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Effect of Fermentation on the Microbial Flora, Ph and Proximate Composition of Garden Egg (*Solanum Melongena*)

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Abstract: *Garden egg (Solanum melongena) samples were subjected to uncontrolled fermentation. The microbial loads, flora, pH and proximate compositions of the fermenting produce were monitored for a period of 15 days. Standard methods were used for the microbiological analysis and proximate composition of the sample. Samples were taken at 3 days intervals and analyzed. The results of the analysis of the proximate composition showed no significant (P ± 0.05) differences between the unfermented and fermented garden egg for all the proximate composition parameters measured. The pH of the fruit and brine solution decreased from 6.7 and 6.1 to 3.6 and 3.7 respectively while the microbial load decreased from 4.12 x 10^3 to 1.59 x 10^3. The mould populations in both the fruit and brine solution decreased as the fermentation progressed and finally inhibited. No indicator organisms and Salmonella/Shigella species were isolated from the internal tissues of the fruit but these organisms were found in the brine solution but were finally inhibited by the end of the fermentation. The lactic acid bacteria (LAB) though not present initially in the fruit but were after 24 hrs observed to increase in the fruit and maintained steady growth to the end of the fermentation. The LAB in the brine solution observed from the first day continued to decrease as the fermentation continued. This fermentation has shown its drastic effect on the microbial flora and pH and also minor effect on the proximate composition of the produce.*

Keywords: *Fermentation, Lactic acid bacteria, brine solution, proximate composition, preservation.*

1. INTRODUCTION

Fermentation of plant material results from activities of the lactic acid bacteria which eventually predominate from a large and varied epiphytic flora. The diversity of microorganisms on plant provided for several microbial interactions, which influence the activity of certain lactic acid bacteria at different stages and are of great importance for product quality (Daeschel *et al.*, 1987). The key to a successful fermentation is to enhance the activities of the desired fermentation microorganisms and to suppress the growth of pathogenic and spoilage microorganisms.

Egg plant, commonly called garden egg (*Solanum melongena*) is a popular vegetable. It is also known as African egg plant. The vegetable contains numerous small seeds (Giuliani and Smale, 2000). It is commonly served and eaten as desert mostly with groundnut in this part of the world. Despite the low interest in garden egg in North American, it is a particularly important vegetable in China and Japan (Daunnau *et al.*, 1999). The nutritional content of this vegetable is comparable to that of tomato, but it has a lower content of vitamin C. Diet rich in fibre contents lower serum cholesterol concentration in animal models (Zhang *et al.*, 1994). As apples and oats form important contributors to the nutrient intake in Western societies, the same is true for garden egg in Nigeria where it is widely consumed. Medicinally, it is used as carminative and sedative and used to treat colic and blood pressure (Grubben and Denton, 2004). The studies by Edijala *et al.*, (2005); Zhang *et al.*, (1994); Aprikian *et al.*, (2001) and Anderson *et al.*, (1990) showed that garden egg significantly reduced weight gain, reduced total serum cholesterol, triglyceride and increased serum HDL.

Garden egg when matured has a greenish to white appearance. It turns red to yellow when kept for a longtime without proper preservation. The fermentation products of garden egg are not common as no major work has been done in the area. Considering the nutritional and medicinal value of this vegetable and its perishable nature, it will be of great value to subject this vegetable to fermentation to produce pickled garden egg which will extend its shelf life and possibly increase its nutritional value. Also, the effect of the fermentation on the pH and microbial flora such as spoilage and food borne pathogens which are the major concerns of consumers will be determined.

2. MATERIALS AND METHODS

2.1. Collection of Samples

A cool box was prepared a day prior to sampling day by placing some ice blocks into it. On the morning of the sampling, the ice blocks were removed and the temperature in the cool box measured with a thermometer and found to be about 4^0c. The temperature in the cool box remained at 4^0c-8^0c throughout the sampling and up to the period of samples analysis.

Sixty garden egg samples were harvested aseptically using sterile gloves and placed in food grade sampling bags from the garden egg farms in Odukpani Local Govt. Area of Cross River State and placed in the cool box. The other garden egg samples were aseptically swabbed with sterile swabs (Jackson *et al*., 2001), Williamson *et al*., 2003 while they were still on the plants. The swabs were replaced into their containers containing maximum recovery (peptone/saline) to prevent physiological shock of the organisms (Williamson *et al.,* 2003) others were harvested aseptically these were then transferred to the cool box (American Public Health Association, 1998). These were transported immediately to microbiology department laboratory, University of Calabar while others were taken to department of crop science and Biochemistry departments for analysis.

