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INFLUENCE OF PRESERVATION METHODS ON PH AND MICROBIOLOGICAL QUALITY OF TIGER NUT (*Cyperus esculentus*) MILK

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ABSTRACT
The deteriorating effect of microorganisms on tiger nut (Cyperus esculentus) milk has hampered its large-scale production and profitability. To study the effect of preservation on the pH and microbiological quality of the milk, big yellow tiger nuts were soaked in 3 L of tap water at 60°C for 6 hours, additives such as coconut, date, cinnamon and ginger were added and blended. The extracted milk was divided into twenty five portions and subjected to the following treatments: pasteurization, sterilization, ultraviolet light, freezing and sodium benzoate. The pH and microbiological quality of both the fresh and preserved samples were investigated over 9-day storage period. The pH of the milk samples significantly decreased ($p<0.05$) over the period due to microbial activity. There were significant ($p<0.05$) decrease and increase in the pH values of the preserved samples with negative control (FTM) and positive control samples (TMA) respectively. The less acidic the products are the higher the bacterial load. The values of the total bacterial count for the preserved milk ranged from 5.19±0.06 to 6.84±0.03 log_{10}cfu/ml. The values within this range were significantly lower ($p<0.05$) than FTM (6.58±0.05log_{10}cfu/ml) but higher than TMA (4.44±0.02 to 5.85±0.06 log_{10}cfu/ml). The organisms isolated from the samples were Staphylococcus species (16%), Clostridium species (11%), Bacillus species (10%), Acinetobacter species (3%), Enterobacter species (6%), Corynebacterium species (4%), Neisseria species (1%), Vibrio species (1%), Micrococcus species (4%), Aeromonas species (5%), Saccharomyces species (35%) and Rhizopus oryzae (4%). The results suggest that ultraviolet light and sterilization methods were more effective at eliminating most of the bacteria implicated in milk spoilage.
Keywords: Fresh, large-scale production, microbiological quality, preserved, tiger nut milk.

INTRODUCTION

Tiger nut (*Cyperus esculentus)* belongs to the Division–*Magnoliophyta*, Class–*Liliopsida,* Order–*cyperales* and Family–*Cyperaceae*, Species- *Cyperus esculentus* and was found to be a cosmopolitan, perennial crop of the same genus as the papyrus plant (Hankus and Sarret, 1967). The plant was introduced by the Arabs, first in the Valencia region. It is native to most of the Western Hemisphere as well as Southern Europe, Africa, Madagascar, the Middle East and the Indian Subcontinent (Abaejoh *et al*., 2006). The tuber is known by various names in Nigeria, as "Aya" in Hausa, "Imumu" in Yoruba and "Aki Hausa" in Igbo. Tiger nuts can be eaten raw, roasted, dried, or baked (Belewu and Abodunrin, 2006; Oladele and Aina, 2007; Rita, 2009). It can also be used for preparation of "kunu aya" (a local beverage in Nigeria) (Belewu and Abodunrin, 2008). Tiger nut milk or "kunu aya" is mostly consumed in the afternoon to cool the body from the hot weather, it is cheap and popular; available, affordable, drink of both the poor and the rich (Bamishaiye and Bamishaiye, 2011). Tiger nut milk is a nutritive and energetic drink both for old and young people (Abaejoh *et al*., 2006; David, 2010), is rich in energy content (starch, fat, sugar and protein), minerals (phosphorus, potassium) and vitamins E and C (Belewu and Belewu, 2007; Oladele and Aina, 2007). The milk is underutilized due to its short shelf life and lack of information on its nutritional potentials (Omode *et al*., 1995; Cortes *et al*., 2005). Apart from use as a beverage, tiger nut milk is thought to be beneficial to diabetic patients (if sugar-free) (Anderson *et al*., 2009) and those seeking to reduce cholesterol or lose weight (Martinez, 2003; Oladele and Aina, 2007; Borges *et al*., 2008), due to its content of carbohydrates with a base of sucrose and starch (without glucose), and its high content of arginine, which liberates the hormone that produces insulin (Chevallier, 1996; Alegría-Torán and Farré-Rovira, 2003). It was reported that tiger nut is high in dietary fiber content (Alegría-Torán and Farré-Rovira, 2003), which could be effective in the treatment and prevention of many diseases including colon cancer (Adejuyitan *et al*., 2009), coronary heart diseases (Chukwuma *et al*., 2010), obesity, diabetics and gastro-intestinal disorders (Anderson *et al*., 2009) and treatment of flatulence, indigestion, diarrhoea and dysentery (Bixquert-Jiménez, 2003) because it provides digestive enzymes like the catalase, lipase and amylase (Bixquert-Jiménez, 2003; Adejuyitan, 2011).

