

Full Length Research Paper

Microbial load of processed *Parkia biglobosa* seeds: Towards enhanced shelf life

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***Parkia biglobosa* seed is one of the major sources of plant protein in African diet. This work was carried out to improve the shelf-life of the processed seeds of *P. biglobosa*. The microbial count of the micro-organisms (responsible for the fermentation of the processed seed), sensory and physical characteristics were evaluated on fermented seeds preserved with various salt concentration. Our results show that the microbial load was the least in the group with the highest salt concentration at ration 10:3 g/g. Statistical analysis shows a significant difference in the number of colonies formed between the groups at ($p < 0.05$). Salting, as observed in this work, reduced the microbial load, discourage quick spoilage, and encourage longer shelf-life of processed *P. biglobosa* seed in concentration dependent manner.**

Key words: *Parkia biglobosa*, microbial load, African diet, shelf-life, salting.

INTRODUCTION

Parkia biglobosa is a perennial deciduous tree which occurs in the rainforest and the arid zones of some African countries. The tree has the capacity to withstand drought; seeds are embedded in a mealy pulp that is high in energy value. The seeds of *P. biglobosa* have been shown to contain up to 29% crude protein and 60% saccharose; it is also rich in vitamin C and high in oil content (Orwa et al., 2009).

Aside being a good source of plant protein to man, it serves as good source of protein for animal feeds, chick (Obun, 2007, 2008) and fish (Audu et al., 2008). Studies by Esenwah and Ikenebomeh (2008) and Omafuvbe et al. (2004) show some changes in the nutritional and the anti-nutritional constituents of the processed samples of

this important food supplement.

The fermentation of African locust beans (*P. biglobosa*), initiated by *Bacillus* species to produce spices called "iru" or "dawadawa" in Nigeria had been described by several authors (Bridget et al., 2004; Ouoba et al., 2003; Odunfa, 1981, 1985a). The preparation of foods by fermentation has good advantages such as the destruction of undesirable flavours and odours, production of good flavour, increase digestibility, synthesis of desirable constituents, and changes in physical state, longer shelf-life, and destruction of inhibitors (Odunfa, 1985a).

Studies by Antai and Ibrahim (1986) and Odunfa (1985b) show the presence of *Bacillus subtilis*, *Leuconostoc mesenteroides* and *Staphylococcus* species after fermentation of seeds of *P. biglobosa*. They also proposed that several microorganisms are associated with the fermentation, and noted that the most abundant and the major dominant agent of fermentation after 24 h

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was *B. subtilis*.

Previous studies mentioned the food and nutritive value of this important African spices (Alabi et al., 2005; Alabi, 1993; Odunfa, 1986; Fetugal et al., 1974). The processing and the packaging of this product has encountered setback in the past, hence there is need for this study.

The presence of coliform microorganisms after fermentation and the ability of salt, at various concentrations, to reduce the colonization of fermented locust bean by lactic acid bacteria were determined in this work. This research work is geared towards the development of standard method for the preservation of the seeds for a longer shelf life. It is hoped that the findings from these studies will further encourage the use of "iru" as soup condiment and discourage the over dependence of Nigerians on imported food flavours.

MATERIALS AND METHODS

Raw African locust bean seed used for this study was purchased at Bodija Market, Ibadan and processed at the Locust Bean Laboratory of the Forestry Research Institute of Nigeria, Ibadan as previously described by Obun (2008).

400 g of fermented *P. biglobosa* seeds were evenly divided (100 g each group) into four groups: A, B, C, and D (control). The samples were mixed with salts: 10, 20, 30 and control (unsalted), respectively. Thus, the sample to salt ratio in the groups were 10:1, 10:2, 10:3, and 10:0 g/g in groups A, B, C, and D respectively. The seeds were oven dried for 12 h. The samples for each group had five replicate. Each sample was milled into powder. Samples were taken from each group and they were evaluated for total bacterial count at an interval of two weeks throughout the eight-week duration of the experiment. The physical and organoleptic characteristics of the samples were also determined.

