Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

The Effect of Different Starter Cultures on the Protein Content in Fermented African Locust Bean (Parkia Biglobosa) Seeds

Modupe, E. Ojewumi[1], Prof. Abiodun, J. Omoleye[2], Prof. Adesola, A. Ajayi[3]
[1,2]Chemical Engineering Department,
[3]Department of Microbiology,
Covenant University Canaan land, P.M.B 1023, Sango-Ota,
Ogun State, Nigeria

Abstract: **The quality of African locust bean seed fermented with three (3) different types of starter cultures were investigated. Freshly prepared *Bacillus Subtilis*, *Saccharomyces cerevisiae* and the mixture of the two starter cultures were used for the fermentation *Parkia biglobosa* seeds for five (5) days (120 hours). The proximate composition, sensory evaluation and physiological properties of the starter culture aided and naturally fermented samples were determined.**

Sample fermented with *Bacillus subtilis* increased the protein composition from 32 % to 52 %, *Saccharomyces cerevisiae* to 39 %, the mixture of the 2 microorganisms to 35 % while 40 % was obtained in the naturally inoculated sample. Fat, moisture and ash contents also increased in the same trend. However there was a decrease in crude fibre composition and total % carbohydrate of the substrate during fermentation.

Sensory evaluation carried out for all the samples indicated that the use of *bacillus subtilis* as starter culture for the production of 'Iru' gave more acceptable products in all the tested parameters. However, products from *Saccharomyces cerevisiae* and the mixture were rated least.

Key words: Bacillus subtilis, fermentation, saccharomyces cerevisiae, starter culture, substrate, parkia biglobosa

INTRODUCTION

Parkia biglobosa is also known as African locust bean seeds. The tree is a perennial deciduous tree of the family Fabaceae and genus of *parkia.* Various parts of the tree are used for medicinal purposes. *Parkia biglobosa* tree is a perennial tree that grows between 7 meters to 30 meters in height. *Parkia biglobosa* pod is as long as 30 - 40 centimeters with up to 30 seeds embedded in it. *Parkia biglobosa* is a multipurpose tree with the bark, roots, leaves, flowers, fruits and seeds useful in traditional medicine to treat a wide diversity of complaints, both internally and externally, this can be used with the combination of other medicinal plants. In the research carried out in healer's conference in Togo, *Parkia biglobosa* was one of the highest cited plants used for treating hypertension (Karou, *et al.*., 2011).

The tree was listed as one of plants having real wound-healing properties in South-Western Nigeria (Adetutu *et al*., 2011). The use of 'Iru' in Africa dates as far back as the 14th century.

Fermentation is the chemical breakdown of substance by bacteria, yeast or other microorganism into alcohol, carbon dioxide or organic acids.

A starter culture is a microbiological culture which performs fermentation.

Parkia biglobosa is processed and fermented into a vegetable protein based condiment known as several names in African. The finished product is known as 'Iru' in Yoruba land. *Parkia biglobosa* seed is rich in protein, therefore the seed is fermented into what can serve as a cheap source of protein for low income earners who cannot afford animal protein.

There is need to apply new technology techniques like introducing starter culture deliberately into fermentation to upgrade the local methods of producing Iru. This is will guarantee the end product safety and as well as reduce variation in quality.

This work aims at getting the best starter culture for the fermentation of this proteineous seed by using two different health friendly microorganisms; *Bacillus subtilis*, *Saccharomyces cerevisiae* and the mixture of the two.

MATERIALS AND METHODS

Raw materials: African locust bean seeds were bought from retailers in Itapaji market in Ekiti state, Nigeria. The starter cultures used were prepared in the Microbiology laboratory in Covenant University, Ota, Nigeria.

Preparation of Starter Cultures

Bacillus subtilis: Previously isolated *Bacillus subtilis* was used in solid inactive form. This was activated by taking some of the bacteria with a loop and mixing it with a freshly prepared nutrient broth and incubated for 24 hours. The required concentration to inoculate 100 g of substrate was calculated.

