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PRODUCTION AND EVALUATION OF MICROBIAL QUALITY AND STORAGE STABILITY OF TIGERNUT (*Cyperus esculentus*) MILK USING LIME (*Citrus aurantiifolia*) JUICE AS NATURAL PRESERVATIVE

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Abstract

Lime (*Citrus aurantiifolia*) juice preservative potentials were evaluated for the microbiological quality and storage stability of tigernut milk. The aqueous extract of tigernut milk (*Cyperus esculetus)* was preserved with lime juice. Pasteurized 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 ranged of 1.53±0.02^b to 1.73±0.01^{a}log10cfu/ml of the samples preserved using lime juice; the fungal counts recorded range from 1.70±0.07^a to 1.93±0.03^{a}log10cfu/ml while the control tiger nut milk ranges from 2.32±0.05^a to 2.55±0.03^{a}log10cfu/ml respectively. The tiger

Introduction

Tigernut (*Cyperus esculetus*) is an underutilized, juicy and sweet tuber crop. Its products (milk, flour) are enjoyed and patronize by most people around the world and northern part of Nigeria in particular due to its palatability when consumed as snacks, beverages and junk food (Belewu et al., 2005). Tigernut is a good source of micro and macronutrients, antioxidants, and sucrose sugar. It is a good source of food for old people with

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nut milk was acidic with a range of 4.50±0.1[a] to 4.60±0.5[a] pH value. At Week 0 the samples containing preservative recorded a low microbial count. There was significant 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. *Staphylococcus aureus, Streptococcus spp, Bacillus spp,* and *Pseudomonas spp* were isolated. While *Saccharomyces cerevisiae, Aspergillus niger, Aspergillus flavus* and *Penicillium spp* were fungi isolated. *Staphylococcus aureus* and *Saccharomyces spp* remained the predominant organisms throughout the storage period.

Keywords: Natural preservatives, lime juice, microbial quality, food preservative, storage stability, tigernut milk.

diabetic due to the presence of sucrose sugar, also ideal to children and sportsmen as healthy drinks (TTSL, 2005). According to Gherbawy, (2018), Aspergillus and Penicillium spices are common fungal natural contaminant associated with tigernut milk and flour their spoilage could be prevented by hygienic processing of the products.

Storage quality of tigernut milk has brought untold hardships and complications to tigernut milk vendors as well as the consumers. The use of synthetic additives as preservatives by consumers has been associated with various health problems, harmful or even carcinogenic (Bhutani, 2003). George *et al*. (2012) reported synthetic or chemical preservatives contains chemicals - calcium propionate, sodium nitrate, sodium nitrite, sulfites among others. Some chemicals have been shown to aggravate certain diseases, no wonder this world is so packed with serious disease such as diabetes, high blood pressure respiratory problems, behavioral changes, bloating, lethargy, in addition to other health problems like constipation and they always give a false appearance in the name of freshness, when majority of the times the food is stale. However, consumers quest towards ingredients from natural sources has brought the attention of the scientific community worldwide in shifting towards spices and herbs to harness their antimicrobial properties for use as natural

food preservatives. Moreover, this tendency has driven food and beverages industries in further search and development of natural preservatives for storage stability of products and maintain their safety (Bhutani 2003). Hence natural preservatives in tigernut milk are indispensable so as to prevent health problems associated with microbial spoilage or undesirable chemical changes in foods and beverages. Chukwuma (2012) reported the potential of lime juice as preservative on *Zobo* drinks. Likewise, the study investigated by Shan (2012) on the antibacterial efficiency of spices on different form of enteric bacteria. Similarly, preservation using lime on the control of microorganisms has also been investigated by (Plaza, 2004). Also, Adegoke et al., (2000) reported that natural preservatives are believed to have some desirable medicinal impacts on food without any negative influence on the overall organoleptic analysis. Lime juice contain citric acid and have some antimicrobial properties thus it could serve as natural preservative. Hence, the study is to evaluate lime (a natural preservative) on microbiological quality and storage stability on tigernut (*Cyperus esculentus)* milk.

