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Original Research Article

Microbial Dynamics of Fermenting Garden Egg (*Solanum melongena)*

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Keywords

Fermentation, dynamics, indicator organisms, freshly harvested, market retailed.

A B S T R A C T

The microbial dynamics of fermenting garden egg (*Solanum melongena*) was investigated. The initial microbial loads and flora of the freshly harvested and market retailed produce were determined before subjecting the produce to fermentation in 7 % salt solution for 15 days. Also, the microbial loads and types were determined during the fermentation at 3 days interval for a period of 15 days. The results showed initial microbial loads to be 4.12 X 102 and 2.2 X 103 for freshly harvested and market retailed garden egg respectively. The microbial types were *Bacillus* spp, *Leuconostoc* spp, *Pediococcus* spp, *Lactococcus* spp, *Fusarium verticilliodes, Penicillium spp, Botrydioplodia theobromae, Candida spp,Paecilomyces spp* for freshly harvested and *E. aerogenes*, *Salmonella* spp, *E. coli*, *S. aureus*, *Lactobacillus* spp, *Pseudomonas* spp, *shigella* spp, *Bacillus* spp, *Leuconostoc* spp, *Geotrichum spp, yeast spp, Aspergillus niger, Penicillium spp, Mucor plumbeus, Fusarium verticilliodes* for market retailed. The fungi species and indicator organisms decreased jn populations as the fermentation progressed and finally inhibited on the 15th day while salmonella/shigella group was inhibited on the 12th day. The lactic acid bacteria sharply increased in population from 2.3 X 104 to 2.1 3 X 105 and and thereafter decreased gradually and maintained a steady population of 1.5 X 104 to the end of the fermentation. The elimination of the food pathogens during the fermentation is a positive development in rendering this produce safe for consumption.

Introduction

Egg plant, commonly called garden (*Solanum melongena*) is a popular vegetable or fruit. The fruit contains numerous small seeds (Giuliani and Smale, 2000) despite the low interest in egg plant in North America, egg plant is a

particularly important vegetable in China, Japan and developing countries (Daunnay *et al.*, 1999). The nutrient content of garden egg is comparable to that of tomato, but it has lower contents of vitamin C. Even though garden egg is, highly demanded locally and has export potential, there is no official variety for commercial cultivation (Owusa and Lund, 2001). The roots and fruits of garden egg are described as sedative and carminative used to treat coli and high blood pressure (Grubben and Denton, 2004). The studies by Ediyala, *et al.*, (2005) showed that garden egg significantly reduced weight gain, reduced.

Total serum cholesterol, triglyceride and increased serum HDL-cholesterol. This confirms the studies of Anderson *et al.*, (1990), Zhang *et al*., (1994), Aprikian *et al*., (2001). This fruit/vegetable is commonly sold along the streets, major highways and in the open markets infested with flies. People just buy and eat most times without washing them. This could be major cause of foodborne disease outbreaks but in this part of the world there are no documented cases. This is because no agency is established to monitor and document such cases. Knowing the health benefits of this vegetable and the common food borne diseases associated with eating fresh vegetables and fruits, it is ultimate importance to determine the role the microbial flora of the vegetable will play in prolonging the shelf life and also make it safe for consumption.

This can be achieved through bio preservation or fermentation. Spontaneous fermentation could be used to achieve this since different species of microorganisms maybe involved and may play positive or negative roles during the fermentation. This fermentation takes place at ambient temperature for about seven days and the pH may be as low as 2.7. Spontaneous fermentation of garden egg is homogeneous as far as the microbial genera involved, with the most exclusive predominate of lactobacillus (Sanchez *et al*., 2000). This is a main difference from other fermentations such as olives, sauerkraut or cucumbers (in which species of the genera *Lactobacillus, Pediociccus* and *Leuconostoc* commonly occur) as well as capers (Daeschel *et al*., 1987, Montano *et al.*, 2002; Perezpulide *et al.*, 2005).

During the fermentation, the facultative hererofermentative species of *Lactobacillus plantarum* (as well as *Lb. pentosus*) becomes predominant (sanchez *et al*,. 2000, Sesena *et al*., 2001). This is similar to other fermented vegetables (Fleming, 1982, Daeschel *et al*., 1987, OYewole and Odumfa, 1990, Kunene *et al*., 2000, Mugula *et al.*, 2003, PerezPulide *et al*., 2005). The sensory attributes of this pickled product derive mainly from the contribution of *Ib. plantarum* and from a lesser extent from obligate heterofermentation lactobacilli producing lactic acid (Kandler, 1983, Hounhouigan *et al.*, 1993 Sesena *et al.*, 2001). Preservation of garden egg by fermentation is not common as no major work has been done in this area. This has prompted this investigation to determine the role microorganisms play in the fermentation of garden egg.