Saccharomyces cerevisiae: Peptose dextrose sugar manufacturer guide was used to prepare the broth. A pure previously cultured *saccharomyces* was taken and fed in the homogenized solution and left at room temperature for 24 hours. The required concentration to inoculate 100 g of substrate was calculated.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

Laboratory Preparation of *parkia biglobosa* Seeds

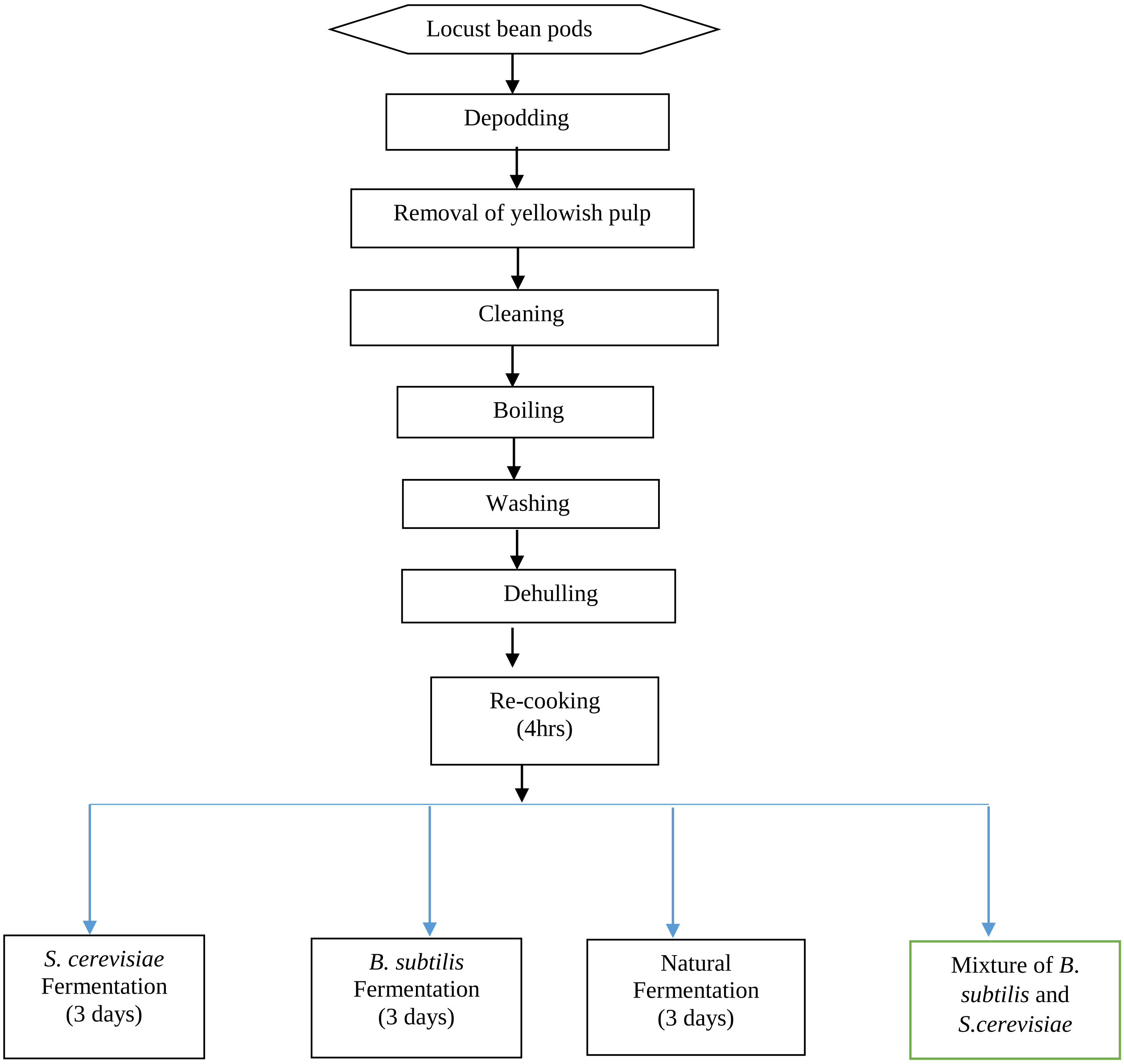

Fig. 1 - Flow diagram of traditional upgraded processing of locust bean seed to food condiment (Iru)

Microbial fermentation

100 g of the processed seed were inoculated using freshly prepared *B. subtilis, S. cerevisiae* and the mixture of the two microorganisms. Five flasks labelled Day 1 to Day 5 were placed in a thermostatically controlled fermenter and fermented for five days. At the end of each day (24 hours) a flask was removed and the sample kept in a freezer for further analysis.

pH Determination

5 g of fermented samples were weighed into 20 ml of distilled water using Unicam pH meter. The pH of each homogenate was recorded.

