EFFECT OF AERATION ON FERMENTATION OF *Mucuna jaspadae* USING *Bacillus subtilis* AS THE STARTER CULTURE

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Abstract: Various efforts are currently being directed at exploiting the vast majority of unconventional protein sources in order to ameliorate the problem of protein deficiency associated with developing communities and of such is the production of *Mucuna* beans condiments through fermentation process. The effect of aeration on the fermentation *of Mucuna jaspadae* beans using *Bacillus subtilis* at three aeration speed of 60, 90 and 120rev/min respectively for 96hours was examined. Both the physicochemical parameters and the sensory evaluations were determined after fermentation. There were observable changes in the odour of the fermented seeds with the highest score at 60 and 120rev/min, while the most acceptable change in colour, texture and sliminess was recorded at 90rev/min. An increase was observed in the pH of the fermented seeds up to the third day of fermentation at 90rev/min (6.88 - 7.76) and 120rev/min (6.25 - 7.37) before declining; while it only increased up to the second day of the fermentation at 60rev/min (6.31 - 6.81), after which it showed a reduced value on the third day but subsequently increased on the fourth day of fermentation. The titratable acidity (TTA) generally increased with fermentation time, while moisture content was observed to decrease. A TTA value of 5% observed after 24 hours of fermentation at 60rev/min was the lowest value recorded while the highest value observed was 19% recorded after 96hours of fermentation at 90rev/min. Fermentation at 60rev/min recorded the lowest moisture content of 40% while a 70% moisture content was observed for beans fermented at 90rev/min. The reducing sugar content showed a decrease after the fermentation process at 60 and 120rev/min from 1.037A to 1033A and 1.021A respectively while it was observed to increase at 90rev/min from 1.037 to 1.039 after fermentation. Locust bean *(Pakia biglobosa)* fermented with *Bacillus subtilis* was used as the standard for this experiment.

KEY WORD: Aeration, *Bacillus subtilis, Mucuna jaspadae,* solid substrate, fermentation.

INTRODUCTION

Fermentation is the oldest of all biochemical process exploited by man in food processing (Cooke *et al.*, 1987, Sasson, 1988). It essentially involves the activities of microorganisms or their product usually in an anaerobic condition to bring about changes in the properties of the fermented substrate (Wood and Hodge,

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1985). It brings about numerous biochemicals, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents, the reduction of anti-nutritional factors in legumes, grains and the synthesis of B-vitamins (Djurtoft and Nelson, 1984; Egounlety and Awoh, 1995). Benefits of fermented foods include improved nutritional value, food preservation, health benefit, etc (Johnsson *et al.*,(2011). Fermented foods constitute a significant component of diet in developing countries; some are served in the main course of meal, others as beverages while others are highly priced condiments. Although the consumption of fermented food is not only associated with developing societies but also to the developed communities. For example, fermented food such as cheese, alcoholic beverages are also important diet of the developed communities (Steinkrauss, 1983). Common legume seed such as African Locust Bean (*Pakia biglobosa*), Soya bean (*Glycine max*), melon seed (*Citrulus vulgaris*) etc are fermented to produce condiment which are important part of diets in the developing countries (Achi, 2005). Various studies has also shown that other not commonly consumed legume like the *Mucuna* species can serve as a potential source of cheap protein in developing countries if properly processed. Although Mucuna species has been established to contain anti-nutrient but there are various projections that an effective optimization of various fermentation conditions involved in the fermentation of *Mucuna* may aid its detoxification to a level safe for consumption. *Mucuna* species like other not commonly consumed legumes exhibit relatively high tolerance to a number of abiotic stress including drought, low soil fertility, high soil acidity etc, therefore they can be easily cultivated although they poses spines on their pods which has itching effect on human skin (Lattwell, 1990). Like most legume, *Mucuna* has the ability to fix atmospheric nitrogen and can also serve as cover crops thereby improving soil fertility. In lieu of the aforementioned reasons, fermented *Mucuna* species is therefore a potential source of economical food product to complement protein deficiency in developing countries if properly harnessed. This work therefore seek to study the effect of aeration on the controlled fermentation of locally available *Mucuna jaspadae* in order to determine if aeration affects its fermentation and to subsequently determine the optimum aeration condition that yielded the best product.