2.2. Microbial Load

The microbial load of the garden egg was determined using the methods of Downes and Ito (2001) and Von Schelhorn (1980). Fifty grams of the sample was weighed and placed in sterile blender previously sterilized with 70% alcohol and rinsed with sterile deionized water. This was blended aseptically and twenty five portion of the produce was placed in 225ml of sterile maximum recovery diluents (peptone/saline) in sterile flask. The homogenate was allowed to stand for 30min and vigorously shaken for 2 to 3min (Mosupye and von Holy, 1999). From the dilution, further ten-fold dilutions up to 10^{-4} were prepared. Pour plate method was used by pipetting 1m1 of the dilutions 10^{-3} and 10^{-4} with a pipette and put into empty Petri dishes. About 15mls each of plate count agar (PCA) cooled to 45^0c (Downes and Ito, 2001) was poured into two Petri dishes. The plates were swirled gently to mix the contents properly mixed (Geyid *et al.,* 1991). These were incubated at 37^0c for 18-24hrs. At the end of the incubation period, discrete colonies (30-300cfu) were counted average taken and computed using the appropriate dilution factor. Microbial load per gram for the sample was determined.

2.3. Microbial Flora of the Freshly Harvested Produce

The swabs were allowed to stand for one hour in their jackets after which they were streaked on Staphylococcus aureus agar, sabouraud dextrose agar (SDA), salmonella-Shigella agar, (SSA), MacConkey agar and MRS agar (Difco, France) and incubated at 37^0c for 24 hrs while the SDA plates were incubated at 27^0c for 3 days. The plates were then observed for growth after the incubation periods.

2.4. Isolation and Identification

The isolates were sub-cultured into fresh media for purification and incubated for 24hrs. The isolated colonies were examined and stock culture prepared. Wet mounts using lactophenol in cotton blue were prepared for the fungal isolates. The bacteria isolates were Gram stained and viewed under the microscope. Additionally, biochemical tests including citrate, urease, oxidase, catalase, sugar fermentation were performed.

2.5. Proximate Composition of the Produce

The methods of Association of Official Analytical Chemists, 1980 (A.O.A.C.,1980) were used for determination of crude protein, crude fibre, fat content, ash content, carbohydrate content, and moisture content.

2.6. Preservation of the Produce by Fermentation

Ten fruits of the garden egg samples were placed in two 1.5 liter wide mouth glass bottles containing 7% sodium chloride solution and one bottle without sodium chloride which served as control were tightly covered. The pH of the solutions in the bottles was taken. Ten millimeter of sample from each bottle was taken and place in 90ml peptone/saline diluents. Further dilutions were prepared. To incubate, 0.5ml aliquots each from the 10^4 and 10^3 dilution were taken and 0.5ml water solution from the control and cultured by spread plate on PCA, deMan Rogosa Sharpe (MRS) agar, MacConkey agar, sabauraud dextrose agar and salmonella-shigella (SS) agar. The microbial types and loads determined. The above was repeated during the fermentation. The fruit samples were monitored daily for changes in colour, texture and signs of spoilage. All these were done in duplicates.

2.7. Determination of pH

The fruits were crushed to get the juice out. A pH meter (model 215, Denver Instruments Co.) was used to measure the pH of the juice and the initial brine solution.

2.8. The pH and Microbial Profile of the Garden Egg Fruits and Brine Solutions During the Fermentation at Room Temperature

During the fermentation, the microbial profile of the fruits, brine solution, pH of the fruits and the brine solution were monitored at 3 days intervals for 15 days. At each sampling, about 2 fruits and 10mls from the containers were aseptically removed and the fruits

Crushed and the pH of both the crushed fruit and brine solutions taken separately.

