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# Effect of Varying Concentrations of Table Salt on the Microbial Fermentation of *Parkia biglobosa* (Jacq.) Benth Seeds

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***Abstract***

*Local processing of Parkia biglobosa seeds for the production of the popular seasoning called Locust bean cake "Daddawa", involves the addition of various substances including common salt. In this study, an attempt was made to investigate the effective concentrations of table salt addition on the microbial, organoleptic, and nutritional qualities of the product. Table salt of 0, 1, 2, 3, 4, and 5% (w/w) was added to a prepared pre-fermented seed of African locust beans (ALBS). A 2cm substrates thickness was prepared and aerobically fermented without stirring at 37$^0$C in an incubator for two days (48 hours) by spontaneous fermentation in clean plastic containers. The fermented products were assessed for microbial and chemical changes, sensory and nutritional properties. The results show chemically and nutritionally significant (at P<0.005), and organoleptically important effect of varying concentrations of table salt in the fermentation of African locust bean seeds. However, this research recommends the use of table salt at a concentration of 1g salt per 100g of the substrate mass for enhancement of desirable properties of the fermented product.*

***Keywords**: Fermentation; African Locust Beans Seeds; Salt.*

## INTRODUCTION

*Parkia biglobosa* (Jacq.) Benth is a perennial deciduous tree from the subfamily Mimosoideae and family Leguminosae . The family Leguminosae is the third largest family of angiosperms (Lewis *et al.,* 2005). Its products are collected and processed into economically essential products useful in various human endeavors including medicinal, handicraft, domestic, medico-magic, veterinary, cultural, food, and commercial products (Belem, *et al.* 2007; Kourouma, 2011).

In African countries such as Benin, Burkina Faso, Niger, Togo, and Nigeria, Fermented African locust beans seeds (FALBS) called '*Daddawa', Iru', 'Ogiri' 'Soumbala', 'Netetu'* comprised one of the essentially consumed traditional food-based condiment serve primarily for seasoning sauces and soups (Belem *et al.,* 2007; Simonyan, 2012; Abdullahi, 2017). The use of the fermented beans of African locust bean seeds (*Daddawa*) dates back many centuries and was already described in the 14$^{th}$ century (Sina and Traoré, 2002). The production of fermented locust bean has remained a traditional family art in a cottage industry, especially in the rural areas. Omokhua *et al.,* (2013) and Rashid *et al.,* (2014) reported that processors involved are middle-aged females with little or no formal education and are dominant in the processing activities.

FALBS have nutritional, organoleptic, and microbiological properties of immense importance in food technology. Nutritionally, it is rich in protein, lipid, carbohydrate, oils, mineral elements, vitamins, antioxidants, and low level of antinutritional factors (Esenwah and Ikenebomeh, 2008; Sadiku, 2010; Elemo *et al.,* 2011); sensorially and organoleptically, it is characterized by a sweet taste, smooth mouth feels, marshy texture and dark brown coloration (Sadiku, 2010); microbiologically, it is a rich harbor of many important microorganisms responsible for its microbial and metabolic changes (Omafuvbe, 1998; Omafuvbe *et al.,* 2004; Simonyan, 2012).

Alkaline solid substrate fermentation of African locust bean seeds is responsible for the nutritional, organoleptic, and microbiological properties of FALBS. Fermentation of African locust beans seeds (ALBS) is characterized nutritionally and chemically by an increase in moisture content, protein and lipid profiles, antioxidants, mineral elements, digestibility, pH and also decrease in ash content, crude fibre, and total carbohydrate, and anti-nutritional factors (Esenwah and Ikenebomeh, 2008; Chukwu *et al.,* 2010); organoleptically and sensorially by an increase in aroma, sweet taste, smoothness of mouth feels, textural marshness, and darkening of brown color (Sadiku, 2010); microbiologically by an increase in the microbial population, production of greyish and slimy mucilage (Omafuvbe *et al.*, 2004). However, it is associated with the microbial production of metabolites from mainly carbohydrate and protein fermentation such as alcohol, glycerol, carbon dioxide, ammonia, etc. (Watson, 1970; Abdulkarim *et al.,* 2009; Simonyan, 2012.

