EVALUATION OF POTENTIALS OF ALLIGATOR PEPPER AS NATURAL PRESERVATIVE ON THE MICROBIOLOGICAL QUALITY AND SHELF LIFE ON TIGER NUT (*Cyperus esculentus)* MILK

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ABSTRACT

The potentials of Alligator as natural preservative were evaluated on the microbiological quality and shelf life on tiger nut milk. The aqueous extract of tiger nut milk (*Cyperus esculetus*) was treated with Hydro-methanolic extract of Alligator pepper. Pasteurizes at 75^{0}C for 20 minutes and stored at ambient temperature (28^{0}C) for 14 days followed which was investigated by determining pH, total microbial counts and determination of percentage occurrences of microorganisms. Bacterial counts was at the range of 1.54 ± 0.07^b to $1.72\pm0.07^a \log_{10}$cfu/ml of the samples preserved using Alligator pepper extract; the fungal counts recorded the range of 1.56 ± 0.02^a to $1.83\pm0.05^a \log_{10}$cfu/ml while the control tiger nut milk ranges from 2.31 ± 0.01^a to $2.55\pm0.03^a \log_{10}$cfu/ml respectively. The tiger nut milk was acidic with a range of 3.00 ± 0.2^a to $3.36\ \pm0.5^a$ pH value. At Week 0 the samples containing preservative recorded a low microbial count. There was drastic reduction in microbial load as the effects of the preservative became evident at Week 1 and Week 2. Moreover, the antimicrobial activities of the preservative revealed that samples at concentrations 300mg/ml were most effective against microbes than the 200mg/ml and 100mg/ml. This shows that the active ingredients in the preservative exhibited more potential at higher concentration than at lower concentration. Samples without preservatives recorded high microbial counts throughout the storage period. Five bacterial species, namely *Staphylococcus aureus, Streptococcus* sp, *Bacillus* sp, *Psuedomonas sp and Escherichia coli* were isolated. While five fungal species, of *Saccharomyces, Rhizopus* sp, *Aspergilus niger, Aspergillus flavus and Penicillum* sp were also isolated. *Staphylococcus aureus* and *Saccharomyces* remained the predominant organisms throughout the storage. The role played by the organisms on microbiological quality of tiger nut milk likewise the potentials exhibited by the preservative on the organisms had been discussed. Conclusively, the use of Alligator pepper as natural preservative should be encourage because the study has shown its potentials on the shelf – life and quality on tiger nut milk. More so, the preservative is relatively cheap, accessible and available, its nutritional and medicinal values are generally acceptable by communities and void of unwanted side effects.

Keywords: Alligator pepper; Microbiological quality; Food Preservative; Shelf life; Tiger nut milk.

INTRODUCTION

Tiger nut (*Cyperus esculentus)* is a nut that is juicy and sweet. This crop has not enjoyed maximum patronage owing to its wide consumption as a junk food or at most as a snack, although it enjoyed general acceptability by many people because of its palatability but it is still underutilized (Belewu *et al.*, 2005). Tigernut is a good source of phosphorous, potassium and iron. It also contains magnesium, calcium, zinc, copper, sodium and manganese (TTSL, 2005). Tigernuts are suitable for diabetic

persons, ideal for children, older persons and sportsmen and are very healthy. There are no reported cases of tigernut toxicity. However, Ochratoxin A (OTA) mycotoxin produced by different species of *Aspergillus* and *Penicillium* has been found as a natural contaminant in tigernut and many foodstuff but they are consumed directly in herbal plants (Adebajo, 1993). However, the tiger nut milk is still underutilized and that the milk produced naturally from it has few hours shelf life, hence the need for incorporation of a preservative to extend its shelf life. The preservative effects of Alligator pepper, Lime and Ginger have been variously reported (Ben–Nwadia, and Ndukwu 2005).Their antioxidant properties of spices have been recognized about six decades ago when it was demonstrated that spices effectively increased the antioxidant capacity of foods and that their effects depended on food matrices. The use of local spices to control the activities of micro-organisms in food has been reported (Akpomodaye, and Ejechi 1998). Apart from antimicrobial activities, spices are believed to have medicinal value (especially in African settings) and have desirable determinative influences on the overall organoleptic analysis when used (Adegoke *et al.*, 2000). The aim of this study to evaluate the use of Alligator pepper which is one of locally available spice as a natural preservative on microbiological and shelf life on tiger nut (*Cyperus esculentus*) milk.