Tiger nut milk has a very short shelf life of often less than 24 hours depending on the condition of storage (Akoma *et al*., 2006).

High temperature and humidity significantly reduce the shelf life of the product (Nutso, 2014). As a result, tiger nut milk is often associated with significant microbial contamination, including bacteria and molds (Onovo and Ogaraku, 2007; Nutso, 2014). The short shelf life of raw tiger nut milk hinders widespread consumption of the beverage due to the deteriorating effects of some microorganisms on the milk (Abaejoh *et al*., 2006). Although, tiger nut milk has contributed to the upliftment of the living conditions of people especially women, where most women have developed the skills for commercial production of the milk (Musa and Hamza, 2013). Nwobosi *et al*. (2013) have made an attempt to delay its spoilage by addition of natural tropical preservatives and some physical methods such as short temperature long time pasteurization and refrigeration but with little success that could allow long distance transportation of the product and lengthened the storage time. This study was therefore aimed at determining the effects of some preservation methods on the pH and microbiological quality of laboratory scale tiger nut milk.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade.

Sample Collection

Big yellow tiger nut (the most commonly used for preparation of tiger nut milk) was obtained from Maggi Market in Sokoto, Sokoto State, Nigeria. The nuts were taken to the laboratory in a clean polythene bag for processing and analysis.

Sample Identification

The tiger nut was identified by a taxonomist in Botany unit of Usmanu Danfodiyo University, Sokoto and voucher number was assigned (UDUH/ANS/0082) and voucher sample was kept in the herbarium for future reference.

Sample Preparation

Tiger nuts were sorted out to remove broken, rotten, stones, pebbles, and other dirt materials before rinsing in water to remove adhering soils. Other ingredients used in the milk preparation (coconut, date, cinnamon and ginger) were also processed before use. The shell of the coconut was removed using knife and the water was discarded, the coconut flesh was cut into smaller pieces. The seed of the date was removed and discarded. These entire ingredients were thoroughly washed in warm water.

Tiger Nut Milk Preparation

One kilogramme (1 kg) of tiger nuts was soaked in 3 litres of boiled water at 60^{o}C for 6 hours according to modified method of Djomdi and Ndjouenkeu (2006). After washing the nut was mixed with 300 g of coconut, 150 g of date, 15 g of ginger and 3 g cinnamon, and then the mixture was blended with 6 L of cooled boiled water several times into slurry with engine moteur (GX 160) The slurry was pressed using muslin cloth to extract the milk. To the extracted milk 60 g of refined sugar were added. The extracted milk was transferred into sterile container.

Experimental Design

The milk was divided into twenty five portions and packaged into sterile cork bottle as 50 mL portions (each group has three representative samples except group 1 with one sample) and subjected to the respective treatments after 15 to 20 minutes of preparation as follows:

Group 1: Fresh tiger nut milk without treatment (FTM; Negative control).
Group 2: Tiger nut milk stored at 28 to 32^{o}C (TMA; Positive control).
Group 3: Tiger nut milk stored at 0^{o}C (FRTM).
Group 4: Tiger nut milk treated with 0.05% Sodium benzoate and stored at 28 to 32^{o}C (TMS).
Group 5: Tiger nut milk treated with 0.05% Sodium benzoate and stored at 0^{o}C (FSTM).
Group 6: Tiger nut milk irradiated with ultraviolet light and stored at 28 to 32^{o}C (UVTM).
Group 7: Tiger nut milk Pasteurized at 70 to 75^{o}C for 30 minutes and stored at 28 to 32^{o}C (LLPTM).
Group 8: Tiger nut milk Pasteurized at 90 to 95^{o}C for 15 to 30 seconds and stored at 28 to 32^{o}C (HSPTM).
Group 9: Tiger nut milk Sterilized at 121^{o}C for 15 minutes and stored at 28 to 30^{o}C (STM).

Microbiological Analysis

Nutrient agar and Sabouraud dextrose agar were prepared according to manufacturer's (Titan Biotech Ltd) instruction. The spread plate method of inoculation after serial dilution of the sample by 10^{-4} dilution factor was applied as described by Manga and Oyeleke (2008) a drop of the diluted sample was placed on the surface of solidified agar and bent glass rod was used to carefully spread the sample onto the agar. Different colonies of bacteria were selected and sub-cultured from the mixed cultures before they were incubated at 37^{o}C for 24 hours. Colonies that were grown on nutrient agar where gram stained in accordance with standard Gram staining procedure described by Tortora *et al*. (2003).