MATERIALS AND METHOD

SAMPLE COLLECTION

The *Mucuna* beans used were obtained from the pods of *Mucuna* collected from Akungba forest and farmland in Akungba-Akoko, southwest, ondo state, Nigeria. The beans were identified at the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo state, Nigeria, to be *Mucuna jaspadae*. Likewise the *Parkia biglobosa*, (locust beans) used were obtained from the farmlands in Akungba-Akoko community.

STATER CULTURE

The starter culture used for the fermentation was part of the pure cultures isolated from previous fermentation of *Mucuna* bean labeled BA-081002 (Gabriel-Ajobiewe *et al.*, 2011). The isolated cultures were then

characterized and identified through Gram staining reaction, sugar fermentation, starch hydrolysis, casein hydrolysis, catalase, motility and indole test. The organisms were subsequently identified through the aid of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Bacillus subtilis, isolates was subsequently activated and harvested by sub-culturing them on Nutrient agar (LAB M, Lancashire, UK) for 24 hours. The young cultures were then inoculated in a sterile broth of Nutrient broth (NB) and incubated for 18h at 35^0C. The cells were centrifuge at 4000 rpm for ten minutes with the sediment re-suspended in 5ml sterilized recovery diluents bottles of normal saline and peptone water (BIOTEC, Suffolk, UK); until a viable turbidity population of 10^8cfu/ml comparable to that of the 0.5 McFarland standards was obtained. The harvested cells were kept in the refrigerator at -8°C until when needed.

FERMENTATION

The *Mucuna* used were dehulled by boiling in water for 2 hours and subsequently hand-dehulled. The dehulled beans were soaked in water for three days with the water decanted daily to reduce the L-Dopa constituent of the seed. The locust bean seed used was dehulled by boiling in water for 8 hours and subsequently hand-dehulled (Gabriel-Ajobiewe *et al.*, 2011).

The dehulled seeds were fermented as follows; the seeds were pressure cooked at 121°C for 30 minutes and subsequently sterilized by washing in a 10% solution of hypochlorite. 100g of the sterilized seeds were weigh into an aluminum foil plate which has been surface sterilized by cleaning with 85% alcohol. Four medium sized holes were bored on the coverlid of the aluminum foil plate and plugged with cotton wool to prevent the influx of microorganism but allow for the entrance of air. The *Mucuna jaspadae* seed samples were inoculated with 2ml of the harvested cells of *Bacillus subtilis* (a concentration of 10^8 cfu / ml), while the locust bean was also inoculated with 2ml of *Bacillus subtilis* to serve as the standard for the fermentation process all in triplicates respectively. Fermentation was carried out in a water bath shaker at 37°C for 96 hours using three different revolutions of the shaker (60rev/min, 90rev/min and 120rev/min) respectively. Examination of samples was carried out under aseptic condition at 24h interval throughout the period of fermentation.

Bacterial count

One gram of the fermenting seed was ground and dissolved in 9ml of sterile distilled water in a test tube. One milliliter of the mixture was also taken and dissolved in another 9ml of sterile distilled water. This procedure was repeated until the sixth test tube to obtain the 10^{-6} diluents. One milliliter of the sixth diluents was inoculated using the pour plate technique with sterile Nutrient agar (LAB M, Lancashire, UK). The colony was counted using a colony counter and the subsequent result obtained multiplied by 10^6.

Physical Sensory Parameters

Sensory characteristics of fermenting samples were assessed by 10 trained members of the department of microbiology of the Adekunle Ajasin University of Akungba Akoko Nigeria. Physical states of the substrates were assessed for the changes in color,

sliminess, texture, odor and overall acceptability during the fermentation period. The panelists were instructed to sip water and to wash their hands 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: 7 – Excellent, 6 – Very good, 5 – Good, 4 – Average, 3 – Dislike, 2 – Poor, 1 – Very poor. Each treatment was evaluated three times by each panelist.

Physiochemical parameter

At every 24h interval for the whole 5 days, samples were taken during the combined fermentation and analyzed for, titratable acidity, temperature and pH (AOAC, 2000).

pH and temperature

The pH and temperature reading were taken by using portable pH meter (Hanna HI 98127 pHep®4 pH/Temperature Tester, USA). Five grams of sample was mixed in 45ml sterile water. The anode was put into beaker containing the mixture and the reading was taken when it is stable (AOAC, 2000).