A survey by Abdullahi (2018) recorded about twenty-two (22) additives out of which seventeen (17) are of botanical origin and five (5) are of non-botanical origins used as cooking catalysts; local starter culture of microbial inoculums; softeners; foaming agent; antifoaming agent; supplementary additive; taste and flavor enhancers; flavor retainers; binders; preservatives; color and texture control agents; deodorants; cooking, dehulling and fermentation facilitators. These additives are prepared differently and are preferentially added during cooking, parboiling, fermentation, pounding, and tableting/cubing.

Additives such as table salt which are used as external inoculants provide an additional mineral requirement for the optimum growth and metabolism of microorganisms. They also act as a fermentation-texture control agent, which also could trigger the formation of good desirable qualities and improve consumer's preference (Watson, 1970; Abdulkarim *et al.,* 2009; Yusuf and Rahji, 2012; Sadiku, 2010). Similarly, at some certain amount, increasing concentration of table salt addition is likely to suppress the microbial activities (Watson, 1970; Abdulkarim *et al.,* 2009; Ademola *et al.,* 2011).

Most consumers of FALBS use it not because of its nutritional compositions but for particular preferences on its sensory qualities (Abdullahi, 2017). However, despite the nutritional values of FALBS, some of its kind is rejected or neglected or challenged by some individuals due to the strong, pungent and irritating aroma; and lost in aroma or taste due to deterioration and poor fermentation (Rhoda, 2004; Abdullahi, 2018). Therefore, in search for a product that could meet the different preferential needs of consumers, this study is designed as a food biotechnological approach for the improvement of consumers' preference qualities, by finding out the effective concentrations of table salt for enhanced microbial, organoleptic, and nutritional qualities.

## MATERIALS AND METHODS

**Source of seeds**

Good seeds of African locust beans (ALB) were purchased at Bindawa Local Government Area (L.G.A) weekly market throughout the study and these were used in the production of FALBS. The seeds were further sorted out in the laboratory to ensure only healthy seeds are used.

**Fermentation**

The healthy raw seeds of *Parkia biglobosa* were sorted and depulped manually in a mortar with a pestle, washed thoroughly with clean water. The depulped seeds were hydrothermally boiled for about 6 hours until the cotyledons became softened. The hydrothermally boiled seeds were dehulled in a mortar with a pestle, and the hulls/seed coats from the seeds cotyledons were manually separated by floatation mechanism using a perforated calabash until the cotyledons were cleaned. The cleaned cotyledons were parboiled for nearly 2 hours and allowed to drain (Sadiku, 2010). A table salt ($Nacl_2$) of 0, 1, 2, 3, 4, and 5g was added per 100g of the cleaned cotyledons and thoroughly and gently mixed to make the supplemented substrates. The supplemented substrates were then placed in clean, relatively wide absorbent plastic containers and then covered in a thin membrane of tissue paper and muslin cloth and fermented aerobically without stirring at $37^0$C in an incubator for two days (48 hours) by spontaneous fermentation. The thickness of the fermenting substrates was maintained as 2cm throughout as recommended by Kankara and Bindawa (2020). The formation and intensity of visible microbial colonies were observed, then homogenized for chemical (pH) analysis, and then was oven-dried (at 60°C for about 12 hours) to preserve the products for sensory assessment. The dried samples were kept in tight containers.

**Assessment of Microbial Macrocolony**

The formation and intensity of microbial macrocolony of the fermented products at undisturbed states were observed, ordered based on its intensity, and their pictures were taken.

**pH Determination Method**

Five (5) grams of freshly fermented condiments were dissolved into 20 ml of distilled water and a reading was taken using pH meter. The procedure of Ojewumi *et al.,* (2016) was adopted.

**Sensory Evaluation of the Fermented Substrates**

Twenty panelists (ten males and ten females) who are conversant with the condiment were selected and were briefed about the aim of evaluation and how it should be conducted. All the participants voluntarily consented to participate in the sensory evaluation. They were presented with well-dried samples each of which was labeled appropriately. The attribute of the samples evaluated by the panelists was: Taste, aroma, mouth feels, texture and color based on 3-scale rankings. The evaluation was based on the highest consensus of the panelist. The results obtained were tabulated and presented. The procedure of Sadiku, (2010) was adopted for the sensory evaluation.

