



# EFFECT OF STORAGE PERIOD ON FERMENTATION POTENTIAL OF BACILLUS SUBTILIS A2

Ibrahim, T.A., Agbaje, R.B., Orungbemi, O.O. and Ajimuda, A.M  
Department of Food Science and Technology,  
Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria

**Abstract - *Bacillus subtilis* A2 stored over a period of time (15, 30 and 45days) was used as starter culture to ferment African locust bean seeds to produce iru. The proximate composition and the sensory attributes of the fermented African locust bean seeds (iru) were determined using standard methods. The result of proximate composition showed that the protein value ranged from 38.54 – 41.63%, ash (2.32 – 3.38%), fibre (4.43 – 5.53%), fat (18.78 – 19.62%), moisture (11.74 – 12.00%) and total carbohydrate (by different) (20.00 – 22.97%). The results of the proximate composition revealed that the storage period had little or no effect on the fermentation potential of the starter culture when compared with the control with 24 hours old starter culture. The sensory evaluation of all the samples indicates that the use of the starter culture for the production of iru gave production that is acceptable in all the tested parameters.**

## I. INTRODUCTION

Fermented vegetable protein seeds used for condiments constitute a significant proportion of African diets where they serve as flavoursome and culinary components in various dishes [Achi, 2005]. The vegetable protein seed used in production in West

Africa include African locus bean (*Parkia Biglobosa* (Jacq. Benth)), melon seeds (*Citrullus vulgaris* (schrad)), castor oil seeds (*Ricinus communis*). Fluted pumpkin seeds (*Telfaria occidentalis*), African yam bean (*Stenophyllis stenocarpa*), cotton seeds (*Gossypium hirsitium* (L.)), roselli seeds (*Hibiscus sabdariffa*) and baobab seeds (*Adansonia digitata* (L.)) [Onoba *et al.*, 2008, Parkouda *et al.*, 2010]. They include iru or dawadawa in Nigeria and Ghana; soumbala, bikalga, and maari in Burkinafaso; afitin and sonru in Benin Republic; netetou in Senegal; kinda in Sierra Leone; dawadawa botso in Niger

Republic; datou in Mali; mbuja in Cameroon and furundu in Sudan [Parkouda *et al.*, 2010].

However, the vegetable seeds used for the production of these condiments differ considerably from one region to another [Achi, 2005]. Nonetheless, the production process for iru are generally characterized by spontaneous solid substrate alkaline fermentation with increase in pH because of extensive hydrolysis of the proteins into peptides, amino acids and ammonia, which favours the dominance of *Bacillus* spp. As the fermenting organisms [Kurs *et al.*, 2000., Sarkar *et al.*, 2020]. The production of iru locally is also carried out in exclusive uncontrollable environmental conditions thereby yielding products with variation in quality and organoleptic properties [Sanni, 1993 and Steinkraus, 1997]. This necessitates the need for the development of appropriate starter cultures to initiate fermentation for the production of consistent products with acceptable qualities.

The processing operations of African locust bean to produce iru involve cleaning and sorting, boiling, dehulling, and fermentation ambient temperature [Odunfa, 1985]. Investigations on the microbiology of iru using selective isolation and cultivation based on traditional culture-dependent methods including the biochemical changes that occur during fermentation have been documented [ Odunfa, 1985, Omafube, 2004]. These techniques often fail to account for minor microbial populations; stressed and injured cells that are present in low number [Kesmen *et al.*, 2012] and have been observed not to give a complete representation of food microbial communities [ Kesmen *et al.*, 2012]. Also, they are bias, unreliable and lack accurate detection of population dynamics and microbial diversity [Amann *et al.*, 1995, Jany and Barbier, 2008]. To determine the effect of storage period on the fermentation potential of *Bacillus subtilis* A2 .



**II. MATERIALS AND METHODS**

**Source and Preparation of Locust bean Seeds for Fermentation**

The African locust bean seeds (*Parkia biglobosa*) used for the laboratory study was bought from Oja Oba, in Ado Ekiti, Ekiti State. The reagents and equipment employed in the process of production and analysis of the iru was obtained from the laboratory of Microbiology Laboratory of Department of Food Science and Technology, Rufus Giwa Polytechnic, Owo, Ondo State. The *Parkia biglobosa* seeds were weighed, boiled and dehulled to wash off the testa. The seeds were steamed again with little water and then drained aseptically.

**Preparation of Starter Cultures**

Previously isolated *Bacillus subtilis* A2 was obtained from the Department of Microbiology, Ekiti State University, Ado Ekiti activated by taking some of the bacteria with a sterilized wire loop and mixing it with a freshly prepared nutrient broth and incubated for 15, 30 and 45 days before being introduced into the fermenting medium for iru production. A 24 hours old culture serves as control.

**Proximate Composition**

**Results and Discussion**

**Table 1: Proximate compositions of African locust bean seeds fermented with *B. subtilis* A2**

Sample	Protein	Ash	Crude fibre	Fat	Moisture	Carbohydrate
A	38.34	3.38	5.53	18.78	11.6	22.97
B	40.97	2.42	4.69	18.96	11.9	21.06
C	41.63	2.32	4.43	19.62	12.00	20.00

Key: Sample A: African Locust Bean Seeds fermented with 15 days old culture  
 Sample B: African Locust Bean Seed fermented with 30 days old culture  
 Sample C: African Locust Bean Seeds fermented with 45 days old culture

**Table 2: Sensory evaluation of African locust bean seeds fermented *B. subtilis* A2**

Samples	Colour	Aroma	Taste	Texture	Overall acceptability
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The proximate composition of samples was carried out using the methods described by the Association of Official Analytical Chemist [AOAC, 2005]. The parameters determined were crude protein, crude ash, crude fibre, crude fat and carbohydrates (by difference).