Chemical Analysis evaluation

The proximate composition of samples were carried out using the methods described by the Association of Analytical Chemist, AOAC, 2000.

Sensory Evaluation

10 people who were familiar with the taste of fermented African locust bean seeds were used for the organoleptic test.

RESULTS AND DISCUSSION

Figure 1 shows the pH of samples while figure 2 to 5 shows the proximate compositions of both unfermented and fermented African locust bean seeds. pH values of samples ranged from 5.36, 6.03, 5.42, 5.16 to 8.70, 7.01, 8.30, 7.99, *B. subtilis*, *S. cerevisiae*, mixture and natural samples respectively.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

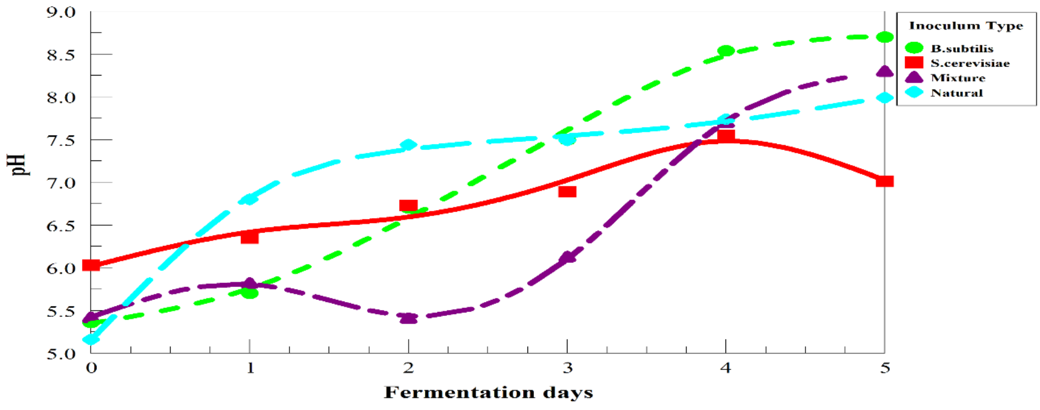

Figure 1 - pH composition of African locust bean seeds

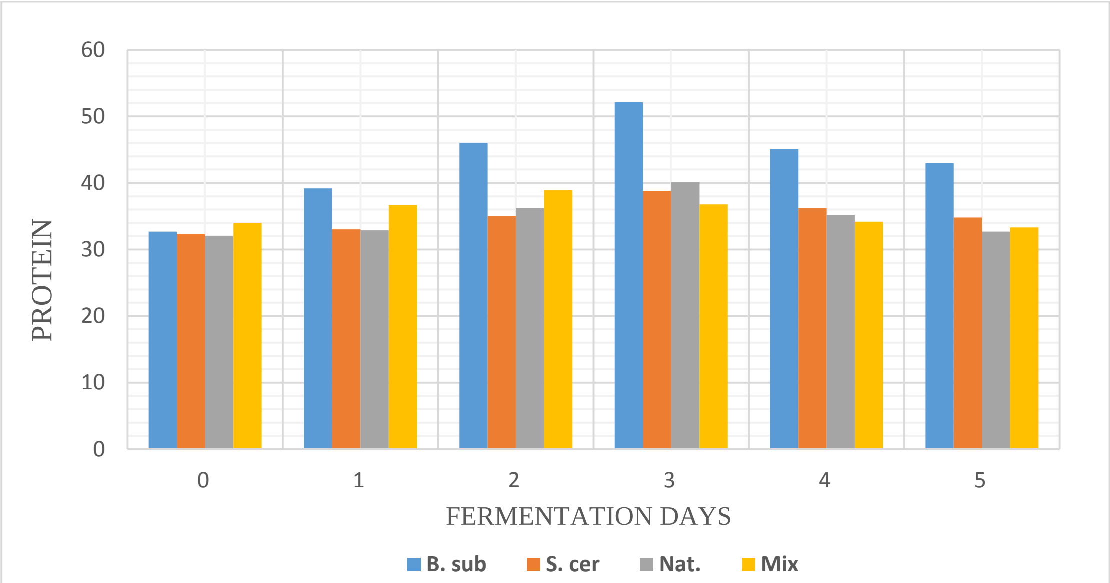

Figure 2 - The effect of using different starter cultures on Protein composition of African locust bean seeds.

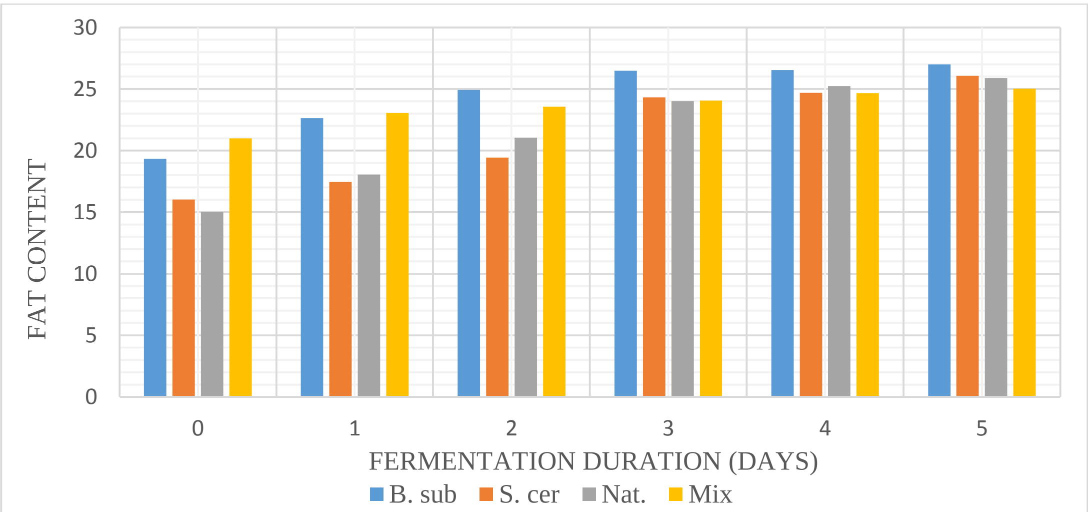

Figure 3 - The effect of using different starter cultures on Fat content of African locust bean seeds.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