MATERIALS AND METHODS

Materials

Material used in the production, preservation and analysis of tigernut milk includes the following; tigernut, lime fruits, kitchen utensil, muslin cloth, cooking pot, blender, nutrient agar, potato dextrose agar, peptone water, MacConkey agar, Blood agar, reagent and chemicals.

Sources of Materials

Fresh tigernut and lime fruits were procured from Owode market Offa, Kwara State and identified by IITA, Ibadan Oyo state. It was later brought to Nigerian Stored Products Research Institute Ilorin, Kwara State Laboratory for further studies. Media and chemicals used were prepared and standardized.

Preparation of Lime Juice

Hundred (100g) of lime fruits were surface- sterilized with (70%) ethanol and peeled using a pre-sterilized knife. The fruits were squeezed aseptically de-seed and transferred into a 100ml sterile sample bottles.

Preparation of Tigernut Milk

Two hundred and fifty-gram (250g) tigernut tuber was steeped for 4-8 hours and later grinded into mash using a sterilized Mammomlex Electric Blender. Cold water was added in a ratio of 3ml per unit gram of tigernut and the mixture was macerated. The macerated tigernut milk was pressed, sieved, and dispensed into graduated sterilized sample bottles (Okafor et al., 2003).

Sample Preparation

The sample bottles containing tiger nut milk were divided into two main groups thus: Control (no treatment) and Tiger nut milk + Lime juice. The Tiger nut milk + lime group was subdivided into 3 group with each group, sub grouped of 1ml, 2ml and 3ml of the lime juice added to 100ml of tiger nut milk. Five samples were prepared per group to serve the required analyses and maintain a reasonable margin for error. After the various treatments, all samples were pasteurized in a water bath set at 75^0C for 20 minutes and were stored at ambient temperatures (28^0C) for two weeks.

Microbiological Analysis on Tiger Nut Milk

The microbial analysis was done as described by (Gebremedhin et al., 2022). The samples bottles were aseptically opened and 1ml aliquot was collected by means of a micropipette and it was then transferred into 9ml of sterile peptone water. The samples from each treated and untreated bottle were serially diluted to 10^{-3} dilution strength and 1 ml of the diluent was transferred into sterile petri dishes using the pour plate method. The plates were carefully swirled, allowed to mix and solidify under UV light. The cultured nutrient agar plates in triplicate per treatment were then incubated at 37°C for 48 hours while the PDA plates were kept at 28°C for 72 hours.

Processing and Maintenance of Isolate

Axenic bacteria culture was obtained by as described by (Olutiola *et al.,* 1991). Aseptic streaking representative isolate of different morphological types from previous cultured plates onto freshly prepared nutrient agar slant which were later incubated at 37°C for 24 hours and refrigerated. The isolates were maintained on the slant until when required.

Identification of microbial isolates

The microbial isolated was identified as described by (Oniya et al., 2023). Gram staining, spore staining and Biochemical tests such as catalase, indole, methyl red, hydrogen sulphide, sugar fermentation tests for further identification of bacterial isolates. After which viable bacteria were counted, calculated and expressed as colony forming units (cfu) per ml. The types of the colonies grown were recorded with respect to the colours, shape elevation edges and opacity on the growth agar.
Fungal isolates were identified using lactophenol stain, microscopy for comparison of diagnostic septa. Coloured monographs and identification book of microfungi was employed (Vwioko et al., 2013).

Chemical Analysis on Tiger Nut Milk

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). Ten milliliters (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 (Mugula, 2003).
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 the table 1 below shows the pH and viable counts of bacteria and fungi (log_{10} cfu/ml).