Characterization

Biochemical tests including motility, indole, catalase, citrate utilization, Methyl Red (MR), Voges- Proskauer (VP), Triple-Sugar Iron agar (TSI), starch hydrolysis, urease and hydrogen sulfide production were carried out in accordance with standard methods described by Cheesbrough (2002). The bacterial isolates were characterized and identified on the basis of their cultural, morphological and biochemical properties as described by Holt *et al*. (2000).

Isolation and Identification of Fungi

A sterile syringe was used to transfer 1 ml of 10^{-3} diluted sample onto the surface of prepared Sabouraud Dextrose Agar. The inoculum was then spread out thinly and evenly on the surface using a sterile bent glass rod.

The plates were then incubated at 25 to 28^{o}C for 72 hours. Colonies were identified on the basis of colonial and microscopic characteristics based on taxonomic schemes described by Ainsworth *et al*. (1973).

Data Analysis

The analysis was done in triplicate; results were expressed as Mean ± Standard error of mean. All microbial counts were converted to the base -10 logarithm of the number of colony forming units per mL of tiger nut milk samples (log_{10}cfu/ml).

Data was subjected to one way Analysis of Variance (ANOVA) and Dunnet compare all versus control was used to test for the level of significance between mean. Statistical significance was accepted at $p<0.05$. The results obtained from microbial screening were compared with FAO/WHO (2002) microbial limit for dairy milk.

RESULTS

Figure 1.0 presents the pH of fresh and preserved tiger nut milk. The pH of the preserved samples varied from 2.58±0.01 to 6.16±0.01. The values within this range are higher than the pH of positive control samples TMA (2.53±0.01 to 2.67±0.01) but lower than that of negative control FTM (6.75±0.02). FSTM after day 2 has the highest pH value (6.16±0.01) while TMS after day 9 has the least value (2.58±0.01). The pH of the preserved tiger nut milk decreases as the storage time (day) increased. There were significant decrease ($p<0.05$) in the pH value of the preserved tiger nut milk with FTM and significant increase ($p<0.05$) when compared with TMA with respect to the respective days except TMS which was not significant ($p>0.05$) with TMA throughout the storage period. Frozen tiger nut milk with and without sodium benzoate (FSTM and FRTM respectively) have a pH range (6.08±0.01 to 6.16±0.01) near the neutral pH (6.70 to 7.20) while samples treated with other preservatives, but stored at ambient temperatures (28 to 32^{o}C), have pH ranging from 2.58±0.01 to 4.36±0.01 (in the acidic range).

