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Effect of Natural and Controlled Fermentation Using *Saccharomyces cerevisae* as Starter Culture to Enhance the Nutritional Qualities of Locust Beans (*Parkia biglobosa*, *Robert bam*)

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Abstract: Locust beans were fermented to assess its effect on the nutrient composition as well as the qualitative and quantitative determination of the microorganisms involved in the process. Chemical analysis was carried out on the beans to obtain its proximate composition, the average mean composition of carbohydrate was found to decrease from 19.30% of the raw sample to 17.09% of the fermented locust beans; while the moisture content increased from 12.00 to 42.65%, the fat content decreased from 21.02% of raw sample to 10.10%; ash content decrease from 4.47% of unfermented locust beans to 4.31% of fermented locust beans. The crude fibre also decreased from 13.06 to 8.53 in the fermented locust beans. Protein content decrease from 30.14 of unfermented locust beans to 17.32 of fermented locust beans. The pH of the locust beans also increase to 7.93 in the fermented sample from the 5.31 in the raw sample. The identities of the organisms involved in the fermentation were found to be *Staphylococus aureus* and *Bacillus* Sp. for bacteria and *Fusarium*, *Aspergillus* and *Penicillium* sp. for fungi.

Key words: Mineral composition, *parkia biglobosa*, microbial load

INTRODUCTION

The genus *Parkia* to which the locust beans belong is large in the family leguminoseceae. The pods are flat, large, irregular clusters from which the locust bean seeds are obtained[1,2]. The species of the genus include *Parkia filicoidea*, *Parkia biglobosa*, *Parkia bicolor* and *Parkia clappertoniana*. The seeds of *P. biglobosa* and *P. filicoidea* had been successfully fermented for the production of food condiments in seasoning foods in Nigeria and other West African countries. The locust bean tree is planted mainly because the fruit is rich and provides valuable protein in the dry season[3] It is also used for medicinal purposes and as a source of mouth wash to relieve toothache. The bean husk (seed coat) are used with indigo dye to improve the luster of fabrics while the tree bark yield a red tannin for dying leather. Mugo Park, a Scottish surgeon, first described this tree in his “Travels in the interior district of Africa” and was named after him by Robert Bran in 1826.

The traditional name for fermented locust bean in Hausa land in Nigeria is dadawa which is an important food condiment in the entire Savannah region of West and Central Africa including northern areas of Nigeria, Ghana, Togo, Chad, Benin, Sierra Leone, Burkina Faso, Gambia, Cameroon, Ivory coast, Guinea, Mali, Senegal and Niger. It is known as Iru in Yorubaland of Nigeria, Oghiri in Iboland, Kpahgu among the Kusasis ad Dagombos of Northern Gambia. All these condiments are strong smelling products with taste and used as soup or stew flavoring. Low-income families use them as a low-cost meat substitute and added to soup or stew and sorghum or millet based douphing and porridge.

A lot of foods products have been prepare by fermentation process with the action of microorganisms and this had led to development of characteristics flavours, textures and changes in nutritive properties of the food usually from different carbohydrate rich food substances such as tubers cereals and legumes.

Since fermentation of locust beans have been found to produce food condiments[2], then it is necessary to know the effect of fermentation on nutritional qualities of the seeds and also access the microbial safety with respect to shelf stability. Thus the objective of this research is to determine the proximate composition of naturally fermented, inoculated fermented using *Saccharomyces cerevisae* as starter culture and unfermented locust beans to evaluate the effect of fermentation on improvement of the nutritional qualities of the seeds. Qualitative and quantitative microbial analysis will also be investigated in order to correlate the effect of pH change on the microbial load and types on the naturally fermented, inoculated fermented and unfermented locust seeds sample.

MATERIALS AND METHODS

Locust beans: The locust beans seeds were purchased from Oja-Oba Market, Ondo State, Nigeria. They were those harvested from the previous season. The seeds were sun dried to reduce the moisture content.

Fermentation: The dried locust bean were fermented by soaking in water for 15 minutes and boiled for six (6) hours. The seed were then dehulled and washed thoroughly. The washed beans cotyledons were boiled for 1 1/2 h with potassium bicarbonate. Fermentation proceeds for thirty 6 h in a fermentor under carefully controlled environment conditions of temperature, pH, pressure and humidity. The controlled fermentation was carried out by inoculating part of the dehulled beans wit pure strain of *Saccharomyces cerevisiae* as a starter culture.

Chemical characteristics: The chemical characteristic that was determined for the naturally fermented, inoculated fermented and unfermented locust beans include moisture content, crude content, crude protein, ash content and crude fibre using AOAC[4]. The mineral content was evaluated for using Atomic absorption, Spectrophotometer. The minerals are Iron, Calcium, Magnesium, Potassium and Ascorbic acid[5].

The soluble salts of the metals determined were used for the preparation of the standards. Ascorbic acid was determine by titrating the sample with 1% ascorbic acid solution using dichlorophenol-indolphenol solution as standard dye indicator. Phosphorus was determine as hydrolysable phosphate using HACH DR/2000 direct reading spectrophotometer at 490 nm using phosover 3 phosphate powder pillow containing ammonium molybdate and as ascorbic acid[6].

Potassium, calcium, magnesium and Iron content were measured with Atomic Absorption spectrophotometer (Unicam 929 model) using the potassium, calcium, magnesium and Iron lamps with prepared standard solution of each model[5].