**Sensory Evaluation of the Iru Samples**

Sensory evaluation was carried out using semi trained taste panelists to assess the sensory attribute (colour, aroma, taste, texture and overall acceptability) of the produced iru. The panelists were selected randomly cutting across students and workers of the Polytechnic community which include people who are used to eating iru and those who are not used to it. The samples were presented in coded identical plates. The panelists were instructed to rate the sample for the parameters based on a 9- point hedonic scale ranging from 9-liked extremely to 1-disliked extremely. The raw scores was statistically analyze using the method described by [Ihekoronye and Ngoddy, 1995].

**Statistical analysis**

Data obtained from the study of the sensory evaluation will be subjected to Analysis of Variance (ANOVA) and the means will be separated using Fisher LSD and judged significantly different at 95% confidence level (i.e.  $P < 0.05$ ).



A	7.60	7.50	7.34	8.00	7.00
B	8.50	7.80	9.02	8.80	9.23
C	3.62	6.32	5.61	8.30	5.34

Key: Sample A: African Locust Bean Seeds fermented with 15 days old culture  
 Sample B: African Locust Bean Seed fermented with 30 days old culture  
 Sample C: African Locust Bean Seeds fermented with 45 days old culture

Table 1 showed the effect of storage period on the performance of starter culture *B. subtilis* A2 in relation to proximate composition of African locust bean (iru). The protein content ranged from 38.34 – 41.63, ash 2.32 – 3.38, crude fibre 4.43-5.53, crude fat 23.78 – 26.62 and total carbohydrate 20-22.97%. The percentage moisture content ranges from 11.00-12.00. The protein content of iru produced by the preserved starter culture could be generally considered very rich in protein (38.34 – 41-63%). The high amount of protein in the iru samples may be due to the ability of the starter culture to digest protein and change the small size peptide as well as functional properties [Awor, 2008]. Obviously, report, fermentation of legumes and cereal can increase in the amino acid content [9]. The increase in the protein content of fermented products is excepted of the fermentation organisms (especially *Bacillus* sp.) even stored over a period of time that are known to be proteolytic and possess high level of free acid. This implies that one of the desirable effects of the fermentation process is the increment in the protein content.

The increase observed in the moisture content of the fermented affirm locust bean seeds using the preserved/stored *Bacillus subtilis* A2 after fermentation is in line with the report of [Anti and Ibrahim, 1986 , Omodara ] who reported an increase in the moisture content o bambara nuts after fermentation. This result differs for the report of [Enujiugba and Badejo, 2007] who reported a decrease in the moisture content of a African locust bean after 72hours of fermentation. The crude fibre of the iru samples were relatively ions but less than 8.8% value reported for iru samples by [Enujiugba and Badejo, 2007] 10% for soybean [Omafube et al., 2004] but higher than 3.6% value reported for cowpea [Eka, 1990]. Boiling and Dehulling the African locust beans seed required it's crude fibre. This pattern agrees with the observation of [Latunde-Dada, 2000]. At the end of the boiling process, the boil water of the African locust beans was more viscours than it was at the beginning of the process. This is an indication of the

presence of mucilaginous material in the boil water, which willed explain in part the reduction of the crude fibre content of the African locust been seeds on boiling.

The ash content of the iru samples decreased with the storage time of the starter culture. The value reported for African locust bean agrees favourably with 5.1% (only for iru sample fermented with 15days stored *B.subtilis* A2 as reported by [Adebayo et al., 2010]. Boling and soaking in water and dehulling of locust bean led to a loss of about 40% ash. This mean that the percentage of the total minerals reside in the hull of the seed or leached during processing [Latunde-Dada, 2000].The fat content of the iru samples showed that lipase activity is low in fermentation African locust bean seed so some short chain fat are known to enhance the flour of food. This may have some contribution to the descended aroma that the condiment imparts in soup [Oyeyiola,1988]. On the other hand, the carbohydrate content reduces as the days of storage of the starter culture increase. The main substrate in the sample that serves as food (energy) for the fermenting organism is the carbohydrates.

Table 2 shows the sensory evaluation of African locust bean seeds fermented which preserved *B.subtilis* A2 culture. The colour ranged from 3.62 – 8.50, aroma 6.32 – 7.80, taste 5.61 – 9.02, texture 8.00 – 8.80 and the overall acceptance 5.35 – 9.23. All the iru samples produced was accepted in term of colour (except sample C) aroma, taste, texture and overall acceptability (except sample C). The work agrees with the work of [Omodara and Aderibigbe, 2013] that noted that the sensory attributes of African locust bean has been widely accepted mostly those produced by starter culture.

### III. CONCLUSION

From this work, it has been confirmed that storage had little or no effect on *Bacillus subtilis* A2



used as starter culture and the iru produced were desirable nutritionally since the process increases the nutrients. The local use of the condiment is expected to increase the intake of the essential daily allowable nutrients of the body.

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