Total titratable acidity

Aliquots from the fermented samples were drown with 20ml pipette into conical flasks and titrated against 0.1M NaoH. The titre values were determined with the help of phenolphthalein as the indicator. This was done in triplicates at 24h interval for the whole fermenting periods (AOAC, 2000).

Determination of moisture content

Pre-weighed clean dishes were used to dry 10g of the fermented samples each in a well ventilated oven at 105°C for 4h. The dried samples were transferred into the silica gel desiccators to cool before re-weighing. This was repeated intermittently until a constant weight was achieved for each sample. The constant (dried) weight was subtracted from the weight of the clean dish and wet sample, the loss in weight was then reported as moisture content.

Reducing sugar test

Fifty percent ethanol was prepared by mixing 50ml of absolute ethanol with 50ml of water. Two grams of the fermented seed was weighed into a mortar and ground to a fine pulp. 20ml of the 50% ethanol was added to the fine pulp and mixed. The slurry was filtered into a McCartney bottle using a What-man No1 paper. This was done for each of the samples and the filtrates were kept in the refrigerator until when needed.

The ethanol filtrate was diluted to 10^4. Out of the 10^4 dilution, each was put into test tubes. A 5% phenol solution was prepared by adding 95ml of water to 5ml of phenol. Zero point five milliliter of the 5% phenol was added to each of the test tubes and was allowed to stand for 10minutes. Then 0.5ml of concentrated sulphuric acid (H_2SO_4) was added. It was shaken thoroughly and allowed to cool. The optical density reading was taken at 490nm using a UV visible spectrophotometer (WPA, Linton Cambridge UK, type S104D No 254). The blank was prepared by adding 0.5ml of 5% phenol to concentrated sulphuric acid.

RESULTS AND DISCUSSION

There were observable changes in the physical sensory parameters (odour, colour, texture and sliminess) of the

fermented *Mucuna jaspadae* and the locust bean which served as the standard. These generally increase with fermentation time with the changes in odour reducing with the aeration speed as shown in Table 1. The change in colour, sliminess and texture was also observed to be affected by aeration with the highest change occurring at 90rev/min. The bacterial count of the fermented *Mucuna jaspadae* generally increase with the time of fermentation for both shaker's speeds of 60 and 90rev/min while at the revolution speed of 120rev/min, its' increased up to the 72nd hour after which it slightly decreased during from the 74 to 96h of fermentation as shown in Table 2. The bacterial count was observed to be highest at the fourth day of 90rev/min while it was lowest at 120rev/min. The bacterial count of the locust bean fermented with *Bacillus subtilis* which served as the standard also increased throughout the fermentation period.

Total titratable acidity generally increase with time but was higher in the fermented *Mucuna jaspadae* than in the locust bean as shown in Figure 1a and b.

The moisture content was also observed to be highest at the revolution of 90rev/min as shown in Table 3. The reducing sugar content of fermented *Mucuna jaspadae* decreases at the end of the fermentation period. This observation was not in line with the submission of Khetarpaul and Chauhan (1990), on the changes in the nutritional value of fermented foods especially for cereal fermentations. Contrary to that submission, the fermented locust beans under the same fermentation condition had an increased level of reducing sugar. Increase in pH with fermentation time was observed throughout the period of fermentation at the revolution of 60 and 90rev/min while it also increased until the 72h before it slightly decreased on the 96h of fermentation at 120rev/min as shown in Figure 2a and b. The pH of the fermented locust bean also increased throughout the period of fermentation for the three revolutions used. This is in accordance with the findings of Egounlety (2003) on the processing of velvet bean by fermentation.

CONCLUSION

This study revealed that aeration affects the fermentation process of *Mucuna jaspadae* and the locust bean which served as the standard. This study revealed that aeration affects the fermentation process of *Mucuna jaspadae* and the locust bean which served as the standard. The highest microbial load and pH was observed at the revolution of 72rev/min. This is the highest microbial activity and signifies the most favourable aeration condition used and subsequently most suitable for the optimization of the fermentation process. This position is also supported by the changes in the physical sensory parameters which were observed to be most acceptable also at the 72rev/min. The prevailing pH (6.81 - 7.76) at the different revolutions of 60, 96 and 120rev/min is suitable for the growth of the *Bacillus subtilis* used as starter culture, which according to Anchalee *et al.*, (2002) was the best range (5.5-8.5) for the proteolysis activities of most strains.