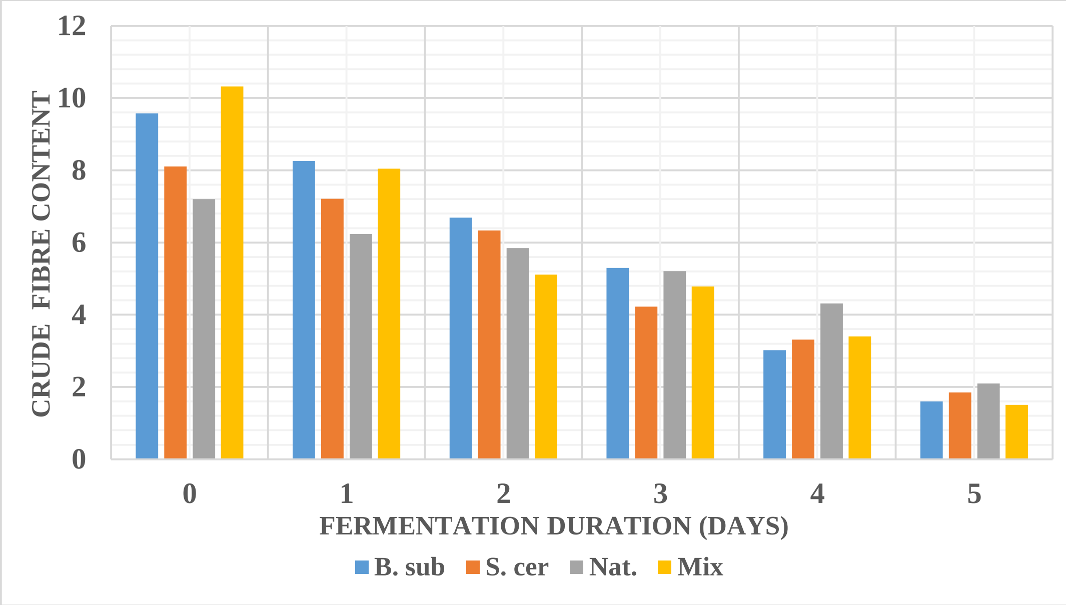

Figure 4 - The effect of using different starter cultures on Crude fibre content of African locust bean seeds

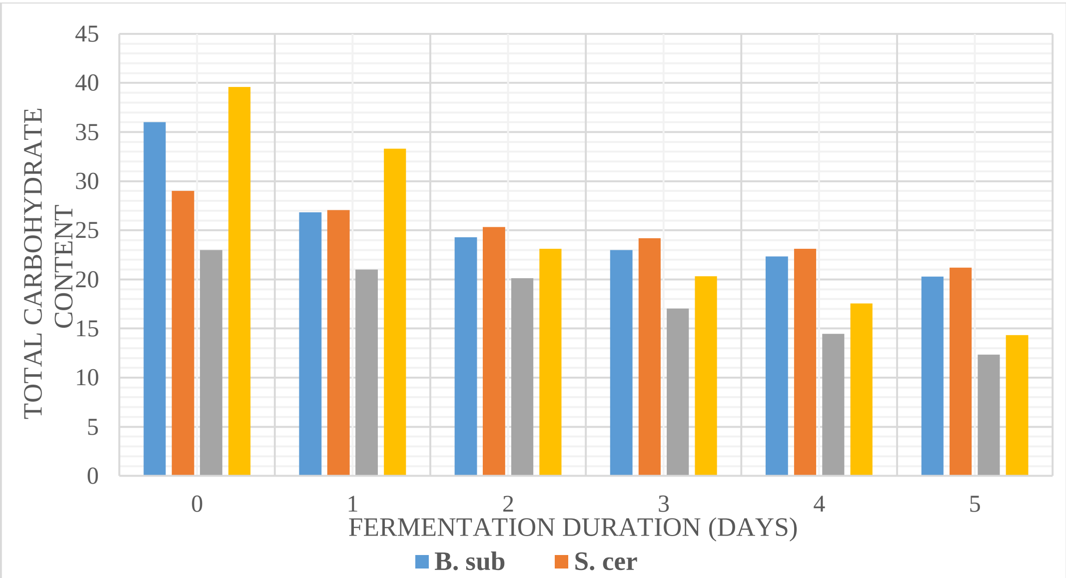

Figure 5 - The effect of using different starter cultures on Total carbohydrate of African locust bean seeds.

KEY

B.sub	-	*Bacillus subtilis*
S.cer	–	*Saccharomyces cerevisiae*
Nat.	-	Natural fermentation (Without inoculum)
Mix	-	Mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae*

Figure 2, agreed with previous work done on the selection of starter culture for the fermentation of African locust bean seeds. Various research work had been carried out on the isolation of the organism responsible for the fermentation of African locust bean seeds and *B. subtilis* had been discovered to be the dominant microorganism, Odunfa 1981a; Antai *et al*., 1986; Popoola *et al.*, 1985; Ogbadu *et al*., 1988; Sanni *et al*. 1993; Omafuvbe *et al*. 2004; Ouaba *et al*. 2004; Diawara *et al*., 1992; Odunfa and Adewuyi 1985.

The protein content increased for all the fermented samples. In the naturally fermented samples we could relate the increase in the Protein content to the fact that the natural inoculant worked together in proper ratio of right proportions since the fermentation process was not aided. This could be related to microorganism synergism. In the mixture of *B.subtilis* and *S.cerevisiae* lower yield was noticed, this could be due to microorganism antagonism (antibiosis) whereby, there is inhibition of metabolites caused by fermenting microorganisms as they compete for the substrate active site.

