Open Journal of Engineering Research and Technology
Volume 1, Issue 1 (2016)
www.ojert.com

The Study of the Effect of Moisture Content on the Biochemical Deterioration of Stored Fermented *Parkia Biglobosa* Seeds

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Research Article **Open Access**

Abstract
The biochemical and physiological changes in the highly proteinous stored fermented African locust bean parkia biglobosa seeds were studied as well as the sensory evaluation. This study was carried out to improve the shelf life of this fermented protein based condiment, known as 'Iru' in Yoruba land. Major functional parameters were used to compare deterioration in stored fermented seeds. Bacillus subtilis was used as a starter culture and fermentation was carried out for 72 hours. Samples were dried to various moisture content. The dried condiments were stored for various days at room temperature in an air tight plastic container. At the end of each storage period, samples were and assessed for pH, titratable acidity (TA), peroxide value (POV) and % crude protein. There was an increase in pH, peroxide value and titratable acidity towards acidity, while the % crude protein decreased with storage. Total dryness of 0 % moisture content was achieved after 12 hours of drying, which made the stored condiment to last for more than 31 days.

Keywords: - *Bacillus subtilis, deterioration, moisture content, parkia biglobosa, shelf life*

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INTRODUCTION

Parkia biglobosa (African locust bean seeds) is a perennial deciduous tree that grows from 7m to 20 meters high (Teklehaimanot, 2004). The pods are flat and have irregular cluster of up to 30 seeds. Fermented African locust bean seed (Iru) is rich in minerals and provide valuable protein (Odunfa, 1983).

It is known by different names in different countries - *kinda* in Sierra Leone and Iru or dawadawa in Nigeria and Ghana (Azokpota *et al.*, 2005; Odunfa, 1981), A*fintin* and *sonru* in Benin republic (Azokpota *et al*., 2005), while in Japan it is known as *natto* (Beaumont, 2002; Azokpota *et al*., 2005). Fermented *P. biglobosa* seeds is produced by either natural fermentation or inoculated fermentation. The consumption increases in urban areas progressively, since the condiments are considered as a functional food which can regularize blood arterial pressure.

In order to improve the shelf life, a major challenge to its stability, we have carried out the effect of moisture content on its deterioration. 'Iru' has a shelf life of 2 to 3 days without additives, which implies that the producer has to dispose of the product

within three days if not consumed. The bacteria, yeast and molds responsible for deterioration need moisture for their metabolism.

Food preservation is the process by which food is been treated in order to stop or slow down spoilage such as loss of quality, edibility or nutritional value. Lack of sufficient food preservation skills is a major problem contributing to food insecurity in many parts of the world, especially Africa. The application of many advanced food preservation methods such as refrigeration, freezing, canning and irradiation have greatly reduced because of the high cost in their operations (Ibe *et al*., 2009). Food preservation is the extension of the shelf life of food, its acceptability and safety, nutritive value, economic viability and availability (Anon, 2012). This study focuses on moisture reduction as preservation method for the fermented condiment, this method is found to be very efficient.

Table 1. *Minimum Water activity requirement of microorganisms (a_w)*

Group of microorganisms	Minimum water activity of growth
Most Gram-negative bacteria	0.97
Most Gram-positive bacteria	0.90
Halophilic bacteria	0.75
Most yeasts	0.88
Osmophilic yeasts	0.62
Most filamentous fungi	0.80
Xerotolerant fungi	0.71
Xerophilic fungi	0.61
Xeromyces bisporus	0.60

Source: www2.univet.hu> feltoltott

For food to be protected from the actions or activities of microorganisms, the water activity (a_w) must be less than 0.7. When microorganisms starts to grow in food it changes the level of available moisture by releasing metabolic water which aids deterioration.

There have been a lot of reports on the methods of preservation for fermented African locust bean in other to increase its shelf life but no one ever considered total dryness through moisture content removal.

MATERIALS AND METHODS

2.1 Collection of Sample: African locust bean seeds for this study were procured from the open market in Itapaji, a town in Ekiti state. All reagents used were of analytical grade from Sigma manufacturing industry. The Broth used was manufactured by BIOTECH Laboratory Ltd. Lanwades Business Park, Kentford. The *Bacillus subtillis* used for the inoculation was prepared freshly in the Microbiology Laboratory of Covenant University Ota.

2.2 Laboratory Preparation of Iru: The seeds were processed and fermented in Covenant University, Chemical Engineering department laboratory using the fabricated isothermal fermenter.