TABLE 1: SENSORY EVALUATIONS OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis*

		60 Rev/min				90 Rev/min				120Rev/min			
	HRS	Odour	Colour	Texture	Sliminess	Odour	Colour	Texture	Sliminess	Odour	Colour	Texture	Sliminess
Mucuna jaspadaea	24	3.5	2.7	2.5	3.8	4.1	3.4	2.5	4.1	3.3	2.6	2.7	4.2
	48	4.3	4.2	2.8	4.6	4.6	4.3	4.2	4.5	4.2	3.3	3.2	4.7
	72	5.0	5.3	4.2	5.5	5.1	5.6	5.4	5.1	5.3	4.2	4.5	5.4
	96	6.8	5.8	5.4	6.9	5.7	6.8	5.9	5.6	6.4	5.5	5.6	5.8
Locust bean (Standard)	24	2.9	2.3	3.2	1.9	3.2	2.8	3.3	2.1	2.6	2.1	2.8	1.7
	48	4.1	4.1	4.5	3.8	4.3	4.5	4.7	4.2	3.1	3.8	3.2	4.1
	72	5.3	5.5	5.7	4.5	5.2	5.4	5.9	4.7	3.9	4.6	4.7	4.3
	96	6.6	6.7	6.5	4.8	5.7	6.9	6.8	5.1	5.4	5.8	5.3	4.6

LEGEND: 7 – Excellent, 6 – Very good, 5 – Good, 4 – Average, 3 – Dislike, 2 – Poor, 1 – Very poor. Each treatment was the mean of all the panelists.

TABLE 2: BACTERIAL COUNT OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis*

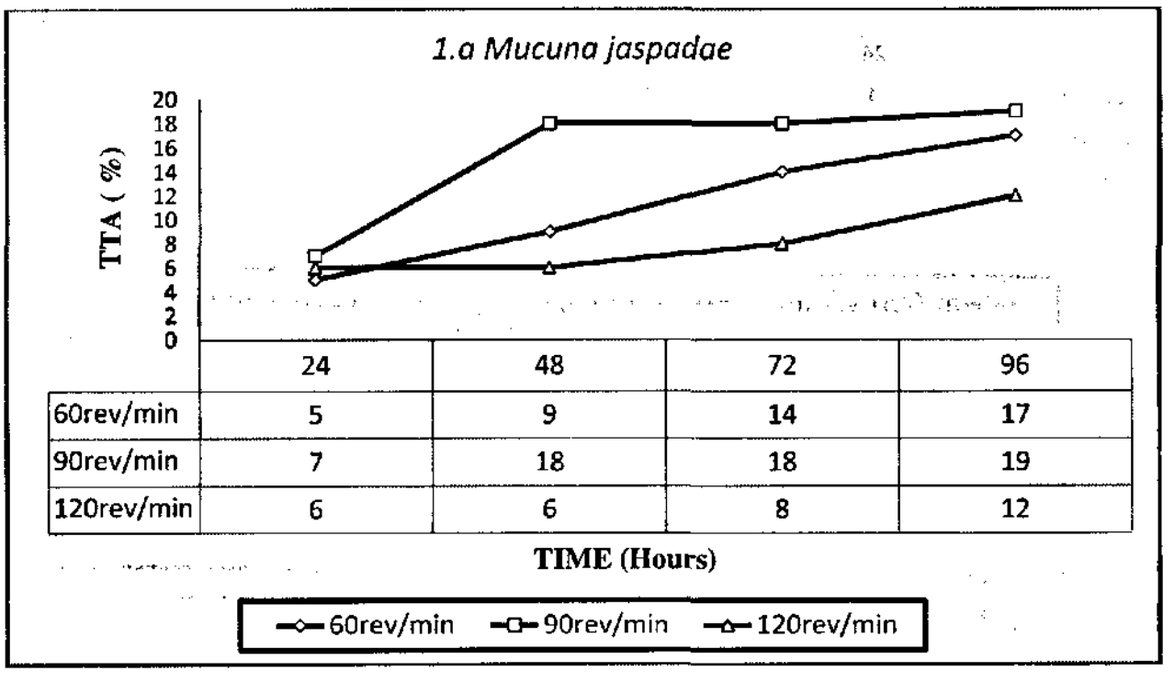
	60 Rev/min				90 Rev/min				120Rev/min			
	24	48	72	96	24	48	72	96	24	48	72	96
Mucuna jaspadae	4.20×10^{7}	5.80×10^{7}	7.20×10^{7}	8.10×10^{7}	6.10×10^{7}	6.50×10^{7}	8.50×10^{7}	8.80×10^{7}	3.20×10^{7}	4.30×10^{7}	5.20×10^{7}	4.20×10^{7}
STANDARD (Locust Bean)	5.10×10^{7}	6.80×10^{7}	8.10×10^{7}	8.80×10^{7}	7.10×10^{7}	7.80×10^{7}	8.30×10^{7}	9.20×10^{7}	4.30×10^{7}	4.90×10^{7}	6.30×10^{7}	7.10×10^{7}