**Table 1:** Scale of Ranking for the Determination of Physical Quality

| Property | Qualities and their scale | | |
|---|---|---|---|
| Taste | Sweet (1) | Sour (2) | Bitter (3) |
| Mouth feel | Very coarse (1) | Coarse (2) | Smooth (3) |
| Colour | Creamy brown (1) | Dark brown (2) | Greyish brown (3) |
| Texture | Slightly marshy (1) | Marshy (2) | Very marshy (3) |
| Aroma | Very mild (1) | Mild (2) | Strong (3) |

Source: Sadiku (2010)

**Proximate Analysis of Fermented African Locust Bean Seeds**

Proximate Analysis of each well-dried samples of the fermented product was conducted in triplicate for Ash content, Crude protein (or Kjeldahl protein), Crude lipid, Crude fibre, and Nitrogen-free extracts (digestible carbohydrates) using a standard Methods of Analysis by the Association of Analytical Chemists (AOAC, 2000). The proximate analysis of the samples was carried out at relatively uniform moisture content and carbohydrate determination was by difference.

*Data and Statistical Analysis*

The pH values and proximate results were analyzed using Graphpad Prism statistical software, version 7.03. The data were subjected to a normality test and variance (ANOVA). The means were compared using Tukey's comparison test. The mean pH values, sensory assessment scale values, and the proximate compositions were presented in tables, the formation of microbial macrocolony was presented in photographs.

## RESULTS AND DISCUSSION

*Chemical and microbial changes during fermentation of African locust ban seeds*

The results on the effect of table salt addition on chemical changes during fermentation of ALBS are shown in Table 1. There was significant variation (P<0.0001) in pH from an alkaline medium to a more acidic medium with increasing concentrations of table salt. The reduction in alkaline fermentation affected the rate of fermentation and this might slow the complete fermentation of the substrate. The formation of microbial colonies, the formation of grayish, slimy mucilage of heterogeneous microbial macrocolonies (Plate 1) and the alkaline medium all of which diminished or was lost with increasing concentrations of table salt are indicators of suppressed microbial activities that bring about the complete fermentation of the substrate. Omafuvbe (1998) reported that the desired state of fermentation of condiments is indicated by the formation of mucilage and overtones of ammonia produced as a result of a breakdown of amino acids during fermentation. The drastic decrease in alkalinity and acidity and the decrease in or absence of microbial macrocolonies at high concentrations of table salt was due to high osmolarity which was likely to have resulted in hyperosmotic shock to the microbial cells causing growth suppression. Most bacteria, fungi, and other organisms cannot survive in a highly salty medium due to the hypertonic nature of salt that draws moisture from the hypotonic medium through a process of osmosis. Any living cell in such an environment will become dehydrated through osmosis thus becomes temporarily inactivated or die (Watson, 1970; Abdulkarim *et al.,* 2009; Ademola *et al.,* 2011). The ongoing fermentation coupled with heat accumulation makes the substrate to be putrefied and decomposed. This decomposition may be the reason for the acidic medium.

**Table 1:** Salt concentrations, mean pH value, and the formation of microbial macrocolony of the fermented substrate media

| % Conc. (g/100g | pH | Formation of Macroscopic Colonies |
|---|---|---|
| 0 | $8.45\pm0.11^{a}$ | $P^{4}$ |
| 1 | $8.34\pm0.01^{a}$ | $P^{3}$ |
| 2 | $7.12\pm0.03^{b}$ | $P^{2}$ |
| 3 | $6.45\pm0.06^{c}$ | $P^{1}$ |
| 4 | $5.84\pm0.03^{d}$ | A |
| 5 | $5.45\pm0.03^{e}$ | A |
| **Exact Significance** | P<0.0001 | |

**Note:** Thickness of the fermenting substrates was 2cm;
Results are expressed as mean± standard deviation of triplicate determinations;
Same letters indicate mean values that are not significantly different on the same column;
**[**Formation of Macrocolonies**:** **P=** Present; **A**= Absent];
$^{1, 2, 3, 4,}$ = Order of increasing macrocolony formation.