Microbial load

One gram of each sample was weighed aseptically using sterile weighing paper under the laminar air flow hood. The stock solution was prepared by dissolving the weighed "iru" sample into 10 ml buffered peptone water and allowed to stay for 30 min; this was made the stock. Plate count agar was weighed and the media was melted using hot plate and then autoclave at 121°C for 15 min. It is allowed to cool between 45 and 50°C before pouring into sterile Petri dishes. A 1 ml of diluents was pipette into sterile Petri dishes and sterile agar was poured, rocked and allowed to gel before incubating at 37°C for 24 h. The bacterial colonies were counted and recorded for interpretation in colony-forming unit (cfu/ml). The result obtained was tabulated and graphically presented.

Sensory evaluation

Twenty panellists (ten males and ten females) conversant with the condiment were selected and were briefed about the aim of evaluation and how it should be conducted. They were also instructed on the attributes of the samples on their questionnaire.

The panellists which were drawn from Forestry Research Institute of Nigeria were then presented with the labelled samples A, B, C, and D. The attribute of the samples evaluated by the panellists were: Colour, texture, aroma, taste, and overall acceptance. They compared its properties according to the methods of Njoku et al. (1991) and Wokoma and Aziagba (2002). The result obtained was

tabulated and presented as shown in Table 2.

Statistical analysis

The data generated from these investigations were analysed using Statistical Package for Social Sciences (SPSS) version 15.0. They were subjected to analysis of variance (ANOVA) and test of significance were carried out using Duncans multiple range tests (DMRT).

RESULTS AND DISCUSSION

The number of microbial colonies formed by the samples in each was obtained and presented in Table 1. Within each group, there was an increase in the number of microbial colonies as the experiment progresses, except in group B; where a slight decrease was observed in the 8th week of the experiment (Figure 1).

The plot of microbial load accumulation against time shows an increase in number of colonies formed with time. This is consistent with the findings of Ogunshe et al. (2006). Across the groups, the number of microbial colonies formed decrease with a corresponding increase in the concentration of the salt applied. Group C had an all time low record of microbial count against group D which had the highest record all through (Figure 1). Group D (control) had the highest number of bacterial colonies against group C with sample to salt ratio of 10:3 g/g. This is actually due to the fact that the sample in group C experience drastic moisture reduction which discourage microbial growth and proliferation (Ademola et al., 2011). These findings are consistent with the works of earlier investigators (Essien, 1983; Yabaya, 2006; Owen et al., 1997) who also observed that active microbial metabolism is required in order to bring about the changes observed in locust beans during fermentation. Microbial fermentation of "iru" have been found to involve only bacteria since fungi found have been regarded as incidental and does not play any notable role in its fermentation (Ikenebomah and Ingram, 1986).

The physical characteristic observed in the organoleptic assessment include: colour, taste, aroma, and texture (Table 2). These were summarized after 2 months of production. As observed, these qualities improved with quantity of salt added. The control (unsalted) lost virtually all its good qualities and turned bad, while the best quality is observed at the group C in which the salt to sample ratio was 10:3 g/g. Owen et al. (1997) on studying the aroma and flavour characteristics of *Bacillus*-fermented *P. biglobosa* noted the presence of active aldehydes, ketones, and acids. Odunfa (1985b) and Campbell-Platt (1980) also noted a high level of proteolytic activity during fermentation, which culminated in the formation of peptides and amino acids. Pelig-Ba (2009) provides evidence that the fermentation process involve deamination during which volatile compounds are released together with ammonia which results in

Table 1. Comparison of the number of colonies of bacterial formed for two months.