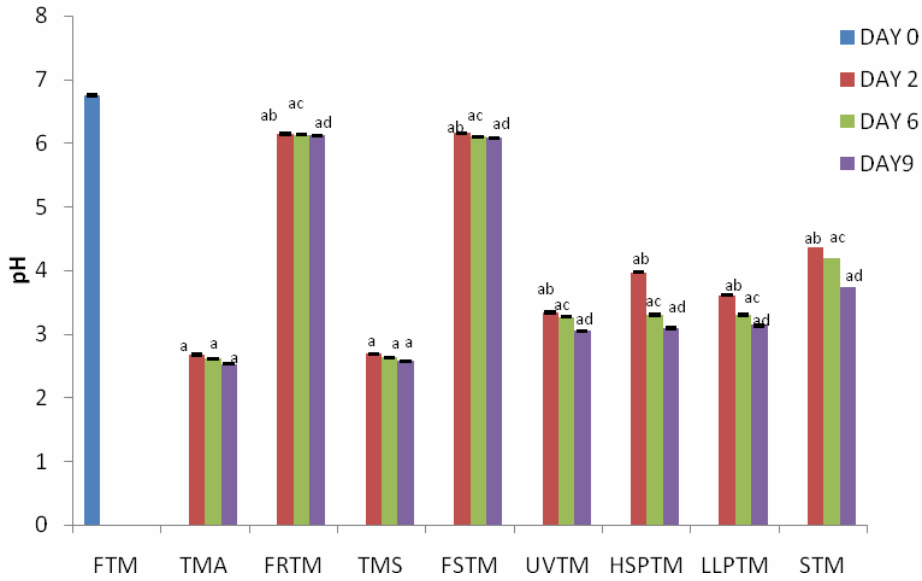

Figure 1.0: Effect of Storage Time (Day) on the pH of Fresh and Preserved Tiger Nut Milk
Key: FTM= Fresh Tiger Nut Milk (negative control), TMA= Tiger Nut Milk stored at 28 to 32^{o}C (positive control), FRTM= Tiger Nut Milk at 0^{o}C, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0^{o}C, UVTM= Tiger Nut Milk Irradiated with Ultraviolet Light, HSPTM= High Temperature Short Time Pasteurized Tiger Nut Milk, LLPTM= Low Temperature Long Time Pasteurized Tiger Nut Milk, STM= Sterilized Tiger Nut Milk. (^{a} denotes $p<0.05$ compared to negative control (FTM); ^{b,c,d} denote $p<0.05$ compared to positive control (TMA) at day 2, 6 and 9, respectively).

The effect of storage time (day) on total viable bacterial count of fresh and preserved tiger nut milk is presented in Figure 2.0. The values range from (5.19±0.06 to 6.84±0.03 $\log_{10}$cfu/mL) for the preserved milk. The values in this range are significantly lower ($p<0.05$) than that of FTM (6.58±0.05$\log_{10}$cfu/mL) and significantly higher ($p<0.05$) than TMA (4.44±0.02 to 5.85±0.06 $\log_{10}$cfu/mL). FSTM after day 6 has the highest number of viable bacterial count (6.84±0.03 $\log_{10}$cfu/mL) while LLPTM after day 6 has the least count (5.19±0.06 $\log_{10}$cfu/mL).

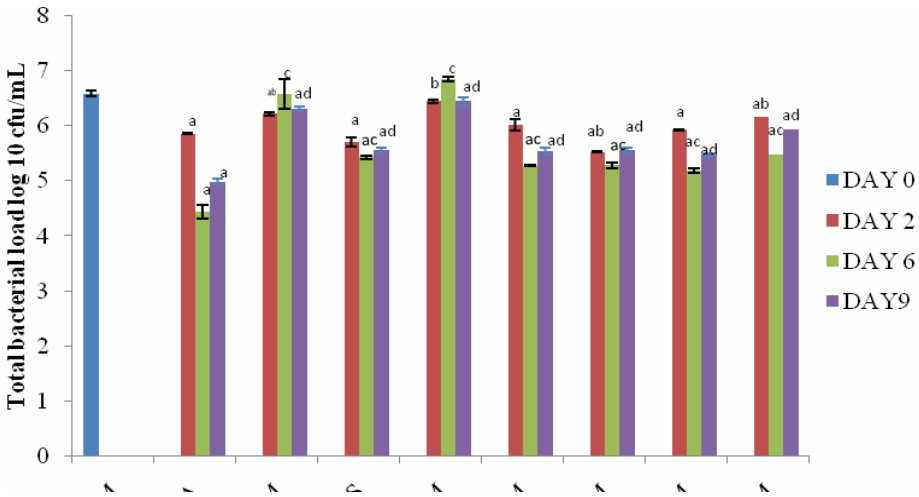

Figure 2.0: Effect of Storage Time (Day) on the Total Bacterial Count of Fresh and Preserved Tiger Nut Milk
Key: FTM= Fresh Tiger Nut Milk (negative control), TMA= Tiger Nut Milk stored at 28 to 32^{o}C (positive control), FRTM= Tiger Nut Milk at 0^{o}C, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0^{o}C, UVTM= Tiger Nut Milk Irradiated with Ultraviolet Light, HSPTM= High Temperature Short Time Pasteurized Tiger Nut Milk, LLPTM= Low Temperature Long Time Pasteurized Tiger Nut Milk, STM= Sterilized Tiger Nut Milk. (^{a} denotes p<0.05 compared to negative control (FTM); ^{b,c,d} denote p<0.05 compared to positive control (TMA) at day 2, 6 and 9, respectively).

Table 1.0 presents the isolated bacteria from tiger nut milk samples. A total of three species of bacteria were isolated from FTM they include *Bacillus cereus*, *Clostridium sordelli* and *Staphylococcus simulans*, while five species of bacteria were isolated from TMA which include *Acinetobacter calcoaceticus*, *Bacillus stearothermophilus*, *Clostridium sporogenes*, *Serratia fonticola* in addition with *Clostridium sordelli*. TMS and HSPTM have the highest number of bacteria species (five) isolated from each sample whereas; UVTM and STM have the least number one specie each. *Clostridium sordelli* is the dominant bacterial specie isolated from four samples such as FTM, TMA, FRTM and TMS.