Microbial analysis: Quantitative and qualitative microbial analysis of the locust beans were carried out using the standard methods of Madigan[7]. Bacteria were isolated and characterized using the gram reaction, colonial morphology and biochemical characteristics. Fungi were identified using the colors on potato dextrose agar, staining and reproductive structures using lactophenol cotton blue.

Table 1: Proximate composition of unfermented, naturally fermented and inoculated fermented locust beans

Nutrient	Unfermented	Naturally fermented	Inoculated fermented
Moisture	12.0±0.0020	42.65±0.200	45.0±0.44
Protein	30.15±0.046	37.32±0.140	38.0±0.22
Ash	4.47±0.180	4.31±0.022	4.91±0.48
Crude fibre	13.00±0.036	8.3±0.0010	6.20±0.56
Fat	21.02±0.360	10.10±0.002	18.00±0.004
Carbohydrate	19.30±0.420	17.09±0.001	15.00+ 0.006

Table 2: Mineral Composition of unfermented, naturally fermented and inoculated fermented locust beans (mg 100 g^{-1})

Minerals	Unfermented	Naturally fermented	Inoculated fermented
Ascorbic acid	2.86±0.005	3.56±0.016	1.32±0.001
Calcium	10.82±0.002	12.68±0.014	13.64±0.410
Potassium	210.40±0.007	250.40±0.460	300.40±0.280
Phosphorus	86.25±0.450	80.12±0.240	82.61±0.460
Magnesium	51.20±0.028	48.46±0.420	56.50±0.780
Iron	2.68±0.042	5.69±0.060	7.20±0.030

RESULTS AND DISCUSSION

The results of the proximate composition of raw, naturally fermented and inoculated fermented are shown on Table 1. The moisture content was found to be higher in the two fermented samples than the unfermented. Increase in moisture content may be due to addition of water and soaking during fermentation. This was in agreement with the report of Omafuvbe *et al.*[2], while carrying out similar research on African locust bean and melon reported an increase in moisture content.

Naturally and inoculated fermentation with *Saccharomyces cerevisae* give an increase in the crude protein value over the unfermented This might be due to reduction in crude fiber and carbohydrate in the unfermented. Another reason for the increase in protein content may be due to the structural protein that are integral part of the microbial cells[8]. The apparent increase in growth and microbial proliferation of microorganisms in the form of single cell protein of the starter culture and the normal floral may account for the observed trend in crude protein[9].

There was significant reduction in the crude fibre, fat and carbohydrate. The reduction may be attributed to the ability of the fermenting micro floral to hydrolyze and metabolize them as carbon source (substrate) in order to synthesize cell biomass[7]. There was no significant difference in the ash content between the fermented and unfermented locust bean sample. This was at variance with the report of Omafuvbe *et al.*[2] that showed a reduction in the ash content. The disparity might be due to the processing of the seeds during fermentation; which involve boiling and dehulling in their own investigation whereas this stage was excluded in this study.

The mineral compositions of fermented, naturally fermented and inoculated fermented locust bean are presented on Table 2. Ascorbic acid value was shown to be reduced in the inoculated fermented sample than the unfermented. This may result from the ability of the starter culture to metabolize the ascorbic acid thereby leading to its reduction. The growth of the Saccharomyces may be an exothermic reaction which ultimately leads to increase in temperature .Ascorbic acid has been shown to be heat labile.

Phosphorus and magnesium were also found to be significantly reduced in both types of fermentation and the same reason for the ascorbic acid reduction may be advanced for the observed patterns. Also during the processing some of the minerals may be leached into the growth medium. Calcium and iron was found to increase in natural fermentation and that with inoculated culture. This may result from the insolubility of these mineral in water and hence could not be easily leached into the growth medium. It may also be that they are relatively heat stable.

The microbial count and pH of the three samples are shown on Table 3. The count was highest for the naturally fermented probably due to proliferation of microbial cells as a result of availability of essential nutrients and minerals in the seeds during processing. The inoculated fermented bean was also lower due to the boiling in order to eliminate the other microbial floral during the control fermentation. The unfermented has the least value because of unavailability of essential minerals that form the component of the seeds for metabolism and subsequent conversion to biomass.

The pH value revealed that the inoculated fermented beans are acidic whereas the unfermented was near neutral or slightly alkaline respectively. The acidity in the controlled fermentation may be due to the nature of *Saccharomyces* with respect to pH requirement for optimum growth which has been shown to be acidic[7]. The naturally fermented and the unfermented samples may be populated by organisms that may be predominatly bacteria which has been shown to thrive better at near neutral or alkaline pH.

The identities of the bacteria involved in the fermentation process were found to be *Bacillu cereus, Bacillus subtilis Staphylococcus aureus* and *Lactobacillus plantanum.* Fungi isolated and identified include *Penicillium species, Aspergillus niger, Fusarium species* and yeast. These isolates can be normal floral of the seeds or contaminants from the processor and environment during fermentation. Similar organisms had been isolated in earlier work by Oke and Umoh,[1] and Odunfa[3].

Table 3: Microbial count and pH of unfermented, naturally fermented and inoculated fermented locust beans

Sample	Count (cfu mL^{-1})	pH
Unfermented locust beans	6.0×10^5	6.8
Naturally fermented locust beans	1.0×10^6	7.2
Inoculated fermented locust beans	3.5×10^5	6.0

CONCLUSION

From the study, the use of starter culture was shown to play important role in nutrient enhancement of African locust beans. There was significant increase in the amount of minerals from the naturally fermented and inoculated fermented over the unfermented locust beans. The research therefore demonstrated a strategy for optimal utilization of Africa locust beans for food and nutrition.

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