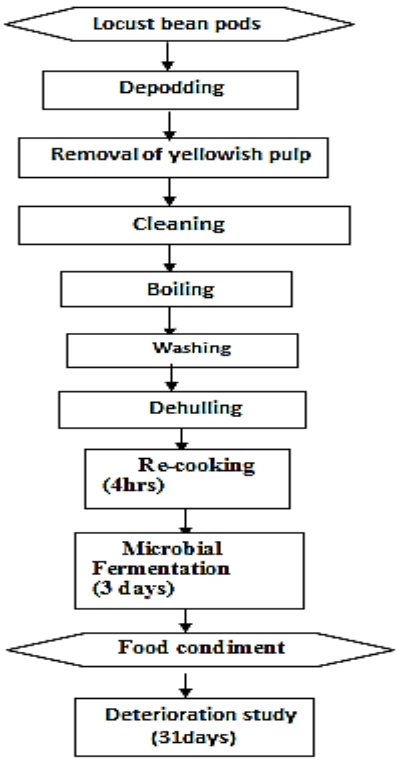

Fig. 1: *Block diagram of traditional upgrade processing of locust bean seed to food condiment (Iru)*

2.3 Preparation of Starter Culture

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.

2.4 Deterioration study and Physio-chemical analysis

Samples were taken after the third day of fermentation and dried to various percentage moisture contents. The % moisture content was calculated until constant weight was gotten at 12 hours which is 0 % moisture content. Samples were allowed to cool in a desiccator and stored in an air tight container for 30 days. Samples were taken every 5 days for analysis.

2.4.1 Determination of pH

5 grams of samples were weighted into 20 ml distilled water and the pH probe was dipped into the suspension to take the readings

2.4.2 Determination of Titratable Acidity (TA)

The remaining suspension from the determination of pH was filtered using 18 cm diameter filter paper after mixing. 2 to 3 drops of phenolphthalein indicator was added to the filtrate and titrated with 0.1 N NaOH until the pale pink endpoint, which persisted for 30 seconds was noticed.

Titratable acidity (TA) was determined twice for each sample, and the average values were calculated.

TA (g lactic acid/L) = {(N NaOH) × (mls NaOH) ×75×1.2}/ mls of sample

2.4.3 Peroxide Value Determination (POV)

A blank determination of the reagents was conducted first.

2.00 g of sample was weighed into a 100 ml glass stoppered Erlenmeyer flask. 12 ml of the acetic acid - chloroform solution was added into the flask with a measuring cylinder. The flask was Swirl until the sample was completely dissolved. It was then careful warmed on a hot plate.

1 ml Mohr pipette was used to add 0.2 ml of saturated potassium iodide solution. The flask was stooped and the contents swirl for exactly one minute.

12 ml of either distilled or deionized water was added immediately, it was stopped and shook vigorously to liberate the iodine from the chloroform layer. The burette was filled with 0.1N sodium thiosulfate. Titration was carried out slowly with mixing until the colour lightens.

A dispensing device was used to add 2 ml of starch solution as indicator. Titration continued until the blue grey colour disappears in the aqueous (upper layer).

The mls of titrant used was accurately recorded to two decimal places.

Calculations:

S = titration of sample

B= titration of blank

$$\text{Peroxide value} = \frac{(S-B)*N\ \text{thiosulphate}*1000}{\text{Weight of sample (g)}}$$

2.4.4 Determination of Percentage Crude protein

The Micro-Kjeldhal method was used for the determination of crude protein content. The methods involved three stages: Digestion, Distillation and Titration. The digestion process involves the conversion of nitrogen of the sample into Ammonium sulphate by boiling with concentrated H_2SO_4. 10 g of Copper sulphate and Potassium sulphate mixture (10 g $CuSO_4$ and 150 g K_2SO_4) was weighed into digestion flask. One gram (1 g) each of the dried samples was added to it. 20 ml of concentrated H_2SO_4 was added into each of the flasks and it was placed on the block digester in a fume cupboard for 30 minutes. It was then allowed to cool for

one hour. 80ml distilled water was added to it and the digests were allowed to cool down. The digests were transferred into the distillation set up after dilution with water. 50 ml of the digest and 50 ml 0.1 M NaOH was placed in a conical flask, 5 ml of 2 % boric acid and 5 drops of mixed indicator (0.016g methyl red + 0.083 g bromocresol green in 100ml alcohol) was added to it and it was distilled using the kjeldahl distillation apparatus. Distillation continued until 100ml of the distillate had condensed into the receiving flask. This was done for all the samples. The distillates were then titrated against 0.01 M HCl to an orange end point. The total nitrogen content of the samples was calculated using the formula;

