Optimum Fermentation Temperature for the Protein Yield of *Parkia biglobosa* Seeds (Iyere)

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***Abstract*— African Locust Bean (*Parkia biglobosa*) seeds were fermented with the aid of a starter culture - *Bacillus subtilis* to a vegetable protein based food condiment known as 'Iru' at various temperatures between 40 °C and 70 °C. Fermentation was carried out for five days (120 hours). The maximum % protein content yield of 52.7 % was obtained after 3 days (72 hours) at an optimum fermentation temperature of 40 ^{0}C. Other parameters like % crude fibre, % ash content, % carbohydrate and % fat content decreased with fermentation temperature and duration. The physiological test carried out showed that fermented sample at the optimized condition were generally acceptable for all parameters tested. The Scanning Electron Microscope (SEM) result shows the effect of temperature on the morphological structure of both fermented and unfermented samples.**

Keywords*— Parkia biglobosa; starter culture; Inoculum; SEM; Bacillus subtilis

I. Introduction

The botanical name *Parkia biglobosa* was given to African locust bean tree (Igba in Yoruba land) by Robert Brown, a Scottish botanist in 1826. He described the tree as genus of flowering plants in the legume group which belongs to the sub - family *Mimosoideae and Leguminosae* (Abdoulaye, 2012). Other known species of the family of this plant are *Parkia bicolor, Parkia clappertoiana*, and *Parkia filicoidea.* These species can be fermented to produce an outstandingly rich vegetable protein based condiment which serves as good seasoning and aroma in food. *Parkia biglobosa* tree has application in both medicine and food additives to the indigenous people of Africa. The tree serves various medicinal purposes such as cure of diarrhea and hypertension since it contains a deposit of histamine which dilates the blood vessels and allows free flow of blood (Ojewumi, Omoleye and Ajayi, 2016). The extract of the bark of the tree is applied for the treatment of wound, bronchitis, pneumonia and malaria (Sherah, Onche, Mbonu, Olotu, Lajide, 2014). Fermentation is the oldest technology known to man (Omafuvbe, Olumiyiwa, Falade, Osuntogun, & Adewusi. 2004). Fermentation is the biological conversion of complex substrates such as starch or sugar into simple compounds by various microorganism such as fungi and bacteria (Eze, Onwuakor, Ukeka. 2014). Diawara, Sawadogo, Amoa AMuwam (1998) and Campbell-Platt. (1980), reported fermented African locust bean seeds to have 39 - 47 % of protein, 31 – 40 % of fat of lipid and 12 – 16 % of carbohydrate. Researchers identified *Bacillus spp*. as the main microorganism involved in the fermentation of most legumes especially *P. biglobosa*. *B. subtilis* was discovered to act the biggest role in the fermentation process (predominat microorganism) others like *B. pumilus, B. megaterium, B. licheniformis* were later discovered (Ouoba, Rechinger, Barkholt, Diawara. Traore and Jakobsen (2003). Several works have been done on the fermentation of African locust bean seeds, biochemical and physiological analysis but little or no research have been carried out on the operating temperature suitable for the optimum yield of protein in this underutilized seed.

II. Materials and Methods

A. *Raw Materials Procurement*

The *P. biglobosa* seeds used were purchased from the open market in Itapaji Ekiti, Ekiti state. All the chemicals used were of good quality and analytical grade. The Inoculum used was prepare in the Microbiology Laboratory of Covenant University Ota. Locally produced Iru was purchased from Ota market, Ogun state.

Laboratory Preparation of *Parkia biglobosa*

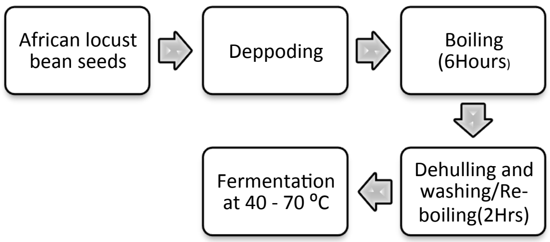


FIG. 1 - FLOW DIAGRAM OF TRADITIONAL UPGRADED PROCESSING OF AFRICAN LOCUST BEAN SEED TO FOOD CONDIMENT

B. PREPARATION OF *BACILLUS SUBTILIS*

6.25 grams of nutrient broth was disperse in 250 litre of distilled water in a sterilized 500 litre conical flask and homogenized in water bath for 40 minutes. The homogenized clear solution was autoclaved for 15 minutes at 121 ^{0}C. 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 at 37 ˚C.