In figure 3 the sample fermented with *B.subtillis* had the greatest fat content, followed by the mixture of *B.subtilis* and

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

S.cerevisiae, this was possible since *B.subtilis* dominate any environment it's found. Although an increase was noticed in all the inoculum used. The Fat content was almost the same for the four inoculum variation in the third day of fermentation except for the sample with *B.subtilis*.

Figure 4 shows that fermented mixture of the inoculum had the highest Crude fibre, followed by the *B.subtilis* fermented sample. All other samples later decreased progressively with fermentation duration. Fermentation reduced the Crude fibre with respect to time. *S.cerevisiae* followed the same trend as in *B.subtilis*. The Boiling, de-hulling and soaking of the African locust bean seeds during processing was responsible for the reduction in crude fibre, which further decreased by fermentation.

Figure 5 shows a progressive decrease in the total carbohydrate with fermentation duration. Initially, the sample with mixed inoculum had the highest total carbohydrate content, this may be due to the presence of two microorganism which worked against themselves to hydrolise starch into simple sugars, thereby slowing the process down since little or no conversion was taking place. Boateng *et al*., 2014; Nitschke *et al.*, (2006) and Rolfe (2000) reported that all the species of *bacillus* produces several enzymes during fermentation which includes amylase, glucosidase and galactanase which are capable of degrade carbohydrates into simple sugars which are been used by the microorganism as energy source and for metabolic activities.

Comparison of optimal protein compositions

Since fermented African locust bean seeds is consumed for the high protein content and health benefits embedded in it. This study used the percentage protein composition which is the highest composition discovered in the fermented seed to determine the number of days it should be carried out and the microorganism to be used.

Literature confirmed that raw unfermented African locust bean has about 32 % protein content which was supported by this work and others such as Omodara *et al*., 2013; Ajala *et al*., 2013; Soetan *et al.*, 2014 and Omafuvbe *et al*., 2004.

Figure 6 shows that at the third day *B.subtilis* gave the highest yield of protein.

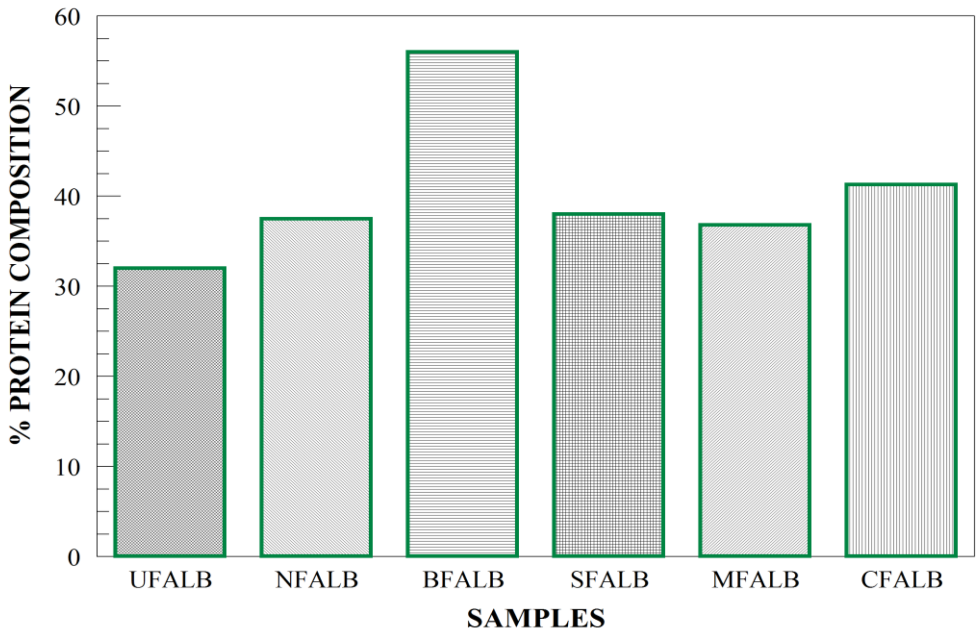

Figure 6 - The comparison of the Protein content in different fermented samples

KEY

UFALB – Unfermented African locust bean
NFALB – Naturally fermented African locust bean
BFALB - *B.subtilis* fermented African locust bean
SFALB - *S.cerevisiae* fermented African locust bean
MFALB – Mixture of Inoculum fermented African locust bean
CFALB – Commercial fermented African locust bean (Bought from the Market)