% Nitrogen (N) = (A ×0.0014 ×100)/ W; where

A = Volume of acid used in titration,

W = Weight of sample used

% Crude Protein = %N × 6.25

Where N is the nitrogen value

2.4.5 Determination of Percentage Moisture content

Washed crucibles were oven dried at 105^0 C for 30 minutes to ensure total dryness. They were then transferred into the desiccator to cool for about 30 minutes. The crucibles were weighed on an electronic balance and the weight recorded as ($W_{1)}$. 5 g of seed sample were weighed into the dried pre weighed crucible (W_2). The crucibles and the content were oven-dried at 105 ^{0}C for 4 hours. The samples were removed from the oven and dried until a constant weight was obtained. After drying, the crucible was transferred into the desiccator to cool for about 45 minutes and weighed (W_3). This analysis was carried out in triplicate.

RESULTS AND DISCUSSIONS

Figure 2 shows the amount of moisture left after drying at 2 hours interval. 0 % moisture content was gotten at the 12th hour.

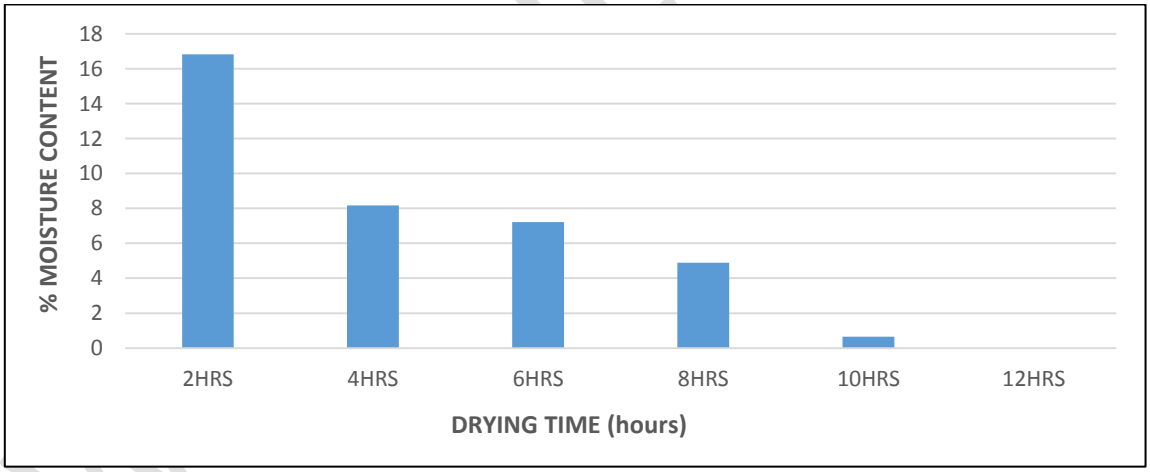

Fig. 2: *The Percentage Moisture content left after drying*

Figure 3 shows the effect of storage time on the pH of the dried fermented condiments. The more the storage days the more the pH towards acidic medium with percentage moisture content.

The more the pH the lower the risk and rate of spoilage, since microorganisms finds it difficult to strive in an acidic medium. Increase in pH towards the acidic range was recorded for drying durations 8, 10 and 12 hours while increase in pH towards alkaline medium was noticed at 2, 4 and 6 hour, this was responsible for the spoilage, as microorganism grows best in an alkaline medium. Spoilage was notice at the 15th day of all the samples with increase alkalinity why spoilage was never noticed in the samples towards acidic medium.

The increase in pH observed in this study is an indication that fermentation still continued after the processing period of African locust bean seeds to 'Iru' (post fermentation operation). This confirmed that the organisms responsible for the fermentation are still present at consumption.

