of the substrates showed a steady decrease between 6.6-5.9, with the exception of the substrate fermented with *R. oryzae* which after an initial drop to 6.1 started a steady increase (Fig. 2). Of all the substrates fermented with starter culture mould, the one fermented with *N. crassa* (33.51 g 100 g^{-1}) had the highest crude protein followed by the one fermented with *P. italicum* (29.17 g 100 g^{-1}), then *A. niger* and *R. oryzae* fermented substrates (27.75 g 100 g^{-1}). While there was a significant increase in all the crude protein compared with the control (26.21 g 100 g^{-1}), the fat content showed a significant decrease in all the starter culture fermented substrates when compared with the control (11.95 g 100 g^{-1}), with the *A. niger*

Table 1: Physical changes in Jack beans (*Canavalia ensiforms* L.) fermented with Single Mould Starter (SEM) culture

	Mean score				
Substrates fermented	Color	Sliminess	Texture	Odour	Overall acceptability
Aspergillus niger	4.5^{d}	3.0^{b}	2.5^{b}	3.9^{c}	4.2^{b}
Neurospora crassa	3.9^{c}	3.0^{b}	2.5^{b}	3.9^{c}	4.6^{d}
Penicillium italicum	3.9^{c}	3.0^{b}	2.5^{b}	3.9^{c}	4.4^{c}
Rhizopus oryzea	3.5^{b}	3.0^{b}	2.5^{b}	3.5^{b}	5.6^{e}
Pressure cooked unfermented	2.2^{a}	2.2^{a}	2.2^{a}	2.2^{a}	2.4^{a}

Means are scores of 10 judges and panelists used 8 point hedonic scale. Values with the same superscript letter(s) down a column are not statistically significantly ($p>0.05$) different

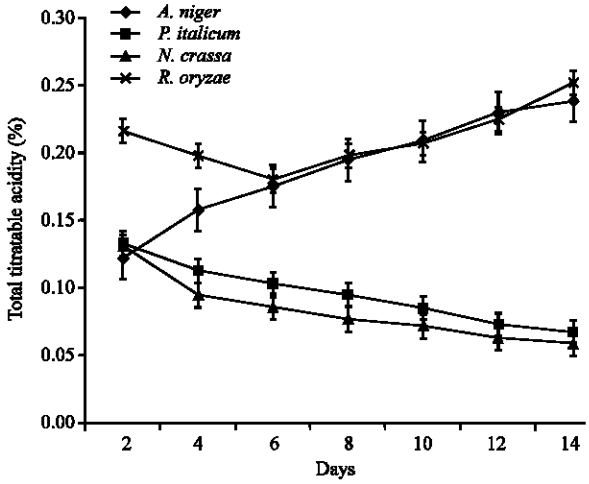

Fig. 1: Changes in the total titratable acidity of Jack beans fermented with single mould starter culture

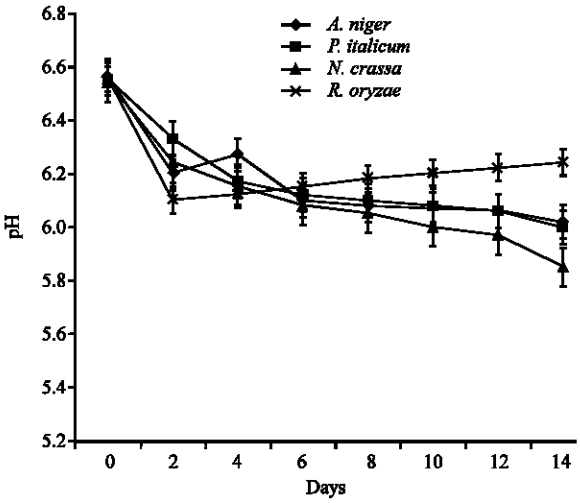

Fig. 2: Changes in the pH of Jack beans fermented with single mould starter culture

fermented substrate (1.95 g 100 g^{-1}) being the lowest. There was a significant difference in the crude fibre, with the substrate fermented with *P. italicum* (3.20 g 100 g^{-1}) being the only one significantly higher than the control (3.00 g 100 g^{-1}). The carbohydrate content for all the fermented samples, was significantly higher than the control (47.52 g 100 g^{-1}) (Table 2).