Table 1.0: Bacteria Isolated from Fresh and Preserved Tiger Nut Milk

Bacteria \ Sample	FTM	TMA	FRTM	TMS	FSTM	UVTM	HSPTM	LLPTM	STM
Aeromonas veronii	-	-	-	-	-	-	+	+	-
Acinetobacter calcoaceticus	-	+	-	-	+	-	-	-	-
Bacillus alvei	-	-	-	-	-	-	+	-	-
Bacillus cereus	+	-	-	-	-	-	+	-	-
Bacillus subtilis	-	-	-	-	-	-	+	-	-
Bacillus stearothermophilus	-	+	-	-	-	-	-	-	+
Clostridium sporogenes		+	-	+	-	-	-	-	-
Clostridium sordelli	+	+	+	+	-	-	-	-	-
Staphylococcus simulans	+	-	+	-	-	-	-	-	-
Staphylococcus schleiferi	-	-	+	+	-	-	-	-	-
Staphylococcus cohnii	-	-	-	+	+	-	-	-	-
Staphylococcus caprae	-	-	-	-	-	-	-	+	-
Corynebacterium xerosis	-	-	-	-	+	-	+	+	-
Micrococcus luteus	-	-	-	-	-	+	-	-	-
Klebsiella pneumonia	-	-	-	-	-	-	-	+	-
Serratia fonticola	-	+	+	-	-	-	-	-	-
Vibrio parachaemolyticus	-	-	-	-	+	-	-	-	-
Neisseria mucosa	-	-	-	+	-	-	-	-	-

Key: FTM= Fresh Tiger Nut Milk (negative control), TMA= Tiger Nut Milk at 28 to 32^{o}C (positive control), FRTM= Tiger Nut Milk at 0^{o}C, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0^{o}C, UVTM= Tiger Nut Milk Irradiated with Ultraviolet Light, HSPTM= High Temperature Short Time Pasteurized Tiger Nut Milk, LLPTM= Low Temperature Long Time Pasteurized Tiger Nut Milk, STM= Sterilized Tiger Nut Milk, + = Present, - = Not Present.

The isolated fungi from both the preserved and unpreserved tiger nut milk samples are presented in Table 2.0. A total of three species of fungi were isolated, including *Saccharomyces pombe,* Saccharomyces *cerevisiae* and *Rhizopus oryzae*. *Saccharomyces pombe* was the dominant fungi isolated. Whereas, *Saccharomyces cerevisiae* was isolated from high heat treated samples (HSPTM and STM) and *Rhizopus oryzae* was only isolated from sterilized tiger nut milk (STM).

Table 2.0: Fungi Isolated from Fresh and Preserved Tiger Nut Milk

Bacteria \ Sample	FTM	TMA	FRTM	TMS	FSTM	UVTM	HSPTM	LLPTM	STM
Saccharomyces pombe	+	+	+	+	+	+	+	+	+
Saccharomyces cerevisiae	-	-	-	-	-	-	+	-	+
Rhizopus oryzae	-	-	-	-	-	-	-	-	+

Key: FTM= Fresh Tiger Nut Milk (negative control), TMA= Tiger Nut Milk at 28 to 32^{o}C (positive control), FRTM= Tiger Nut Milk at 0^{o}C, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0^{o}C, UVTM= Tiger Nut Milk Irradiated with Ultraviolet Light, HSPTM= High Temperature Short Time Pasteurized Tiger Nut Milk, LLPTM= Low Temperature Long Time Pasteurized Tiger Nut Milk, STM= Sterilized Tiger Nut Milk.
+ = Present, - = Not Present.

The percentage frequency of occurrence of isolated organisms from preserved and unpreserved tiger nut milk is presented in Figure 3.0. A total of 12 genera of Bacteria and Yeast are isolated from the samples. *Saccharomyces* species has the highest frequency of occurrence (35%); followed by *Staphylococcus* species (15%) and the organisms with least frequency of occurrence are *Neisseria mucosa* and *Vibrio parachaemolyticus* (1%) each.