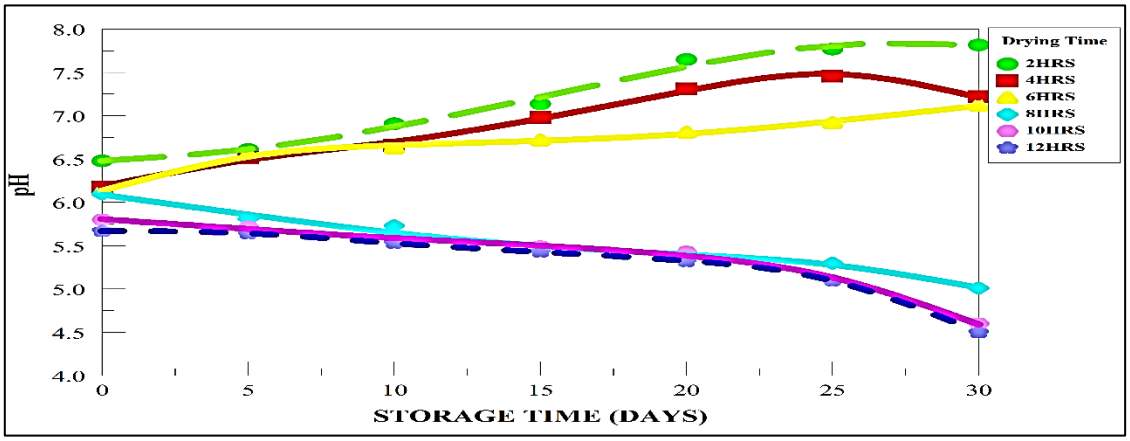

Fig. 3: *The effect of storage time on the pH of the dried fermented condiments*

Figure 4 shows the effect of storage time on the titratable acidity. A significant increase in titratable acidity with storage time was noticed, which is as a result of some acid producing processes going on during the deterioration stage. The significance of the increase in both pH and titratable acidity in stored 'Iru' is the continuation of fermentation process in storage. The simultaneous increase of the two in 'Iru' have been reported by Ikenebomeh *et al.* 1986: Wagenknecht *et al.* 1961 and Popoola *et al.*, 2007. The increase is attributed to the activities of proteolytic enzymes which takes place in the degradation of protein and the hydrolysis of carbohydrates components to sugar and organic acids.

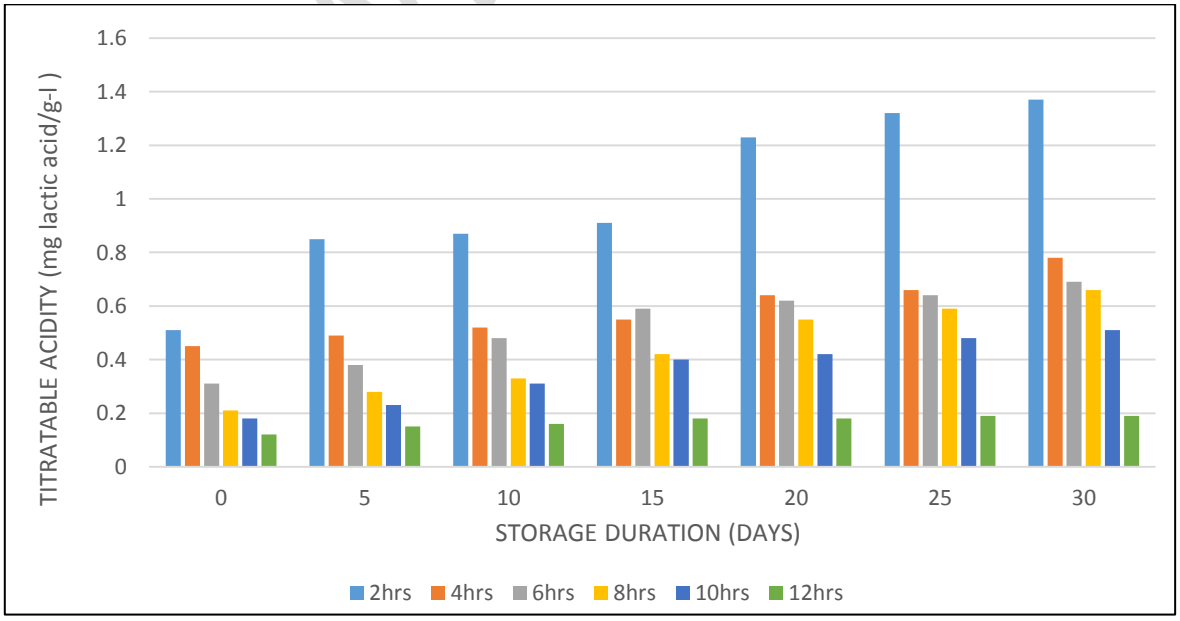

Fig. 4: *The effect of storage time on the titratable acidity (mg lactic acid/g-l)*

Figure 5 shows the effect of storage time on the Peroxide value in stored dried fermented samples of *Parkia biglobosa* seeds.