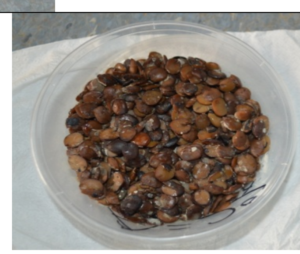
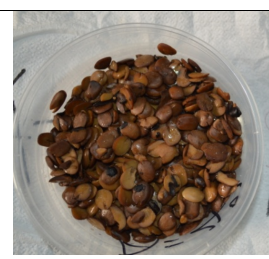
| % Conc. (g /100g) | Images | | |
|---|---|---|---|
| **0%** |  | | |
| **1%** |  | **2%** |  |
| **3%** |  | **4%** |  |
| **5%** |  | | |

**Plate 1:** Formation of microbial macrocolonies (shows by the greyish appearance) of the table salt supplemented and fermented products.

**Sensory qualities of the fermented African locust bean seeds**

The results of macroscopic colony formations and that of the physical (sensory) assessment of the salt-treated samples are presented in Table 2 and Plate 2. The results show that the increase in the concentration of table salt progressively lowered or inhibited the microbial activities and metabolism. The sweet taste, the production of strong irritating aroma, and the smoothness of mouthfeel are directly affected due to suppressed microbial activities induced by high salt concentrations, while the smoothness of mouth feels, the formation of very marshy texture, the appearance of dark brown coloration of the fermented product was indirectly affected as they are the consequential effects of alkaline fermentation. Plate 2 shows the reduction in the dark brown color of the fermented substrates at different increasing concentration of table salt. This finding agreed with the result finding in Abdullahi (2018) that sensory qualities of fermented African locust bean seeds are directly and indirectly affected by microbial fermentation and its produced metabolites respectively. The organoleptically important reduction or loss in the sensory qualities at higher concentrations of table salt was due to high osmolarity which is likely to have resulted in hyperosmotic shock to the microbial cells causing growth suppression (Watson, 1970; Ikenebomeh, 1989; Abdulkarim *et al.*, 2009; Ademola *et al.*, 2011). Ikenebomeh (1989) reported that a well-fermented product has a sweet nutty aroma, soft in texture, and easy to mash between the fingers; while the poorly fermented product is relatively hard in texture, not easy to mash between the fingers, and with a beany aroma. Samples fermented at lower concentrations of table salt greater than 2g per 100g of the substrates were organoleptically rejected for the major qualities, but 1-2g salt per 100g of the mass of the substrate were organoleptically accepted. The modified taste (sweet-saline) at 1g per 100g of the substrate's mass is a similar report by Sadiku (2010) that salt addition improves the taste of the fermented product.

**Table 2:** Salt concentrations and sensory quality assessments of FALBS

| % Conc. (g/100g) | Taste (Ts) | Aroma (Ar) | Mouth Feels (Mf) | Texture (Tx) | Color (Cl) | OMQ |
|---|---|---|---|---|---|---|
| **0** | 1 | 3 | 3 | 3 | 3 | Accepted |
| **1** | 1↔2 | 2 | 3 | 2 | 2 | Accepted |
| **2** | 2 | 2 | 2 | 2 | 1 | Accepted |
| **3** | 2 | 1 | 1 | 1 | 1 | Rejected |
| **4** | 2 | 0 | 1 | 1 | 1 | Rejected |
| **5** | 2 | 0 | 1 | 1 | 1 | Rejected |

**Keys:**
**Ts**= Taste; **Ar**= Aroma; **Mf**= Mouth feels; **Tx**= Texture; **Cl**= Color;
**OMQ**= Objectionability for the major qualities;
**Ts** (1= Sweet; 2= Saline; 3= Bitter);
**Ar** (1= Very mild; 2= Mild; 3= Strong);
**Mf** (1= Very coarse; 2= Coarse; 3= Smooth);
**Tx** (1= Slightly marshy; 2= Marshy; 3= Very marshy);
**Cl** (1= Creamy brown; 2= Lightly dark brown; 3= Deeply dark brown);
**0**= Absence of the quality.
**Note:** The thickness of the fermenting substrates was 2cm.