MATERIALS AND METHODS

Tiger nut and Alligator pepper were bought from Yankaba market located in Nassarawa Local Government Area Kano State. Samples were de-stone, washed thoroughly and air dried. Tiger nut was steeped for 4-8 hours and later grinded into a mash using a sterilized Mammomlex electric blender, cold water was added in a ratio of three litres per unit kilogram and the mixture was left to macerate. The macerated tiger nut milk was pressed, sieved and dispensed into graduated sterilized sample bottles. The Alligator pepper pods were opened and the seeds were separated from the lint-like material in the pod it was washed and air dried. The seeds were grinded to a fine powder using a sterilized mammomlex electric blender (Okafor *et al.*, 2003).

Preparation of the Extracts

Cooled maceration method was used in the extraction of the plant materials. 150g of the resulting powder of Alligator pepper was weighed and soaked in a jar containing 400ml Hydro-methanolic at room temperature for a period of 7 days. The extracts were drained and filtered using Whatmann N0 1 paper and the solvent were evaporated at room temperature. The hydro- methanol extracts of Alligator pepper concentrate were completely air dried at room temperature weighed, labeled and stored in a refrigerator at 4^0C throughout the duration of this study Ayo (1980).

Determination of the Yield of the extracts

An empty clean and dry beaker was weighed using an analytical weighing balance and later the extracts were poured into it. The beaker was weighed after the extracts have been concentrated to constant weight Ayo (1980). The weights of the extracts were calculated as follows.

$$\text{Wt. of extract} = \frac{\text{Wt of beaker and extract} - \text{wt of empty beaker}}{\text{Wt of plant material}} \times \frac{100}{1}$$

The sample bottles containing tiger nut liquor were divided into two main groups thus:

Control (no treatment) and Tiger nut liquor + Alligator pepper extract. Each group was sub grouped of 1g, 2g and 3g of the extracts. Five samples were prepared per group in order to serve the required analyses and maintain a reasonable margin for error. After the various treatments, all the samples were pasteurized in a water bath set at 75°C for 20 minutes. The samples were stored at ambient temperatures (28°C) for two weeks.

Chemical Analysis

The pH meter was standardized using a phosphate buffer of pH 4 and 7. After which the pH of the prepared beverages was carried out on a weekly basis beginning from the time of preparation (Wk 0). 10ml from each of the treated and untreated tiger nut milk were dispensed by means of a sterile calibrated glass

pipette into a sterile 50ml conical flask and its pH was determined with the use of pH meter (Harrigan, 1998).

Microbial Analysis

The sample bottles were aseptically opened and 1ml aliquot collected by means of a micropipette and transferred into 9ml of sterile peptone water. Ten-fold serial dilution was accomplished and the appropriate dilution plated aseptically via spread plate technique on Mac-Conkey Agar (bacteria) and Saboraud Dextrose Agar (Fungi). The media were prepared, autoclaved and incubated strictly according to the manufacturer's instructions, after which the number of viable bacteria and fungi was counted, calculated and expressed as $\log_{10}$ colony forming units per ml (cfu/ml).

Data were analyzed using single factor analysis of variance (ANOVA) in the Statistical Package for Social Science (SPSS, 2003) Software (SPSS version12. 0.1 for windows).

RESULTS AND DISCUSSION

The following are the results of analysis carried out during the storage period. Figure 1 and 2 shows the percentage occurrences of bacteria and fungi, while Table 1 shows the pH and viable counts of bacteria and fungi ($\log_{10}$ cfu/ml).