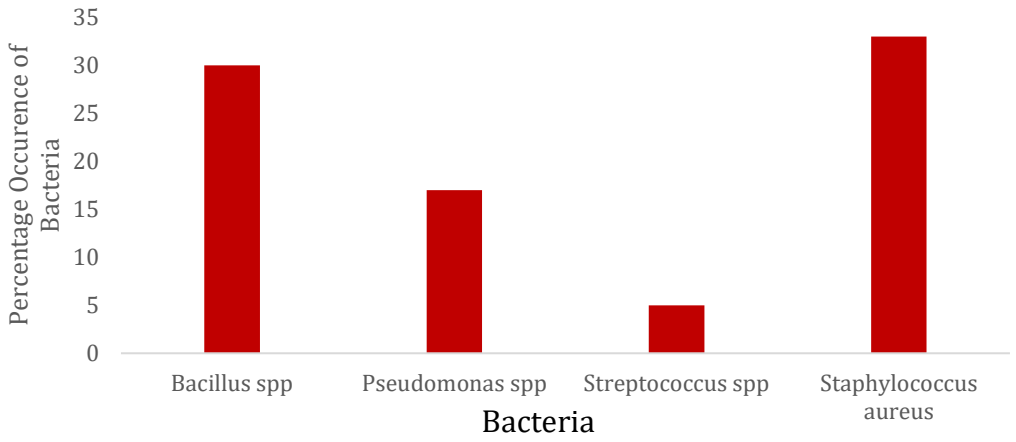

Figure 1: Percentage Occurrences of Bacteria in Tiger nut Milk stored at ambient temperature (28^0C)

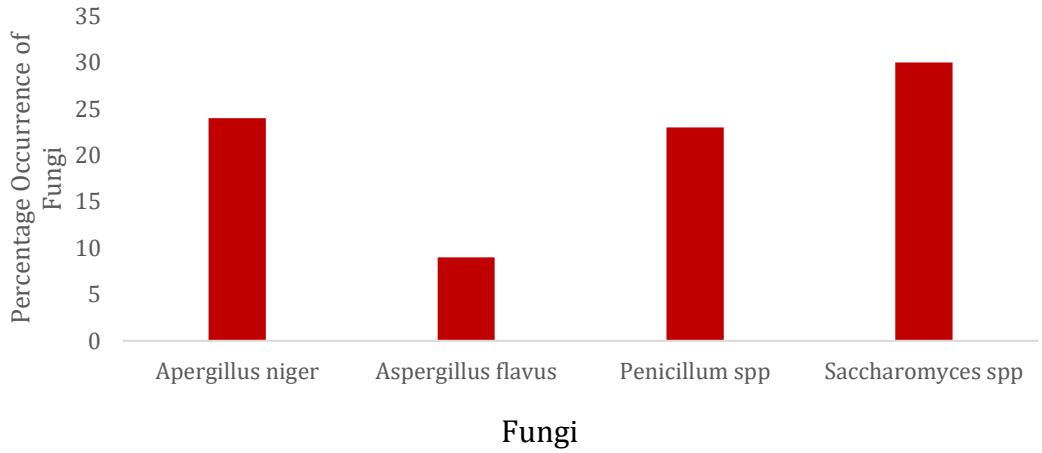

Figure 2: Percentage Occurrence of Fungi in Tiger nut Milk stored at ambient temperature (28^0C).

Table 1: Showing pH values and microbial counts of tiger nut milk treated with lime juice

Analysis	Storage Condition	lime added (ml)	No of Weeks 0	1	2
pH	28°C	1ml	3.40±0.5[a]	3.38±0.4[c]	3.36±0.2[b]
		2ml	3.38±0.3[a]	3.35±0.5[c]	3.33±0.3[b]
		3ml	3.36±0.6[a]	3.34±0.1[c]	3.32±0.7[b]
		(control)	4.50±0.1[a]	4.53±0.2[c]	4.60±0.5[b]
Bacterial	28°C	1ml	1.73±0.01[a]	1.70±0.06[a]	1.67±0.03[ab]
Counts		2ml	1.62±0.04[a]	1.60±0.02[a]	1.61±0.02[ab]
(log_{10}cfu/ml)		3ml	1.59±0.07[a]	1.54±0.05[b]	1.53±0.02[b]
		(control)	2.32±0.05[a]	2.37±0.02[a]	2.39±0.01[a]
Fungal	28°C	1ml	1.93±0.03[a]	1.90±0.01[a]	1.89±0.05[a]
Counts		2ml	1.80±0.03[a]	1.78±0.06[a]	1.76±0.08[a]
(log_{10}cfu/ml)		3ml	1.76±0.09[a]	1.71±0.08[a]	1.70±0.07[a]
		(control)	2.37±0.01[b]	2.43±0.02[a]	2.52±0.03[a]