TABLE 3: CHANGES IN MOISTURE CONTENT OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis*

	MOISTURE CONTENT (%)		
ORGANISMS	60 Rev/min	90 Rev/min	120Rev/min
Mucuna jaspadae	42.0	70.0	60.0
STANDARD (Locust Bean)	48.0	63.0	51.0

TABLE 4: CHANGE IN REDUCING SUGAR OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis.*

	60 Rev/min		90 Rev/min		120Rev/min	
	INITIAL (gL^{-1})	FINAL(gL^{-1})	INITIAL(gL^{-1})	FINAL(gL^{-1})	INITIAL(gL^{-1})	FINAL(gL^{-1})
Mucuna jaspadae	15523.95	15464.07	15523.95	15523.95	15523.95	15284.43
STANDARD (Locust Bean)	15523.95	15613.77	15523.95	15658.68	15523.95	15658.68



	24	48	72	96
60rev/min	5	9	14	17
90rev/min	7	18	18	19
120rev/min	6	6	8	12

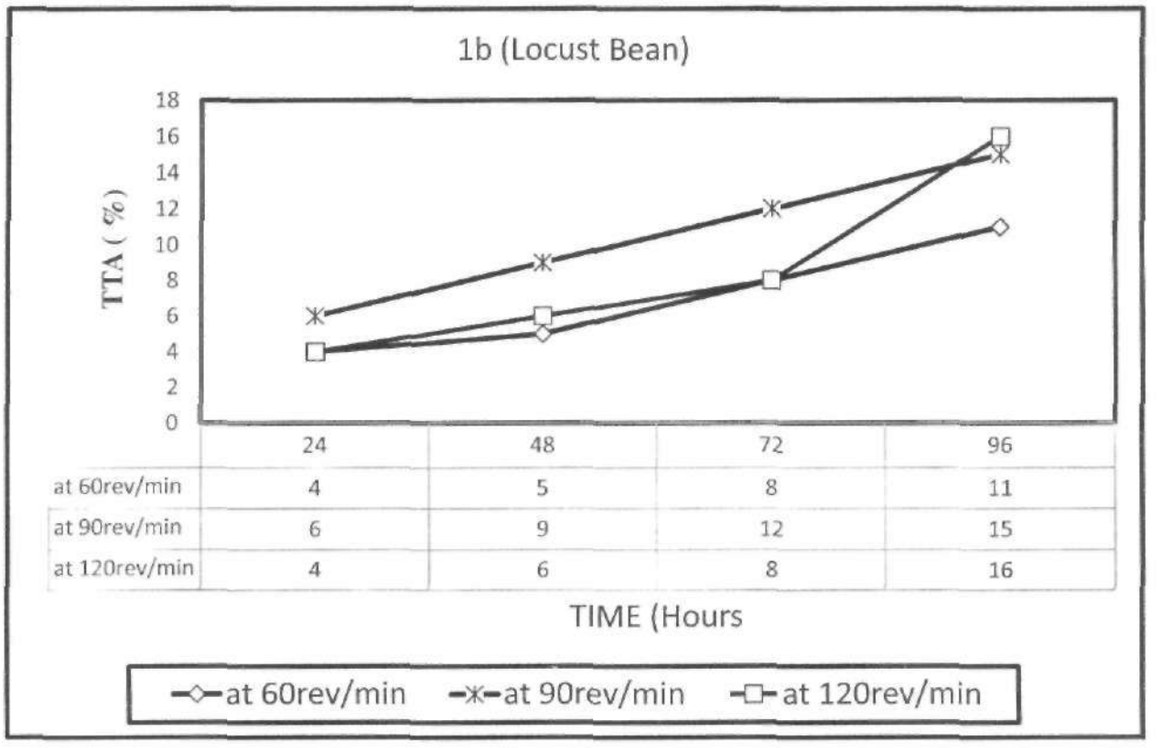
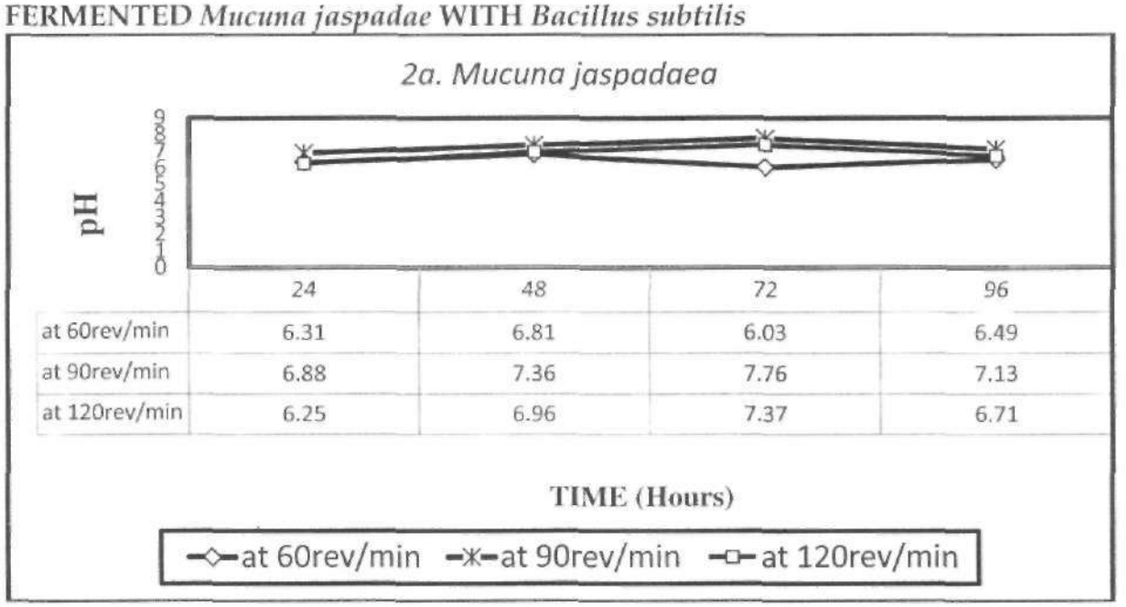
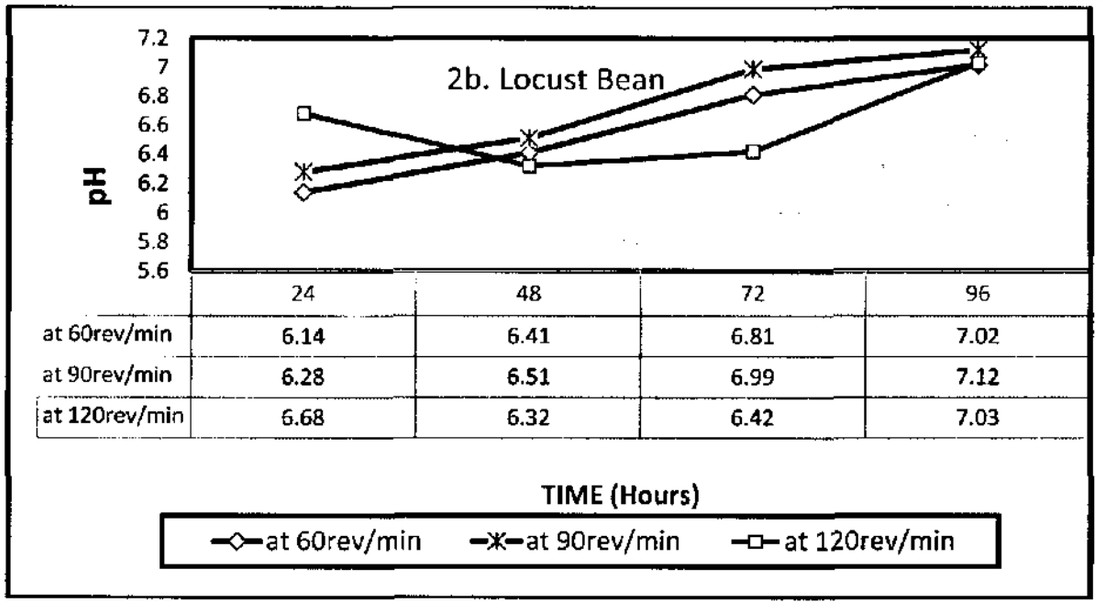