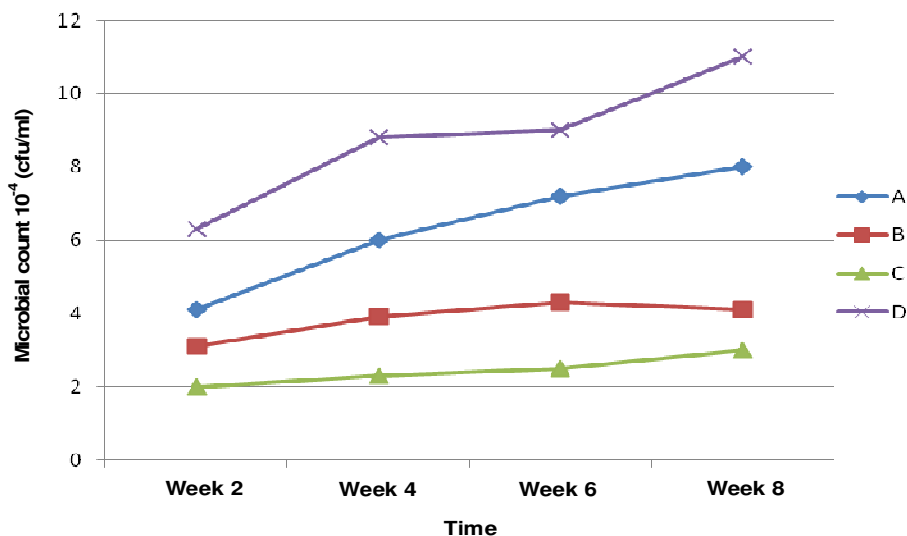
Week	Microbial counts 10^4 (cfu/ml)			
	Group A	Group B	Group C	Group D
2	4.10 ± 0.14^a	3.10 ± 0.16^a	2.00 ± 0.18^a	6.30 ± 0.16^a
4	5.93 ± 0.10^b	3.90 ± 0.14^b	2.30 ± 0.14^b	8.80 ± 0.21^b
6	7.20 ± 0.22^c	4.30 ± 0.18^c	2.50 ± 0.18^b	9.00 ± 0.08^b
8	8.00 ± 0.08^d	$4.10 \pm 0.14^b^c$	3.00 ± 0.08^c	11.00 ± 0.18^c

Mean (SD) followed by the same superscripts in row are significantly difference ($p < 0.05$). Group A = 100 g of sample + 10 g of salt; Group B = 100 g of sample + 20 g of salt; Group C = 100 g of sample + 30 g of salt; Group D = 100 g of sample (Control).

Table 2. Organoleptic characteristic of processed *P. biglobosa* seeds after 8 weeks of production.

Group	Colour	Taste	Aroma	Texture
A	Deep brown	Fresh	Normal	Slightly smooth
B	Chocolate brown	Fresh	Normal	Smooth
C	Chocolate brown	Fresh	Normal	Smooth
D	Dirty brown	Bad	Choking/Offensive	Slippery

Group A = 100 g of sample + 10 g of salt; Group B = 100 g of sample + 20 g of salt; Group C = 100 g of sample + 30 g of salt; Group D = 100 g of sample (Control).

**Figure 1.** Accumulation of microbes against time. Group A = 100 g of sample + 10 g of salt, Group B = 100 g of sample + 20 g of salt, Group C = 100 g of sample + 30 g of salt, Group D = 100 g of sample (Control).

producing odour.

Conclusion

One of the most common local/natural food preservatives is salt, which tends to improve the shelf life of processed *P. biglobosa* seeds by reducing the number of microbial

load on the samples which could have been agent(s) of deterioration or spoilage to the sample and reduce the shelf life. Shelf life is the period of time during which the food product will remain safe, be certain to retain desired sensory, chemical, physical, and microbiology characteristics and comply with label declaration of nutritional data when stored under recommended condition (these should include allowance for some product abuse with

respect to temperature and/or physical handling).

The sensory evaluation carried out shows that the characteristics aroma and appearance are comparable to freshly produced seeds when salt is added. The results of this work have shown the effect of adding salt to improve the shelf life of processed *P. biglobosa* seeds. On the basis of the analysis, it was observed that the addition of salt to processed seeds tend to improve its shelf life as the concentration increases. Processed *P. biglobosa* seeds can stay for months and still be edible if adequate amount of salt is added.

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