3. RESULTS

The microbial isolates from the freshly harvested garden egg were *bacillus* spp., *Leuconostoc* spp, *Pediococcus* spp, and *Lactococcus* spp. While the fungal isolate were *Fusarium verticilliodes*, *Penicillum* spp, *Botryodioplodia theobromae*, *Candida* spp, and *Paecilmyyce* spp. The microbial isolates from the garden egg bought from market were *Enterobacter aerogenes*, *Salmonella* spp., *E. coli.*, S. aureus, *Lactobacillus* spp., *Pseudomonas* spp. *Shigella* spp. *Geotrichum* spp, *Yeast* spp., *Aspergillus Niger*, *Penicillum* spp, *Mucor plumbeus* and *Fusarium* spp. While the fermented garden egg had the following organisms: Pediococcus spp, Lactobacillus plantarum,

3.1. Proximate Composition

The results of the proximate composition of the freshly harvested and fermented garden egg samples are presented in table 1.

Table1. *Proximate composition of garden egg/dry weight.*

	FRESH	FERMENTED
Crude protein	12.7± 0.03	13.6± 0.03
Crude fiber	13.6± 0.01	12.7±0.01
Fat	12.0± 0.01	11.4± 0.03
Ash	2.0± 0.02	2.4± 0.03
CHO	59.7± 0.01	49.9± 0.01
Moisture	90.4± 0.06	88.7± 0.04

Each value is a mean of 3 determinations to standard deviation.

3.2. pH Changes

Effect of fermentation on the pH of the brine solution and garden egg fruit are shown in Fig. 1 while the microbial load change in the fruit and brine solution are shown in Fig. 2

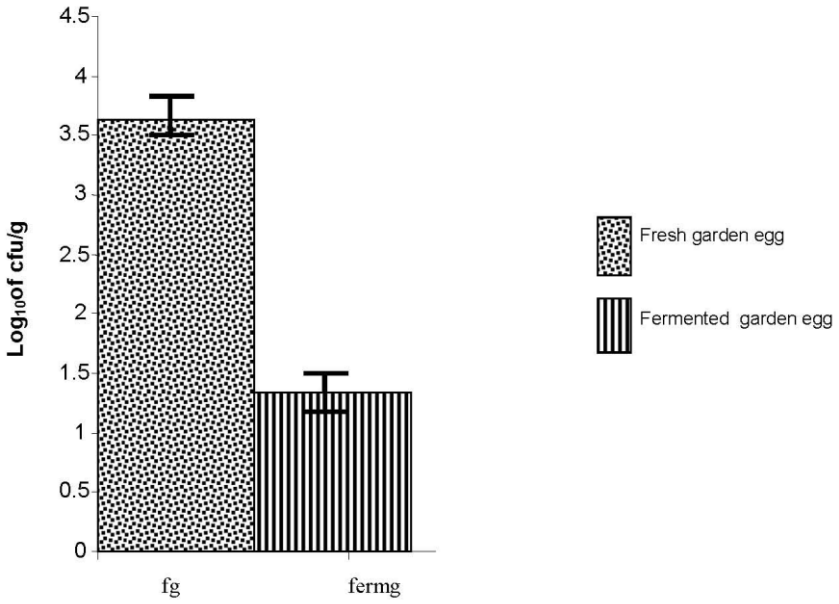
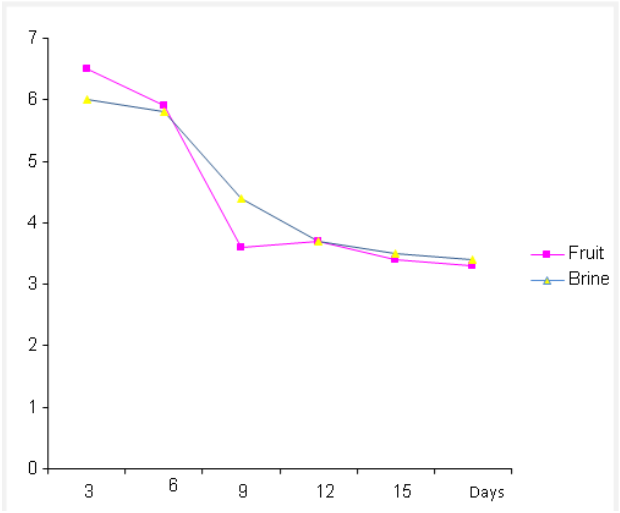


Fig1. *Microbial loads of fresh and fermented garden egg*



pH

Fig2. *pH changes of the brine and garden egg fruit during fermentation*

The microbial changes in the garden egg fruit and brine solution during fermentation are shown in Figures 3-6.