C. Inoculation of Seeds

100 g of the seed sample was inoculated using 0.005 g Bacillus subtilis broth / g seed. Five flasks labelled Day 1 to Day 5 were placed in a thermostatically controlled fermenter. At the end of each day (24 hours) a flask was removed and the sample kept in a freezer for further analysis.

III. ANALYTICAL TECHNIQUE

A. Proximate Analysis of Fermented African Locust Bean Seeds

- The parameters determined were % crude protein, % total carbohydrate, % fat, % ash content and % crude fibre. These were determined using the AOAC analytical method (2000)
- Temperature Monitoring: This was done using the temperature probe fabricated with the fermenter.
- Physiological Analysis: This is majorly the physical qualities of the condiments such as taste, aroma, colour, texture and appearance.

IV. RESULT AND DISCUSSION

The seed underwent spontaneous fermentation to a protein based vegetable condiment identified by its appearance, aroma, and taste. The smell was prominent after the third day (72hours) of fermentation. The substrate became dark and soft with characteristics aroma similar to ammonia.

A. The Proximate Analysis of African Locust Bean Seeds (Figure 2)

- % Fat Content: About 17.33 % and 19.33 % of fat content was recorded in the raw and cooked samples respectively. This confirmed the report of Alabi, (2005) and Omafuvbe, Olumuyiwa, Osuntogun and Adewusi. (2004).
- Although there is an increase in the % fat content with days of fermentation at each temperature, there is a slight but steady decrease in the fat content as temperature of fermentation increases which further increased to 27 % on the last day of fermentation but decreased with increase in fermentation temperature to 20.08 % at 70 ^{0}C.

  The activity of lipolytic enzyme is faster with a slight increase in temperature during fermentation process but an elevated temperature above the optimum operating temperature of the fermenting microorganism- *Bacillus subtils* which is 40 -50 ^{0}C will either make the organism go dormant and reactivated when the condition is favourable or die.

- % Crude Protein: Several research works have been carried out on how fermentation enhances protein value of *Parkia biglobosa* seeds. Figure 2 also shows that at each fermentation temperature protein content increased from the first day up to the third day and declined from the fourth day. The % yield of protein from the fermentation of *Parkia biglobosa* at any given day of fermentation declines with increase in fermentation temperature from 40 to 70 ^{0}C. The protein yield at 40 ^{0}C on the third day of fermentation is 52.7 %, it reduced to 25.6 % on the third day at a temperature of 70 ^{0}C. This confirms the fact that *Bacillus subtilis* functions well at lower temperature (40 – 50 ^{0}C) (Odunfa, 1981; Antai, Ibrahim (1986) and Achi (2005). Thus maximum yield of protein is achieved at the third day of fermentation with fermentation temperature 40 ^{0}C. The low yield of protein at higher temperature is likely due to the fact that the enzymes responsible for fermentation and protein formed get denatured by heat at such a high temperature.
- % Total Carbohydrate (CHO): A decrease in total carbohydrate is expected (figure 2), this confirms that substrate is been consumed by the fermenting microorganisms. The loss in carbohydrate during soaking and boiling is attributed to the leaching of soluble carbohydrates like sugar into the cooking water. Loss in carbohydrate during fermentation may also be as a result of the fermenting organisms utilizing some of the sugar for growth and metabolic activities, Esenwah and Ikenebomeh (2008). The higher the temperature the lower the reaction since the fermenting microorganism cannot function well at high temperature, hence low conversion of carbohydrate to sugar.

- % Crude Fibre: Fermentation reduces the percentage crude fibre of the substrate (fermenting seed) with days of fermentation. Temperature seems to have minimal or no effect on the Crude fibre of *P. biglobosa* seeds during fermentation. An increase in fermentation temperature led to decrease in the percentage crude fibre. Figure 2 shows that protein has the highest composition at fermentation temperature of 40 ^{0}C.

Figure 3 shows the comparison between the proximate analyses of commercial Iru which is usually done at 30 ^{0}C and laboratory prepared samples. This figure shows that fermentation yield of protein at temperature 40 ^{0}C is higher than commercially produced condiment. Hence 40 ^{0}C is the optimum temperature for protein yield of *Parkia biglobosa* seeds.