Materials and Methods

Sixty garden egg samples were harvested in farms in Odukpani Local Government Area of Cross River State and others were purchased from markets in Calabar. These were placed in precooled coolers with ice which maintained the temperature between 40°C and 60°C. The farm produce were

aseptically swabbed with sterile swabs (Jackson *et al*., 2001, Williamson *et al*., 2003) while they were still attached to the plants. The swabs were replaced into their containers containing maximum recovery diluent and also placed in the col box to avoid physiological shock of the organisms (Williamson *et al*., 2003; American Public Health Association, 1998). The cooler was transported to University of Calabar Microbiology Laboratory. Microbiological analysis of the fresh garden egg samples was carried out. Microbial load and types of the garden egg were determined.

Microbial Load of the Freshly Harvested Garden Egg Samples

The microbial load of the garden was determined using the method of Downes and Ito (2001) and Von Schelhorn (1980). Fifty grams of the garden egg was weighed and placed in sterile blender. This was blended aseptically and twenty five portion of the produce was placed in 225mi diluent of sterile maximum recovery diluent (peptone/saline) in a sterile flask. The homogenate was allowed to stand for 30min and vigorously shaken for 2 to 3 min. (Mosupye and Von Holy, 1999). From this dilution, further ten-fold dilutions up to 10-4 were prepared. Pour plate method was used by pipetting 1ml each from the dilutions 10-3 and 10-4 with a pipette into empty sterile Petri dishes and adding 15ml of plate count agar (PCA) of cooled to 450c to each plate. (Downes and Ito, 2001). The plates were swirled gently to mix the contents properly (Geyid *et al.*, 1991). These were incubated at 370c for 18-24 hrs. at the end of the incubation period, discrete colonies (30-300cfu) were counted and computed using the appropriate dilution factor. Microbial load per gram for the garden egg sample was determined.

Microbial Flora of the Freshly Harvested Produce

The swabs were allowed to stand for one hour in their jackets after which they were then streaked on sabouranud dextrose agar (SDA), Salmonella-shigella agar (SSA), MacConkey agar, MRS agar and Manitol salt agar and incubated at 370c for 24 hrs while the SDA plates were incubated at 270c for 3 days. The plates were then observed for growth after the incubation periods.

Isolation and Identification

The isolation were sub-cultured into fresh media purification and incubated for 24 hrs. the isolation colonies were culturally examined and stock cultures prepared. Wet mounts using lacto phenol in cotton blue were prepared for the fungal isolates. The bacterial isolates were Gram stained and viewed under the microscope. Additionally, biochemical tests including citrate, urease, oxidase, catalase, sugar fermentation, and MR-VP were performed on the bacterial isolates (Downes and Ito, 2001).

Microbial Load of Market Retailed Garden Egg

The microbial loads of the samples were determined using the methods described under freshly harvested samples. However, spread plate method was used on plate count agar. These were incubated at 37oC for 18-24 hr. The discrete colonies were counted at the end of the incubation period.

Microbial Flora of Market Retailed Garden Egg

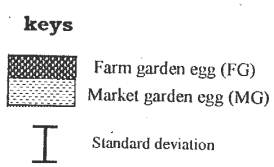
The garden egg samples were placed in 250ml of maximum recovery diluents and shaken thoroughly to dislodge the organisms from the surface making sure that no fruit was damaged to avoid introduction of acid into the diluents. The suspensions were cultured on selective media such as MacConkey agar, SSA, Staphylococcus agar and Sabouraud dextrose agar using 0.5ml of the suspension in each case by pour plate. The plates were incubated at 370c for 18-24 hrs for bacteria and 96 hr at room temperature for fungi. The discrete colonies were sub cultured to purify and stock cultures prepared. The isolates were characterized using different biochemical tests.

Conversion/Preservation of the Produce by Uncontrolled Fermentation

The freshly harvested and market retailed garden egg samples were placed in 2 wide mouth glass bottles containing 7% sodium chloride solution and lightly covered to allow limited air in (Daeschel *et al*., 1987). At intervals of 3 days, samples were aseptically taken for 15 days from these containers, serially diluted and plated on PCA, deMan Rogosa Sharpe (MRS) agar, MacConkey agar, sabauraud dextrose agar, salmonella-Shigella (SS) agar and Manitol salt agar.