PHYSIOLOGICAL ANALYSIS

Colour and Aroma: The processed raw seeds had a normal mild smell with a creamy brown colour. The starting sample was a fine creamy brown coarse with a beany aroma substrate which translated to a more brown substrate and finally to a dark substrate with fermentation duration.

Different flavours/Aroma were noticed during fermentation which can be attributed to the breaking down of some compounds and the formation of microbial metabolites. Ammoniacal flavour was also noticed, this is because the fermentation process resulted from the alkaline degradation of protein component of the seeds by microorganisms during fermentation, Ogueke *et al.,* 2010 and Nwokeleme *et al*., 2015. Other aroma can also come from the breaking down of fat and other available compound since the seed is rich in fat and its compositions increased with fermentation duration.

The colour of the four samples studied in this work were all liked by the assessors. Samples fermented with *B. subtilis* were most preferred (score approx. 9.0), followed by *S.cerevisiae*. Only the taste of 2 samples (*B.subtilis and S.cerevisiae*) were liked (approx. 8). The mixture and natural were neither liked nor disliked (approx. 5).

Texture: The texture of all were liked.

Taste: Sample fermented with *B.subtilis* scored highest in the taste (approx. 9). The mixture of *S.cerevisiae* was neither liked nor disliked.

Overall Acceptability: The samples fermented with *S.cerevisiae* were neither accepted nor rejected (score approx. 5) in the overall acceptability test while the sample fermented with *B. subtilis* received moderate acceptance (score = 7). The remaining 2 were only slightly accepted (score approx. 6). Considering the difference between the commercially and laboratory produced Iru samples analyzed, commercially produced scored 5, with preferred flavor by 5 from some of the assessors. The sample fermented with *B. subtilis* were most accepted among the samples studied including the control. Thus the fermentation of African locust bean with *B.subtilis* for 3 days (72 hours) could be recommended as a new technology for a consistent end product.

CONCLUSION

This work concluded that *Bacillus subtilis* should be used for the fermentation of African locust bean seeds in order to get a higher yield of protein. With the use of *Bacillus subtilis* as starter culture in the fermentation of *Parkia biglobosa* seeds the problem of inconsistency in the end product will be solved.