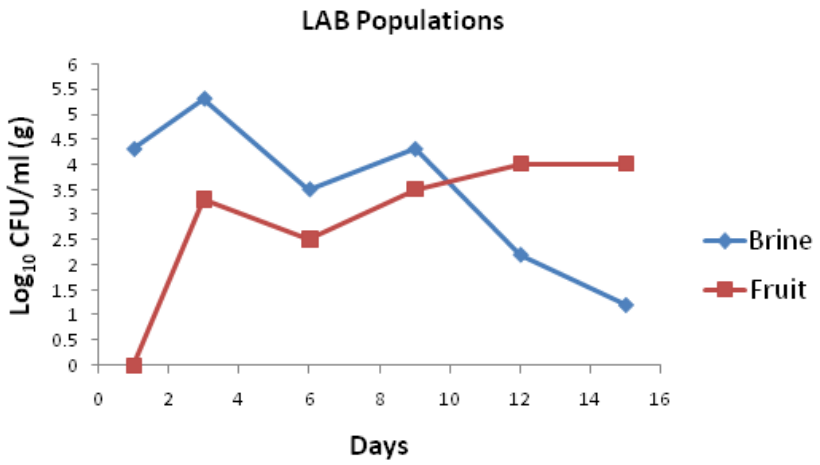


Fig3. *Changes in lactic acid bacteria (LAB) populations garden egg fermentation*

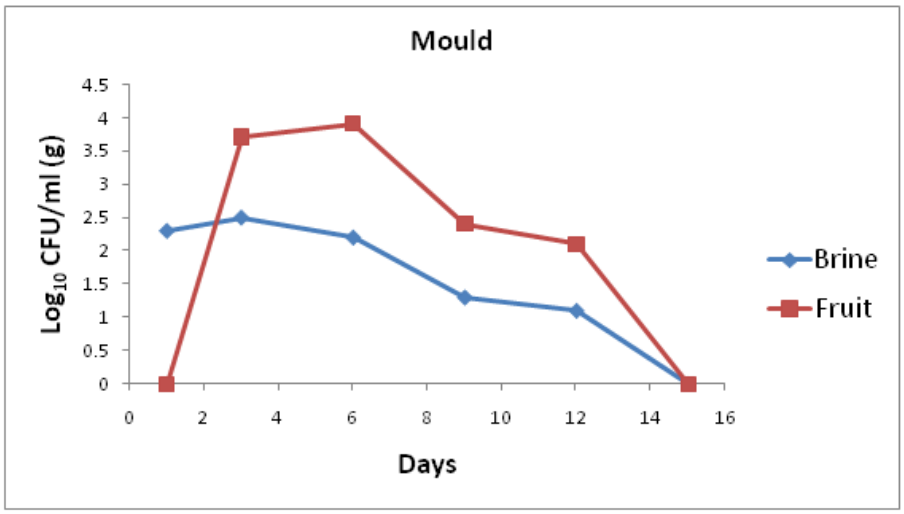


Fig4. *Changes in mould populations during garden egg fermentation*

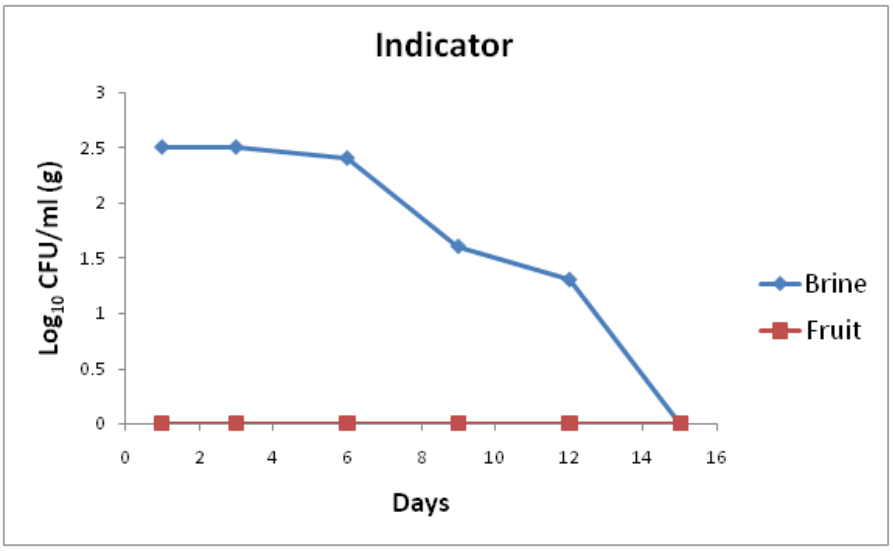


Fig5. *Changes in indicator (E. coli) organism population during garden egg fermentation*