Table 2 also shows that *A. niger* fermented substrate had the highest significant increase in magnesium (112.40 μg g^{-1}), potassium (97.50 μg g^{-1}) and the lowest (0.99 μg g^{-1}) significant decrease in calcium compared to the control (magnesium-30.07 μg g^{-1}, potassium-23.51 μg g^{-1} and calcium-3.55 μg g^{-1}). The substrates fermented with both *P. italicum* (0.14 μg g^{-1}) and *R. oryzae* (0.26 μg g^{-1}) had the highest significant increase for both sodium and iron, respectively. The *A. niger* and *N. crassa* (0.53 mg g^{-1}) fermented substrates had the highest significant decrease of all the mould starter culture fermented substrates when compared with the control (0.79 mg g^{-1}) in canavanine content. In all the fermented substrates there was a significant decrease (Table 3).

Table 2: Proximate composition of jack beans (*Canavalia ensiformis* L.) Fermented with Single Moulds Starter (SEM) culture

Substrates fermented	*Aspergillus niger*	*Neurospora crassa*	*Penicillium italicum*	*Rhizopus oryzea*	Pressure cooked unfermented
Moisture (g 100 g^{-1})	2.21±0.09^{a}	6.76±0.01^{d}	3.66±0.09^{c}	2.88±0.09^{b}	7.83±0.12^{e}
Protein (g 100 g^{-1})	27.73±5.77^{b}	33.51±8.82^{d}	29.17±1.16^{c}	27.75±1.16^{b}	26.20±6.67^{a}
Fat (g 100 g^{-1})	1.95±0.06^{a}	5.18±0.02^{d}	3.05±0.09^{c}	2.37±0.06^{b}	11.95±0.12^{e}
C. fibre (g 100 g^{-1})	2.54±0.02^{b}	2.45±0.09^{a}	3.20±0.06^{e}	2.90±0.02^{c}	3.00±0.12^{d}
Ash (g 100 g^{-1})	4.89±0.09^{e}	2.88±0.02^{a}	4.35±0.06^{d}	3.11±0.09^{b}	3.50±0.88^{c}
CHO (g 100 g^{-1})	60.78±5.77^{d}	49.21±8. 82^{b}	56.57±1.16^{c}	60.99±8.82^{d}	47.52±6.67^{a}
Magnesium (µg g^{-1})	112.40±1.16^{e}	85.31±6.67^{b}	90.23±6.67^{c}	99.76±8.82^{d}	30.07±6.67^{a}
Sodium (µg g^{-1})	32.15±6.67^{b}	39.39±8.82^{c}	64.64±5.77^{e}	41.52±1.16^{d}	18.51±6.67^{a}
Potassium (µg g^{-1})	97.50±5.77^{e}	78.18±8.82^{c}	76.44±8.82^{b}	88.58±8.82^{d}	23.51±8.82^{a}
Calcium (µg g^{-1})	0.99±0.01^{a}	1.52±0.09^{c}	2.66±0.09^{d}	1.45±0.09^{b}	3.55±0.06^{e}
Iron (µg g^{-1})	0.15±0.01^{b}	-	0.12±0.01^{a}	0.26±0.01^{c}	-
Zinc (µg g^{-1})	-	-	-	-	0.14±0.12^{a}

Values with the same superscript letter(s) down a column are not statistically significantly (p>0.05) different. -: Not detected

Table 3: Antinutritional contents of jack beans (*Canavalia ensiformis* L.) fermented with Single Moulds Starter (SEM) culture