Peroxide values gives the initial evidence of rancidity in an unsaturated fats and oil. This is an indication of peroxidation in food.

Findings showed that peroxidation of stored 'Iru' increased with storage duration (Kolapo *et al.*, 2007 and Popoola *et al.*, 2007). An increase in peroxide value is an indication of fat deterioration which brings rancidity. This work reported an increase in the peroxide value of stored 'Iru', which is a good predictor that peroxidation occurred during the storage of 'Iru'. The higher the peroxide value, the more susceptible is the condiments to spoilage, which is caused by fat. Although fat acts as flavour retainer and increase mouth feel of food (Kinsella *et al*., 1976), it can also act otherwise if goes rancid. This work reported an increase in percentage fat content with fermentation days. Pearson (1985) discovered that fatty foods with peroxide value ranging from 20 - 40 meg/kg is rancid, peroxide value should be less than 10 milliequivalents/kg. Oxidation is one of the major causes of deterioration in any protein based foods since they are very rich in fat. 'Iru' becomes susceptible to oxidative deterioration due to its high concentrations of unsaturated fat which always manifests in form of discoloration, formation of toxic compounds, poor shelf life, development of off flavour, nutrient losses, respectively with storage duration (Contini *et al*., 2014; Palmieri & Sblendorio,2007).

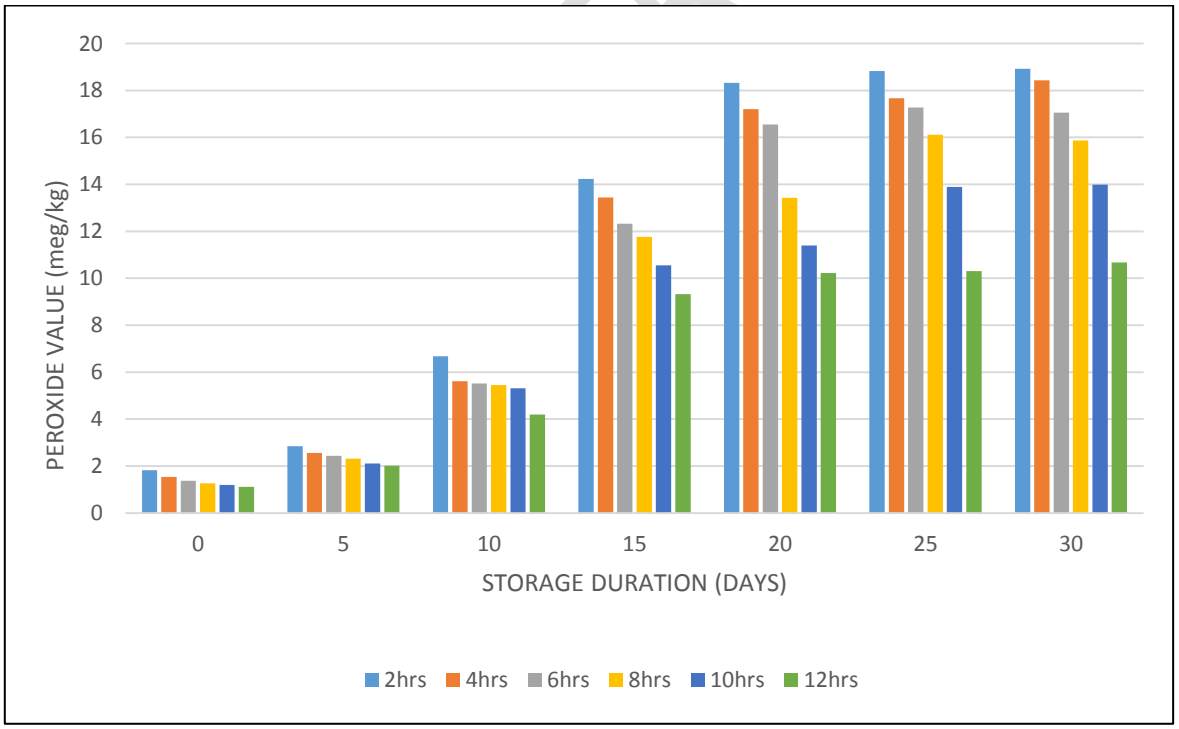

Fig. 5: *Effect of storage time on the Peroxide value*

Figure 6 shows the effect of moisture level The effect of storage days on peroxide POV The protein content increased with drying on the 0 day and later started decreasing with respect to storage days. The long hours of drying probably denatured the protein structure which led to the decrease with storage days.