REFERENCE

[1] Achi, O. K. (2005).The upgrading of traditional fermented foods through Biotechnology. African J. of Biotechnology, 4(5), 375 - 380.
[2] Achi, O. K. (2005). Traditional fermented protein condiments in Nigeria.African J. of Biotechnology, 4(13), 1612 – 1621
[3] Adetutu, A., Morgan, W., & Corcoran, O. (2011).Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria. Journal Ethnopharmacology, 137(1), 50-56. Retrieved November 13, 2013, from the PubMed database
[4] Ajala, C. A., Etejere, E. O. & Olatunji, T. L. (2013). Determination of protein content in the two types of *Parkia biglobosa* (fermented and unfermented) seeds and seedlings. Albanian J. of Agric. Sc., 12(4), 711 – 713
[5] AOAC, 2000. Association of Official Analytical Chemists. Official Method of Analysis. 17th Edn, Gaithersburg, USA.
[6] Antai, S.P., Ibrahim, M.H. (1986). Microorganisms associated with African locust Bean (*Parkia filicoidea* Welw) fermentation for dawadawa production. J. of Appl. Bacteriol., 61: 145-148.
[7] Boateng, M., Okai, D.B., Frimpong, Y.O., Asabere, C.O. (2014). A comparative study of the nutritional and microbial profiles of the raw and processed seeds of theAfrican Locust Bean (*Parkia biglobosa*). Livestock Research for Rural Development, 26, 178
[8] Diawara, B, L. Sawadogo, W.K.A. Amoa Amuwa, and M. Jakobsen, (1992). "(HACCP System for ttraditional African Fermented Foods: Soumbala". Taastrup: WARO, Danish Technology Institute.
[9] Karou, S., Tchacondo, T., Tchibozo, M. D., Abdoul-Rahaman, S., Anani, K., Koudouvo, K., *et al.* (2011). Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo. PharmBiol., 49(12), 1286-1297. Retrieved November 13, 2013, from the PubMed database.
[10] Nitschke, M. and Pastore, G. M. (2006). Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. Bioresource Technology, 97 (2), 336-341
[11] Nwokeleme, C.O. & Obeta, U. J. (2015). Evolution of volatile flavor compounds during fermentation of African oil bean (Pentaclethra macrophylla Benth) seeds for 'Ugba' production. International J. Food Science.
[12] Odunfa, S. A. (1981a). A note on the microorganisms associated with the fermentation of African locust bean (*Parkia filicoidea*) during Iru production. J. Plant Foods, 3, 245-250.
[13] Odunfa, S. A. and Oyewole, O. B. (1998). African fermented foods In Microbiology of Fermented Foods. Wood, B.J.B. 713-746.
[14] Odunfa, S.A. and Adewuyi, E. (1985). Optimization of process conditions for fermentation of Africa locust bean, effect of time, temperature humidity. Food Chem. Microbio. J., 9, 118-121.
[15] Ogbadu, C.O., Okagbue, R.N. (1988). Bacterial fermentation of soybean daddawa production. J. of Appl. Bacteriol., *65,* 353-356.
[16] Ogueke, C.C., Nwosu, J.N., Owuamanam, C.I., Iwouno, J.N. (2010). Ugba, the Fermented African Oilbean Seeds; its Production, Chemical Composition, Preservation, Safety and Health Benefits. Pak. J. Biolog. Sc., 13, 489-496.
[17] Omafuvbe, B.O., Olumuyiwa, S. Falade, B. A., Osuntogun, Steve, R.A., & Adewusi. (2004). Chemical and Biolochemical changes in African Locust Beans (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pak. J. Nutr., 3(3), 140-145
[18] Omafuvbe, B. O., Shonukan, O., & Abiose, S. (2000). Microbiological and biochemical changes in the traditional fermentation of soybean for soy- daddawa -a Nigerian food condiment. J. Food Microbiol., 17: 469-474.
[19] Omafuvbe, B.O., Olumuyiwa, S. Falade, B. A., Osuntogun, Steve, R.A., & Adewusi. (2004). Chemical and Biolochemical changes in African Locust Beans (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pak. J. Nutr., 3(3), 140-145.
[20] Omodara, T.R. & Aderibigbe, E. Y. (2013). Effects of the starter culture on the quality of fermented *Parkia biglobosa*. Inter. J. of Bio-Technology, 3(4), 33 – 40.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

[21] Ouoba, L. I. I., Diawara, B., Amoa-Awua., Traore, A. S., Moller, P. L. (2004). Genotyping of starter cultures of *Bacillus subtillis* and *Bacillus pumilus* for fermentation of African locust bean (*Parkia biglobosa*) to produce Soumbala. Inter. J. of Food Microbiol., 90, 197 – 205.

[22] Popoola, T.O.S., Akueshi, C.O. (1985). Microorganisms associated with the fermentation of soybean for the production of daddawa (a condiment). *Nigeria Food Journal.* 2, 194 - 196.

[23] Rolfe, R.D. (2000). The role of probiotic cultures in the control of gastrointestinal health. J*ournal of Nutrition*, 130: 396S-402S.

[24] Sanni, A. L. (1993). Biochemical changes during production of Okpehe, a Nigeria Fermented food condiment. Chem. Microbiol. Techno. Lebensm, 15, 97 – 100.

[25] Soetan, K.O., Akinrinde, A.S and Adisa, S.B. (2014): Comparative Studies on the Proximate Composition, Mineral and Anti-nutritional Factors in the Seeds and Leaves of African Locust Bean (*Parkia biglobosa*). Annals of Food Science and Technology 15(1):7