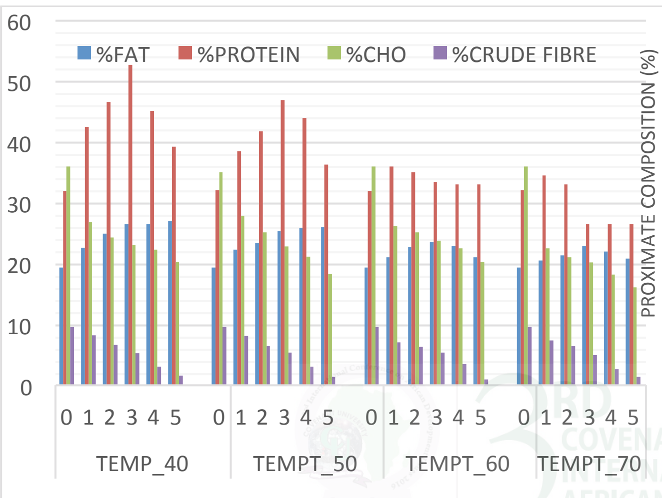


Fig. 2 - The effect of temperation variation on the Proximate analysis of African locust bean seed

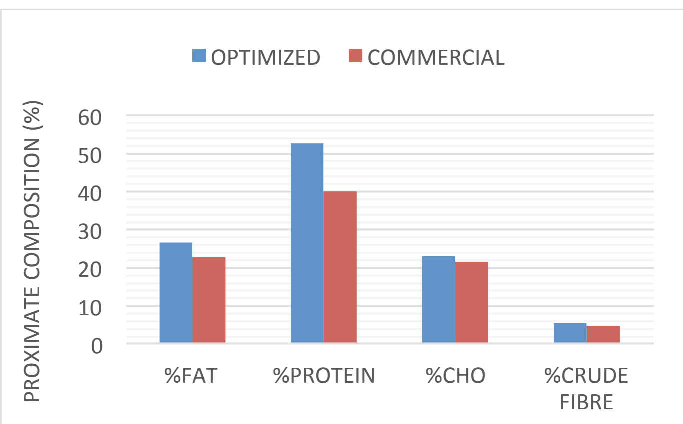


Fig. 3 – Comparison between the proximate analysis of optimised and commercial sample

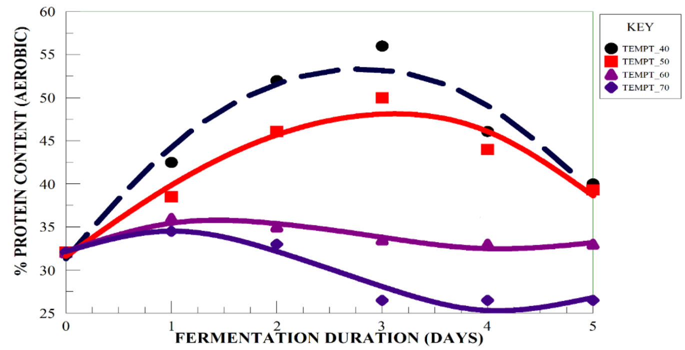


Fig. 4 – The Effect of Temperature on the Protein Content of Fermented African Locust Bean Seed

B. Physiological Test

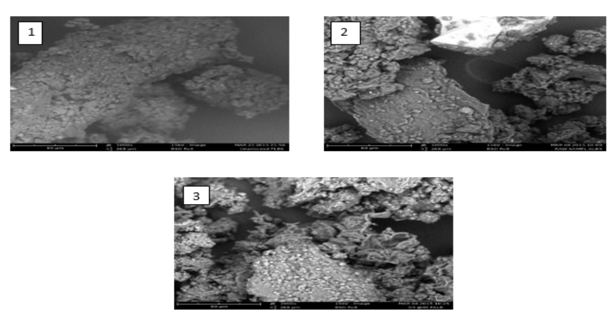
The sample fermented for 3 days with fermentation temperature 40 ^{0}C only was most acceptable with respect to taste, colour, appearance and aroma.

C. Morphological Structure

The morphological structure revealed the differences in processing and fermentation stages

1- The morphology of the unprocessed seed revealed an agglomerated cohering image with a coarse and wrinkled corrugated surface A wider pore, compared to the raw unprocessed sample was noticed. This was due to the processing which is an evidence of structural changes as a result of deformation of structure during processing.

2- Wider agglomerated and non-cohering structure with a wider pores was noticed, this was due to the introduction of heat. Compounds were been broken down into smaller units such as carbohydrates. Granules were still visible, probably protein but adhere more to the surface of the structure.



1 - The morphological structure of unprocessed African locust bean seeds

2 - The morphological structure of processed raw African locust bean seeds

3 - The morphological structure of fermented sample on the third day at 40°C.

Conclusion

The optimal operating fermentation temperature for the production of protein condiment from P. Biglobosa is 40 ^{0}C.

Since Iru was discovered to be very rich in protein, increase in its consumption will reduce the risk of nutrient deficiencies in consumers.

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