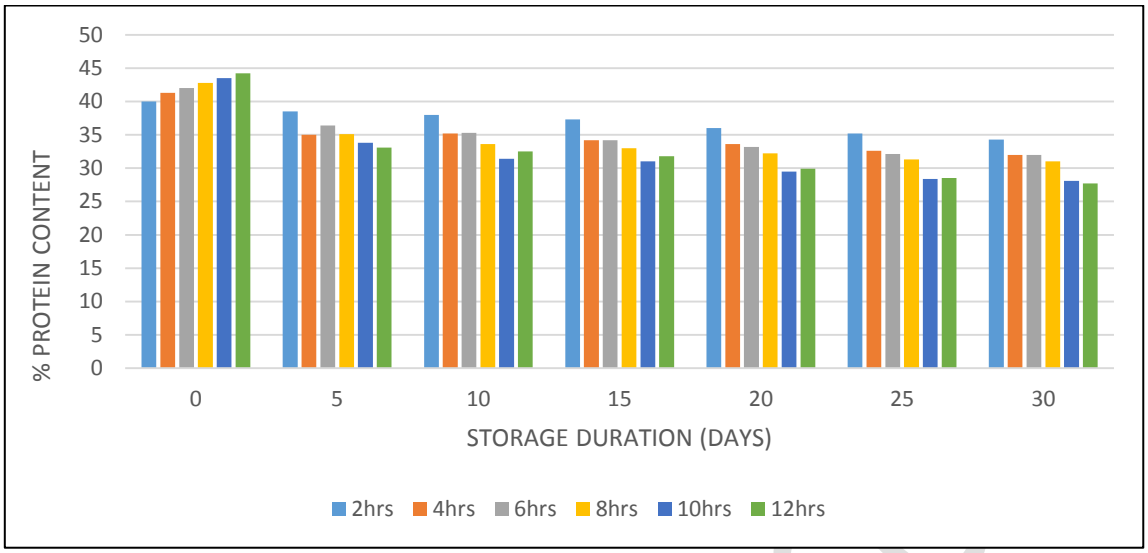

Fig. 6: *The effect of storage time on the Protein content*

The study of the deterioration in stored 'Iru' using moisture difference has never been study by any researcher. However attempt have been made in the past to prolong the shelf life of fermented African locust bean seeds and Soybean seeds with the aids of additives and other preservative, Ogbulie *et al.,* 1998 attempted packaging the processed product inside a high density polyethylene sachets and aluminium foil after treating with 2% Nacl, this method kept the product for only 8 days before spoilage sets in. Other researchers applied pasteurization at temperature of about 100 °C for 30 minutes, with the assumption that all the microorganism had been eliminated due to high heat, including the organism used for the fermentation, (Mbata *et al.,* 2008), this also lasted for 8 days. Returnable and sterilisable bottles for packing the finished products, this was able to keep the product for 4 weeks. The report in this work is therefore an improvement to all the reports on the preservation of "Iru' and will serve as a good method to local producers.

Most of the biochemical deterioration in monitored functional properties of 'Iru' were noticed on the 15th day of storage for the samples with high pH range towards alkaline medium, 20th day for sample dried for 4 hours , while they were never noticed in the samples with lower pH towards acidic medium. Although physiological analysis varies, totally dried sample had an improved (longest) shelf life of up to 9 months.

3.1 The physiological analysis for the deterioration

The colour of unfermented processed African locust bean seed is creamy brown with a characteristic flavour of bean.

Deterioration was noticed in the fermented samples dried for 2 hours on the 10th day while it was noticed for the sample dried for 4 hours on the 20th day.

Fermented sample dried for 6 hours went rancid on the 25th day while all the samples dried from 8 – 12 hours never experienced maggot infestation for the 30 days of monitoring. A darker colour was noticed for the samples dried at higher temperatures.

CONCLUSION

This work concluded that to increase the shelf life of fermented African locust bean seeds, water must be totally eliminated from it. Total dryness of the fermented condiment will preserve the seed and increase its utilization especially in protein deficient food formulation. This will reduce scarcity (due to very short shelf life) and improve its availability all year round. Drying will also get rid of the undesirable smell which have made Iru unpopular in the urban region.

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