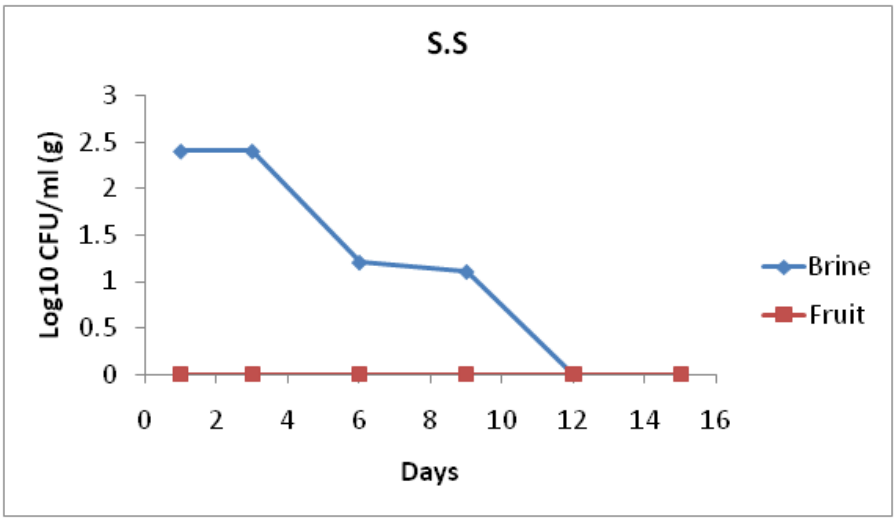


Fig6. *Changes in Salmonella/Shigella species populations during garden egg fermentation*

4. Discussion and Conclusion

Fermentation is often times used to preserve fresh food products, enhance the flavor, improve on the nutrients and eliminate food borne pathogens. The pH of any food product has a lot to do with its shelf life and protection against invading microorganisms such as spoilage and food borne

pathogens. During the fermentation of the produce, the pH of both the garden egg fruit and brine solution decreased to levels that will not allow the survival of most spoilage and pathogenic organisms. This corroborates the findings of Breidt Jr *et al*., (2007) and Daeschel *et al*., (1987). The reduced pH is as a result of lactic acid and probably other metabolites that are usually produced by lactic acid bacteria during fermentation. This could have enhanced the inhibition of spoilage and pathogenic organisms and therefore effectively preserved the produce. The changes in population of the different categories of microorganisms in the fermenting brine solution and garden egg fruit during the fermentation reflect the changes in pH (Lennox and Efiuvwevwere, 2014; Lennox and Efiuvwevwere, 2013). The yeast, indicator organisms, and *Salmonella* and *Shigella* species populations showed steady decline from the first day. The pattern of growth of LAB during the fermentation could have been as a result of the obligate, heterofermentative species (Sanchez, *et al*., 2000; Sesena, *et al.,* 2004). As the results show, the more acid tolerant species probably continued with the fermentation from day 9. Within the period of 15 days fermentation, all the groups of the organisms were eliminated except LAB which survived, though in low numbers to the end of the fermentation period (Lennox and Efiuvwevwere, 2014). It is also of note that no indicator organisms and Salmonella/Shigella species were isolated from the intact fruit throughout the fermentation period. This means that the fruit if properly washed can be eaten without fear of getting sick from food borne disease.

The proximate composition of the fermented produce did not show any significant differences. Crude protein increased by only 7% while crude fiber and fat decreased by 7% and 8% respectively. The component that recorded the highest increase was ash with 20% while carbohydrate and moisture contents decreased by 16% and 2.5% respectively. The decrease in fiber content is worrisome because it plays a major role in maintenance of good health. More research is therefore needed to explore ways of increasing this component during fermentation of this produce.

The microbial isolates were in line with the findings of Lennox and Efiuvwevwere, 2014. This research has shown the role fermentation can play in the preservation of farm produce and also make the produce safe for consumption. Slight effects on the nutrient contents of the produce can be explored further to see its benefits. The pH of any food product also determines its shelf life and the low pH observed in the final product after the fermentation is a great development.

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