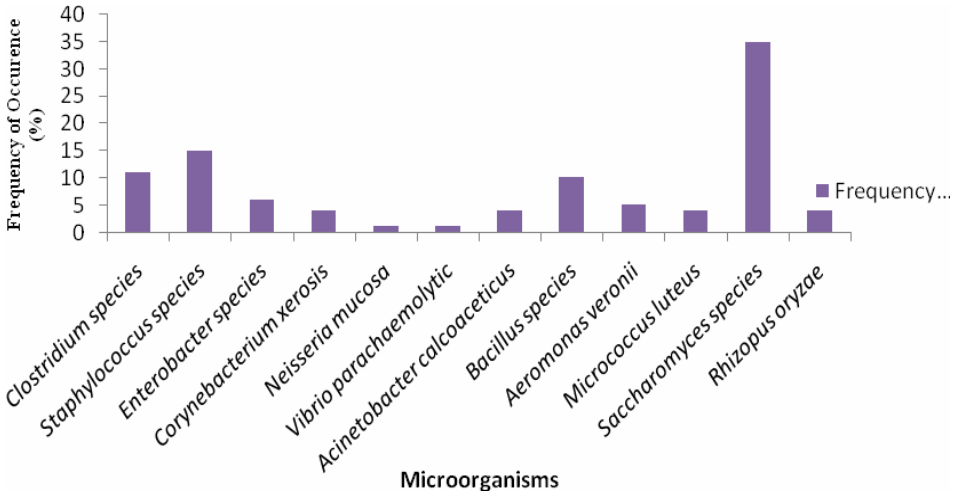

Figure 3.0: Percentage Frequency of Occurrence of Isolated Bacteria and Fungi

DISCUSSION

The effect of some preservation methods on the microbiological quality of laboratory produced tiger nut milk was investigated with a view to extending the shelf life of the milk. Bio-deterioration of the milk was influenced by factors like pH, temperature and microbial load. The significance differences observed in the pH of the products indicate that tiger nut milk owes it acidity or pH to the actions of certain microorganisms. This type of acidity is referred to as biological acidity (James, 2000). It has been well established that most microorganisms grow best at pH values around 7.0 (6.6-7.5), whereas few grow below 4.0 (James, 2000). This might be the reason for high total bacterial count of FTM. Bacteria tend to be more fastidious in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious (James, 2000). This may account for the presence of *Saccharomyces* species in all the samples and further support the decrease in the total bacterial load of acidic samples (ambient stored samples). The acidity of TMS, UVTM, HSPTM and LLPTM tends to increase with increase in fermentation period resulting in spoilage. This level of acidity (pH 3.05±0.01 to 4.36±0.01) of tiger nut milk was similar to that reported by several researchers Efiuvwevwere and Akoma (1995) reported pH of 3.20 for tiger nut milk; Akoma *et al*. (2006) reported a pH range of 3.32 to 4.15 also for tiger nut milk. Musa and Hamza (2013) reported pH range of 3.5 to 4.5 for tiger nut milk consumed by students of Kaduna state University.

The pH of FSTM and FRTM was slightly acidic.

It is a well known fact that foods with higher pH values are subject more to bacteria than fungi spoilage (James, 2000; James, 2012; Paul, 2014). This might be the reason for high total bacterial counts of these samples.