Key:

[1]Each data is the mean ± standard deviation of 3 determinations;

[2]Different letters within the same column are not significantly different ($p<0.05$)

Discussion

The pH value of the preserved milk was higher, than the unpreserved milk, ranged from 3.32-3.40 and 4.50 to 4.60 respectively. The results show that the pH of the tigernut milk were acidic irrespective of the volume and concentration of preservative added and consequently decrease the growth and population of microorganisms. However, the tiger nut milk acidity, coupled with added preservatives in varying concentration could probably have contributed to the inability of the existing microbes to degrade and ferment the treated tigernut beverages the study is related to the study presented by Abenaa et al., (2014) that pH of milk prepared from unirradiated and irradiated tigernut cultivars are slightly acidic and may be harmful to pathogenic bacteria that causes gastrointestinal problems.

Lowest bacteria count of 1.53log_{10}cfu/ml was recorded in week 2 for the tiger nut with 3ml lime addition; while the highest bacteria count of 1.73log_{10}cfu/ml was recorded in week1 for tiger nut with 1ml lime addition. The bacteria count for the control was higher than that of the treated samples and it ranged from 2.32 to 2.39

log_{10}cfu/ml. For the fungal count, 1.70 log_{10}cfu/ml for tiger nut with 3ml lime addition was the lowest in value, while 1.93 log_{10}cfu/ml for tiger nut with 1ml lime addition was the highest in value. Also, the fungal count of the control ranged from 2.37 to 2.52 log_{10}cfu/ml and higher than that of the treated samples. In week 1 and 2 of preservation, samples without preservatives (control) had lost colour (turned dark brown), gone sour and odorous and hence exhibited the highest bacterial and fungal count and consequently had the poorest keeping quality compared with those containing preservatives. This is related to an investigation made by Ngouadjo (2013) that beverage ferment and turns sour when left for two or three days at room temperature and that this could be as a result of fermentation or microbial activities. Fermentation has been attributed to change of taste, nutritional value and increase rate of browning, offensive odour and perhaps presence of cloudy materials at the bottom of the container which is in an acute state after many days of preparation.

At week 0, the samples containing lime juice recorded low microbial count. At weeks 1 and 2, there was significant reduction in microbial load as the effects of the preservatives became evident. Moreover, the antimicrobial activities show that samples 3ml lime addition were most effective against microbes than the samples with 2ml and 1ml addition. Hence, the active ingredients in the preservatives exhibited more potential at higher concentration than at lower concentration. Lime juice (preservative) is acidic. It was found to inhibit the microbes in the tigernut milk. Moreover, Chukwuma et al., (2010) reported the presence of phytochemicals in raw and roasted tiger nut tuber. The reaction of phytochemical compound present in the substrate (tigernut) and citric acid in the preservative (lime) creates an unconducive environment for the microbes to thrive thereby leading to subsequent decrease in the population.

The occurrence of *Streptococcus spp, Pseudomonas spp., Bacillus spp* and *Staphylococcus aureus* observed in this study could be as a result of contamination from additives, water, utensils and the environment where it was processed. According to Brevik, et al., (2020) organisms are found in water and on skin surface as flora to the skin likewise, *Bacillus spp* spores are present in the environment, so their occurrence could be as a result of prevalence of their spores in environment and could survive high temperature of processing. Also, Fasoyiro et al., (2005)

reported presence of microorganism from addition of sugar and other additives after production.

CONCLUSION

This study showed high potentials of lime juice as a natural preservative. Over a period of two weeks addition of one milliliter (1ml) of lime juice was able to reduce the bacteria load by 27.9%, two milliliters and three milliliters (2ml and 3ml) of lime juice reduced the bacterial load by 32% and 34% respectively. In the same manner, 1ml, 2ml and 3ml addition of lime juice reduced the fungi load by 21.8%, 27% and 29% respectively. Hence, its addition in conjunction with proper sanitary practices prolong the storability of tiger nut milk. Thus, lime juice should be encouraged as natural preservative because it is relatively affordable and accessible in Nigeria.

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