The pH value on the preserved samples was higher, range (3.00-3.36). Microbial analysis showed that *Staphylococcus aureus* and *Saccharomyces* remained the predominant organisms throughout the storage. At Week 0 the samples containing preservatives recorded a low microbial count. At Week 1 and Week 2 there was drastic reduction in microbial load as the effects of the preservatives became evident. Moreover, the antimicrobial activities show that samples at 300mg/ml were most effective against microbes than the 200mg/ml and 100mg/ml. Hence, the active ingredients in the preservatives exhibited more potential at higher concentration than at lower concentration. This could be attributed to phytochemical reactions between the preservatives and the tiger nuts milk constituents. This finding corresponds to investigation of Wilkinson (2003) that Alligator pepper phytochemical constituents (zingiberene, 6-gingerol, 6-paradol and shagaols antimicrobial) affects food microbes. Samples without preservatives recorded high microbial counts throughout the storage period.

CONCLUSION

Results of these studies revealed a low microbial counts on the shelf – life of tiger nut milk between 0 - 2 weeks using Alligator pepper extract as a natural preservatives. This conform to Garbutt (1997) that microbial count less than 30 colonies or less than 2.4×14^4 cfu/ml in a mixed culture is insignificant in food quality and safety assessment. This shows that the use of natural preservatives in conjunction with proper sanitary practices would prolong the shelf life and quality of tiger nut milk.

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Table 1. The pH values and microbial counts (log_{10}cfu/ml) of Tiger nut Milk treated with Alligator pepper extract

Analysis	Storage Condition	Conc.	No of Weeks 0	1	2
pH	28°C	100	3.36±0.1[a]	3.33±0.5[c]	3.29±0.3[b]
		200	3.34±0.5[a]	3.32±0.1[c]	3.25±0.7[b]
		300	3.33±0.3[a]	3.30±0.8[c]	3.00±0.5[b]
		(control)	4.33±0.1[a]	4.40±0.6[c]	4.55±0.1[b]
Bacterial Counts (log_{10}cfu/ml)	28°C	100	1.72±0.07[a]	1.69±0.08[a]	1.66±0.05[ab]
		200	1.71±0.02[a]	1.65±0.05[a]	1.60±0.06[ab]
		300	1.63±0.08[a]	1.56±0.01[b]	1.54±0.07[b]
		(control)	2.32±0.05[a]	2.37±0.02[a]	2.39±0.01[a]
Fungal Counts (log_{10}cfu/ml)	28°C	100	1.83±0.05[a]	1.81±0.04[a]	1.79±0.04[a]
		200	1.81±0.05[a]	1.72±0.01[a]	1.70±0.02[a]
		300	1.68±0.03[a]	1.60±0.02[a]	1.57±0.02[a]
		(control)	2.43±0.01[b]	2.45±0.01[a]	2.52±0.03[a]

[1]Each data is the mean ± standard deviation of 3 determinations;
[2]Different letters within the same column are not significantly different ($p<0.05$)

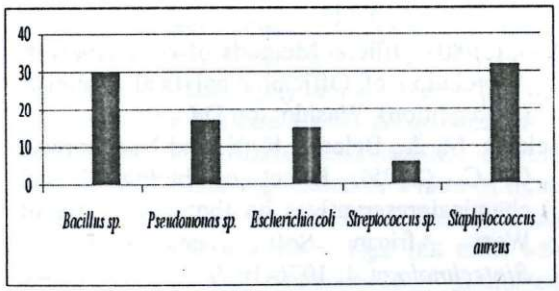

Figure 1. Percentage Occurrences of Bacteria in Tiger nut Tiger nut Milk stored at ambient temperature (28°C)

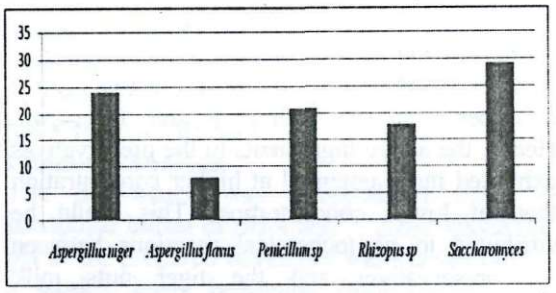

Figure 2. Percentage Occurrence of Fungi in Milk stored at ambient temperature (28°C)