Substrates fermented	Canavanine (mg g^{-1})	Tannin (mg 100 g^{-1})	Phytate (mg 100 g^{-1})	Oxalate (mg g^{-1})
Aspergillus niger	0.53±0.07^{a}	0.26±0.07^{c}	1352.60±6.67^{a}	3.33±0.67^{c}
Neurospora crassa	0.53±0.07^{a}	0.23±0.07^{b}	1579.20±6.67^{b}	2.43±0.67^{b}
Penicillium italicum	0.56±0.06^{b}	0.54±0.07^{d}	1352.60±5.77^{a}	3.60±0.67^{d}
Rhizopus oryzea	0.69±0.07^{c}	0.19±0.06^{a}	2030.40±5.77^{c}	2.43±0.67^{b}
Dehulled raw Jack beans	0.79±0.07^{d}	0.23±0.07^{b}	5865.60±8.82^{d}	2.25±0.67^{a}

Values with the same superscript letter(s) down a column are not statistically significantly (p>0.05) different

Using *R. oryzae* (0.19 mg 100 g^{-1}) for the fermentation of the substrate showed a significant decrease in tannin content when compared to the control (0.23 mg 100 g^{-1}), while the use of *A. niger* (0.26 mg 100 g^{-1}) and *P. italicum* (0.54 mg 100 g^{-1}) showed a significant increase in tannin content. In all the starter cultures fermented substrates, there was a high significant decrease in the phytate content when compared with the control (5865.60 mg 100 g^{-1}). With the use of *A. niger* and *P. italicum* to ferment the substrate, the lowest significant value for phytate content of 1352.60 mg 100 g^{-1} was observed, while the substrate fermented with *R. oryzae* (2030.40 mg 100 g^{-1}) had the highest value for all the fermented substrates. All the moulds fermented substrates showed significant increase in the oxalate content in comparison to the control (2.25 mg g^{-1}) (Table 3).

DISCUSSION

In principle the starter cultures acted in a similar way independent of the plant material to be fermented and the cultivation time which was support by the findings of these works (Rombouts and Nout, 1995; Mbajunwa *et al.,* 1998; Amadi *et al.,* 1999; Sherfi and Hamad, 2001; Egounlety, 2003). In this study, the effect of each starter culture fermentation on the nutritional status of *C. ensiformis* L. was observed. Increase in the total titratable acidity of single starter cultures of *A. niger* and *R. oryzae* showed that secretion of some organic acids might have occurred as was found by the work of Park *et al.* (1996) and Elkhalifa (2005). The steady decrease in the total titratable acidity of *P. italicum* and *N. crassa* is an indication that there was a used up of the organic acid produced and more of proteinase activity (Nout, 1994). The general decrease of the pH of all the fermented substrates was an indication of reduced alkalinity in order to hydrolyse the

available carbohydrate to acid or the break down of protein to low molecular weight amino acids (Pederson, 1991; Caplice and Fitzgerald, 1999; Sahlin, 1999). The significant activeness of starter mould cultures in the fermentation of Jack beans for the desirable physical characteristics of colour, texture, odour and overall acceptability was in agreement with Holzapfel (1997) and Ibrahim *et al.* (2005). This work revealed an increase in crude protein which might be as a result of more active proteolytic mould's involvement and microbial biomass (Yigzaw *et al.,* 2001; Ibrahim *et al.,* 2005). The general significant decrease observed from all the controlled fermentations might be due to usage of the available lipids by the different starter culture moulds. The increase in the carbohydrate content of the fermented substrates was an indication that most of these moulds might have produced metabolites that are carbohydrates. The ash content showing significant difference was an indication that for *N. crassa* and *R. oryzae* fermented substrates, there might have been the usage of some elements while for *A. niger* and *P. italicum* fermented substrates, there might have been some released of trapped elements (Dakwaa *et al.,* 2005; El-Tinay *et al.,* 2005). The significant increase ($p \leq 0.05$) in magnesium, sodium, potassium and iron, of all the fermented substrates was an indication that these minerals were released from chelated complex compounds through the activities of these microorganisms (Gabriel 2002). The fact that calcium and zinc decreased significantly in all the fermented substrates could be an indication that the moulds might have utilised calcium and zinc for their metabolism (Hassan *et al.,* 2005). The significant decrease in canavanine and phytate in all the fermented substrates showed that these microorganisms had enzymes that are capable of hydrolyzing these antinutrients. While it was only *R. oryzae* that showed it had the capacity to hydrolyze tannin (Gabriel *et al.,* 2004).

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