The greatest problem encountered with spoilage organisms in the samples is their survival at freezing temperature. It is known that the higher the concentration of pathogenic micro-flora in milk, the higher chances of spoilage (Brooks and Asamudo, 2003). Lower concentrations of pathogenic micro-flora in the milk samples does not suggest its wholesomeness as low microbial load may be due to bactericidal effect of metabolites of some of the bacteria that leads to low pH which thus inhibit the growth and survival of most bacterial species. The decrease in total bacterial count of TMA could probably be due to the low pH which tends to inhibit the growth of most bacteria. Likewise, the use of 0.05% sodium benzoate produced a decrease in microbial load, indicating that it could be used for preserving unrefrigerated tiger nut milk to some extent. This decrease could be linked to the low pH value and the fact that sodium benzoate exerts its mechanistic action at pH below 5 retarding the growth and survival of most microorganisms that cause food spoilage (Krebs *et al*., 1983). The total bacterial loads of day 6 and 9 TMS samples were fairly good where as that of day 2 was manageable. None of these samples satisfy FAO/WHO (2002) microbial limit (5.30 $\log_{10}$cfu/ml) for dairy milk. Ultraviolet light treated tiger nut milk followed the same trend with 0.05% sodium benzoate treated tiger nut milk. This might be that ultraviolet light was able to destroy some spoilage microorganisms through braking of bonds in the DNA producing thymine-thymine dimer resulting in the death of the organisms. UVTM 6 and 9 were fairly good and day 2 sample was manageable, only day 6 UVTM product satisfies the FAO/WHO (2002) acceptable limit (5.30 $\log_{10}$ cfu/ml). Pasteurized and sterilized tiger nut milk also recorded a significant decrease in microbial load. The bacterial loads of pasteurized and sterilized tiger nut milk reported in this study do not agree with the finding of Ukwuru and Ogbodo (2011) who reported that pasteurized and sterilized tiger nut milk were microbiologically stable for two weeks (storage period). This variation might be due to high temperature (130^{o}C for 10 seconds) applied for sterilization and storage under refrigerated temperature (4 ± 2^{o}C) in the previous study. None of the pasteurized and sterilized samples was good for consumption. All the HSPTM, LLPTM 6 and 9, and STM 6 were fairly good for consumption. The result of HSPTM corroborate with the finding of Nwobosi *et al*. (2013) who reported that the bacterial load of pasteurized tiger nut extract stored at 4±2^{o}C of most treatment were fairly good for consumption. Whereas, LLPTM 2, STM 2 and 9 were manageable, only HSPTM 6 and LLPTM 6 met the FAO/WHO (2002) microbial limit for dairy milk. The decrease could be that pasteurization and sterilization of the milk destroyed some pathogenic and spoilage organisms. In line with the guidelines for microbiological quality of milk and dairy products, none of the frozen tiger nut milk products was satisfactory (FAO/WHO, 2002). Ihekoronye and Ngoddy (1995) states that milk sample containing 5.00 x 10^3cfu/ml (3.70 $\log_{10}$cfu/ml) of bacteria is classified as good for consumption, 1.00 x 10^4 to 4.00 x 10^5 cfu/ml (4.00 to 5.60 $\log_{10}$cfu/ml) is fairly good, 2.00 x10^6 (6.30 log10cfu/ml) is manageable, while over 2.00 x 10^7 cfu/ml (7.30 $\log_{10}$cfu/ml) is bad for consumption.

The rich nutrient and moderate pH of tiger nut milk makes it an excellent culture medium for the growth of microorganisms especially bacteria. Hence, the more the nutrients content of the milk were available, the higher the bacterial load. Milk products are, however, easily perishable because contaminating bacteria may multiply rapidly and render it unfit for human consumption (Ukwuru *et al*., 2011). The results obtained from the microbial analysis of fresh and preserved tiger nut milk show that all the products harboured microorganisms. Most of the bacterial strains isolated were pathogenic while the fungi were basically fermentative microorganism which contributes to the acidity of the milk.

Most of the organisms isolated from the milk samples are implicated in both food spoilage and food-borne diseases (Nester *et al*., 2004; Hubert *et al*., 2011). Their presence might be due to the source of the raw materials purchased from the open market under conditions that allow the organisms in/on them to thrive. The surfaces of harvested grains, legumes, nuts and other food substance retain some of the natural micro-flora they had while growing on the field in addition to contamination from soil, water, insects, and other sources (Edema and Omemu, 2004). The presence of some of these organisms is not surprising as most of them have been isolated from tiger nut tubers (Hubert *et al*., 2011) and are known to thrive in media rich in fermentable substrates such as sugars which often led to the production of acids after fermentation (Essien *et al*., 2011).

Staphylococcus species were the dominant bacteria isolated in the samples. These species include *S. simulans*, *S. schleiferi*, *S. cohnii* and *S. capra*e. These species are known to colonize the skin and upper respiratory tracts of mammals (Kloos, 1980). *Staphylococcus* can cause a wide variety of diseases in humans and animals through either toxin production or penetration (Kloos, 1980). Staphylococcal toxins are a common cause of food poisoning (Kloos, 1980). *Acinetobacter, Aeromonas, Bacillus, Enterobacter* are known to antagonise the growth of *Staphylococcus* species through competition for nutrients and modification of the environment to conditions less favourable (Mossel, 1975). These might be the reason *Staphylococcus* species were not isolated from TMA and most of the ambiently preserved tiger nut milk where these organisms were isolated.

In general, Staphylococci may be expected to exist, at least in low numbers, in any or all food products that are handled directly by humans, unless heat-processing steps are applied to effect their destruction (James, 2000). Although being mesophilic, some species of *Staphylococcus* can grow at temperature as low as 6.7°C (James, 2000). The *Staphylococci* are unique in being able to grow at low water activity (James, 2000); this could explain their survival at freezing temperature.