FIGURE 1: CHANGES IN TOTAL TITRATABLE ACIDITY OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis*





	24	48	72	96
at 60rev/min	6.14	6.41	6.81	7.02
at 90rev/min	6.28	6.51	6.99	7.12
at 120rev/min	6.68	6.32	6.42	7.03

FIGURE 2: CHANGES IN pH OF AERATED FERMENTED *Mucuna jaspadae* WITH *Bacillus subtilis*

REFRENCES

A.O.A.C. (2000). Official methods of analysis; *Association of Official Analytical Chemist.* Seventh Edition, Virginia USA. Pp. 12-98.

Achi, O. k. (2005). The Upgrading Of Traditional Fermented Foods Through Biotechnology. *African Journal of Biotechnology* 4(5): 375-380.

Cooke, R. D., Twiddy, D. R. and Reilly, P. J. A. (1987). Lactic Acid Fermentation as a Low Cost Means of food Preservation in Tropical Countries. *FEM (Federation of European Microbiological Societies) Microbiological Review* 46: 1194-1200.

Djurtoft, R. and Nelson, J. (1984). Increase in Some B-Vitamins during Fermentation of Tempe Produced from Cowpeas and Soybean. *Journal of Plant Food* 13: 125-136

Egounlety, M. (2003). Processing of *Mucuna* Beans by Fermentation. *Tropical and Subtropical Agroecosystem* 1: 173-181

Eqounlety, M. and Awoh, O. C. (1995). Changes on Oligosaccharides during Fermentation of Soybeans, Cowpea And Groundnut with *Rhizopus oligospores. Paper presented at the 9th world congress of food science and technology.* Budapest, Hungary July 30 – August 3, 1995.

Gabriel-Ajobiewe, R. A. O., Ifesan, B. O. T. and Thomas, D. (2011). Involvement of bacteria in the spontaneous fermentation of *Mucuna ghana* and *Mucuna rajada. Nigerian Journal of Microbiology,* 25: 2314 -2321

Holt, J. G., Krieg, N. R., Sneath, J. J, Stanly, J. J. and Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology.* Wilkins Publishers, Baltimore.

Ihekoronye, A. I. and Ngoddy, P. O. (1985). *Integrated food science and technology for the tropics.* Macmillian Publishers Ltd., London; p: 200.

Johnsson, O., Andersson, J. and Johnsson, C. (2011). Probing control in *B. licheniformis* fermentations. *In: Proc. 18th World Congress of the Int. Federation of Automatic Control (IFAC).* Milano, Italy, pp. 7132–7137.

Khetarpaul, N. and Chauhan, B. M. (1990). Fermentation of peal millet flour with yeast and *Lactobacilli;* in vivo digestibility and utilization of fermented flour for weaning mixtures. *Plant Food for Human Nutrition* 40: 167-173.

Latwell, D. (1990). *Legumes green manures, Principle for Management Based on Recent Research.* Cornell University Publishing Co. ISBN 034-3567-86-24.

Panuwan, C., Anchalee, O., Khanungkan, K., Ekachai, C. and Saisamom, L. (2002). Characterization Of Bacillus Subtilis Strain 38 Isolated From traditionally Fermented Soybean In Northern Thailand. *Science Asia 28* : 241- 245.

Sasson, A. (1988). Biotechnologies and the developing countries challenges and promises. *The Courier* 112: 97-100

Steinkraus, K. H. (1983). Lactic Acid Fermentation in the Production of Foods from Vegetable, Cereals And Legumes. *Antonie Van Leeuwenhoek* 49: 337-384

Wood, B. J. B. and Hodges, M. M. (1985). Yeast-Lactic Acid Bacteria Interaction and their Contribution to Fermented Food Stuffs; *M icrobiology of fermented foods* 1: 263-293.