Results and Discussion

These results have shown the wide range of microorganisms associated with the garden egg at the different stages from harvest, handling, transportation, retail market to consumer. This was probably due to the changes in the environmental conditions such as less human activities of the farm areas, unsterile containers used in transporting the produce, and the high human activities surrounding the produce in the market where the produce was displaced for sale. The unhygienic market environments contributed more to the microbial load and diversity. This corroborates the finding of Cantwell (2002) and Appai (1988) which showed high microbial loads of different types of produce sampled from various markets. However, the freshly harvested and market retailed produce attained the satisfactory microbiological quality (<106 cfu/g) when using the PHLS guidelines of microbiological quality for ready-to-eat food (Kam *et al*., 2002). Despite this, the isolation of foodborne pathogens such as *Salmonella* spp, *E.coli*, *Shigella* spp, *Bacillus* spp and *S. aureus* was of great concern to the consumer. This could have been due to the unhygienic environments of the markets and also may be as a result of the water used in washing the produce which must have been contaminated with the pathogens prior to use as observed by Arthur, *et al*., (2007. The findings of the fermentation of the garden egg showed tremendous changes in microbial populations and kinds. The inhibition of the pathogens and the dynamics of other microbial populations during the fermentation could have created an uncondusive environment for other microbes other than the pathogens. The reduced pH during the fermentation must have inhibited the less acid tolerant species and production of antimicrobials by some of the populations such as lactic acid bacteria (LAB) could have affected the other microbial groups. The pattern of growth of lactic acid bacteria (LAB) during the fermentation could have been as a result of obligate, heterofermentative species (Sanchez *et al*., 2000, Sesena *et al*., 2004).



Produce type

Fig.1 Microbial loads of the different types of garden egg.

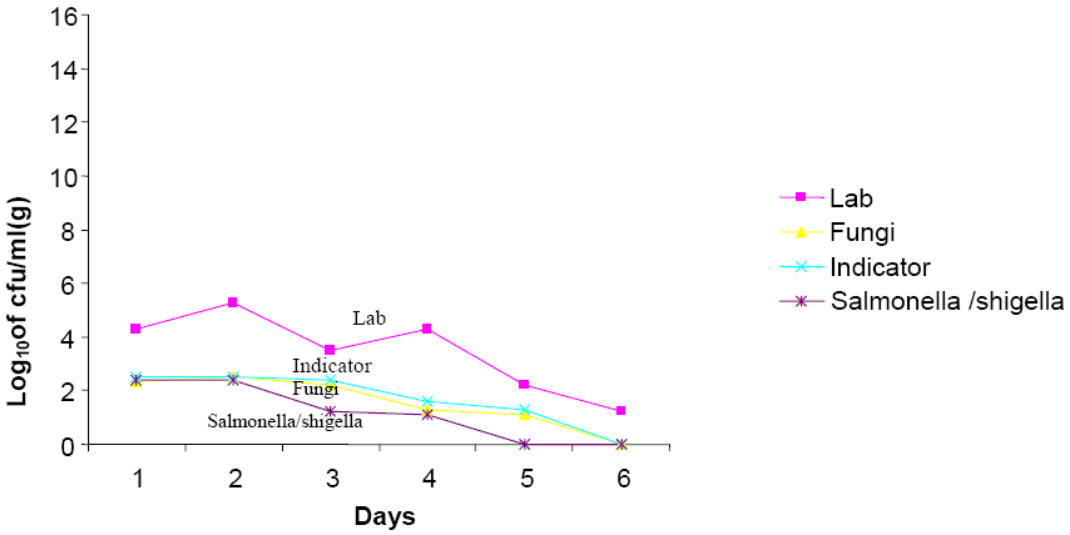


Fig.2 Changes in groups of microbial populations during fermentation of garden egg

Table.1 Bacteria species isolated from freshly harvested and market retailed garden egg.

PRODUCE TYPE	BACTERIA
Freshly harvested garden egg	*Bacillus* spp, *Leuconostoc* spp, *Pediococcus* spp, *Lactococcus* spp
Market retailed garden egg	*E. aerogenes*, *Salmonella* spp, *E. coli*, *S. aureus*, *Lactobacillus* spp, *Pseudomonas* spp, *shigella* spp, *Bacillus* spp, *Leuconostoc* spp

Table.2 Fungi species isolated from freshly harvested and market retailed garden egg

PRODUCE TYPE	FUNGI
Freshly harvested garden egg	*Fusarium verticilliodes, Penicillium spp, Botrydioplodia theobromae, Candida spp,Paecilomyces spp.*
Market retailed garden egg	*Geotrichum spp, yeast spp, Aspergillus niger, Penicillium spp, Mucor plumbeus, Fusarium verticilliodes*

The more acid tolerant species took over from the less acid tolerant species and continued to the end of the fermentation. This work therefore confirms that fermentation process plays an important role in ensuring food safety since foodborne pathogens were eliminated and food security enhancement achieved. This is therefore highly beneficial for millions of people around the world particularly developing countries.

This fermentation process can be exploited further in this part of the world and be extended to other vegetables and fruits.

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