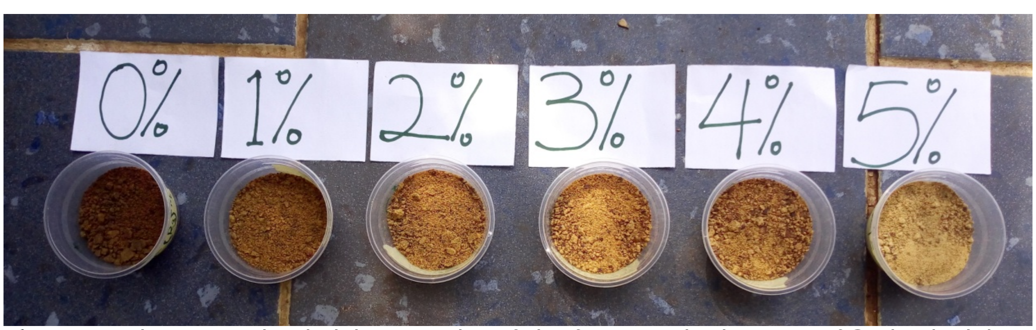



**Plate 2:** Reduction in the dark brown color of the fermented substrates of *Parkia biglobosa* seeds at different increasing concentrations of table salt.

**Nutritional qualities of the fermentation of African locust ban seeds**

The result of the proximate analysis of the salt-treated samples is presented in Table 3. It shows that crude lipid and crude protein are significantly (P<0.0001) lower and total carbohydrate, crude fibre, and ash content are significantly (P<0.0001) higher due to low pH and little or no microbial activities. The highly significant reduction in the nutritional qualities at higher concentrations of table salt may be due to the induced microbial suppression and partial fermentation caused by table salt which slowed or inactivated or destroyed the microbial population (Watson, 1970; Abdulkarim *et al.*, 2009; Ademola *et al.*, 2011). This may have accounted for the reasons for low microbial activities (Plate 1). The highest proportion of crude lipid, crude protein, and the least proportion of total carbohydrate was recorded at 1g salt per 100g of the mass of the substrate. These results are in agreement with Abdulkarim *et al.*, (2009) who reported that a very relatively low addition of salt in the culture media promotes the microbial maximum specific growth rate and increases glucose-intake with increased glycerol production.

**Table 3:** Mean ±SD effect of salt concentrations on the nutritional qualities of FALBS

| % Conc. (g /100g) | Proximate Compositions | | | | |
|---|---|---|---|---|---|
| | Crude Proteins | Crude Lipid | Total Carbohydreate | Crude Fibre | Ash |
| 0 | 40.67±0.63[a] | 35.46±0.64[a] | 15.57±0.72[a] | 4.19±0.09[a] | 4.12±0.04[a] |
| 1 (0.001) | 39.89±0.29[ab] | 37.03±0.25[b] | 13.69±0.23[b] | 4.40±0.16[b] | 4.99±0.16[b] |
| 2 (0.002) | 36.61±0.70[c] | 31.76±0.59[c] | 21.40±0.39[c] | 4.44±0.02[b] | 5.79±0.07[c] |
| 3 (0.003) | 35.04±0.38[dc] | 28.37±0.55[d] | 25.41±0.24[d] | 4.46±0.02[b] | 6.72±0.03[d] |
| 4 (0.004) | 32.18±0.94[e] | 28.12±0.75[de] | 27.53±0.21[e] | 4.47±0.02[b] | 7.70±0.02[e] |
| 5 (0.005) | 30.22±0.15[f] | 27.24±0.35[dfe] | 29.39±0.21[f] | 4.48±0.02[b] | 8.67±0.02[f] |
| **Significance** | P<0.0001 | P<0.0001 | P<0.0001 | P=0.0038 | P<0.0001 |

**Note:** Thickness of the fermenting substrates was 2cm; Total carbohydrate was by difference;
Results are expressed as mean± standard deviation of triplicate determinations;
Same letters indicate mean values that are not significantly different on the same column

## CONCLUSION

Fermentation of African Locust Bean Seeds with a relatively low amount of table salt addition (1g salt per100g of the substrate mass) was sufficiently adequate to enhance the desirable qualities of the fermented product. A chemically (pH), nutritionally significant (P<0.0001), and organoleptically and microbially important and changes in the properties of the fermented substrates of African Locust Bean Seeds was observed with the addition of table salt.

**Recommendations**

I. This research recommends the use of table salt at a concentration of 1g per100g of the substrate mass.
II. Further studies of this kind should focus on other mineral additives (such as wood ashes, ground potash, etc) and their combinatorial effects.
III. The use of specific and selective microbial inoculums rather than random inoculums should be considered.
IV. The activity of extracellular enzymes in fermenting salt added African locust bean seeds need to examine.

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**Conflict of Interest:** The authors declare no conflict of interest.

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