The *Bacillus* species which were isolated from tiger nut milk include *B. cereus*, *B. stearothermophilus*, *B. subtilis* and *B. alvei*. These species were present in FTM, HSPTM and STM. James (2000) states that low numbers of these organisms can be found in a number of food products, including fresh and processed. They are inhabitants of soil and are able to withstand high temperature due to their ability to form spores (Pelczar *et al.,* 1993; Essien *et al*., 2011). The thermoduric nature of the spores of these microbes ensures survival at pasteurization and even sterilization temperatures (Essien *et al*., 2011) and hence their presence in the milk samples was not surprising. The ropiness associated with the fermented tiger nut milk has been associated with the presence of *Bacillus subtilis* (Adegoke *et al*., 1993). The presence of *Bacillus* species in most of the milk samples may be attributed to the fact that their immediate source is usually plant material due to their presence in the soil (Kawo and Abdulmumin, 2009). The source of the organisms might be during processing due to the abundant nature of their spores in air and water (Kawo and Abdulmumin, 2009), but it may also have originated from one of the ingredients (sugar) (Banwart, 1989). *Bacillu*s species are food-borne pathogens associated with health hazards (FAO, 1979; Odu and Adeniji, 2013). The identified *Bacillus* species reported in this study have also been isolated from tiger nut based milk (Adesiyun *et al*., 1995; Onovo and Ogaraku, 2007; Hubert *et al*., 2011; Udeozor and Awonorin, 2014; Sherifah *et al*., 2014).

The fungi isolated were *Saccharomyces pombe, Saccharomyces cerevisiae and Rhizopus oryzae.* Udeozor and Awonorin (2014) also reported the isolation of these organisms from tiger nut-soy milk drink. *Saccharomyces cerevisiae* has also been reported to be isolated from tiger nut milk (Onovo and Ogaraku, 2007). *Saccharomyces pombe* and *Saccharomyces cerevisiae* are harmless organisms, they have an extensive history of use in the area of food processing, especially *Saccharomyces cerevisiae* which is commonly used in bread making and as a fermenter of alcoholic beverages (Battock and Azam-Ali, 1998). *Rhizopus oryzae* is the most common cause of mucormycosis, also referred to as zygomycosis (Julie *et al*., 2000). It is commonly found in dead organic matter and soil (Battock and Azam-Ali, 1998). This microorganism is not only an opportunistic pathogen that cause human disease in immunocompromised people, such as those with diabetes mellitus, cancer, or AIDS, but also used as the source of making fermented foods and alcoholic beverages in Asia (NCBI, 2013).

Preservation prevents or slows down food spoilage, loss of quality, edibility or nutritional value and allow for longer food storage via eliminating the influence of chemical and biological factors.

Pasteurization eliminates some of the microorganisms in pasteurized tiger nut milk, but the presence of the isolated species might be due to their ability to withstand pasteurization temperature and also possibility of recontamination from the immediate surrounding as the products were stored at ambient temperature. The result of the present study on pasteurized tiger nut milk does not agree with the finding of Ukwuru and Ogbodo (2011) who reported no growth of microorganism in pasteurized tiger nut milk for a two weeks storage period. This variation might be due to varied method of milk extraction employed, as in the case of previous study (Ukwuru and Ogbodo, 2011) which used preheating at different stages in the extraction of the milk and the pasteurized milk was stored at refrigerated temperature (4±2^{o}C). Sterilization destroyed most of the spoilage organisms in STM. The isolated microorganisms were quite different from those isolated from the other samples. This might be that the organisms survived sterilization temperature or possibility of post sterilization contamination. This finding does not corroborate the finding of Ukwuru and Ogbodo (2011) who reported no bacteria isolation in sterilized tiger nut milk kept for two weeks. This variation might be due to higher temperature (130^{o}C) used in previous study and the fact that the sterilized milk was stored at 4±2^{o}C against 28 to 32^{o}C used in the present study. Preservation by freezing maintained all the isolated organisms from FTM, but was effective at eliminating *Bacillus*, although, some of the species isolated from TMA were also isolated from FRTM and FSTM. Sodium benzoate was only effective at eliminating *Bacillus*. This ineffectiveness of sodium benzoate might be due to very low concentration of the salt used and also, the fact that the salt was added at the time when the pH of the milk could not favour the un-dissociated form of the salt that was required to enter the cell of the organism to initiate changes that could kill or disable the organisms. Ultraviolet light was able to destroy some of the microorganisms as only two microorganisms were isolated from UVTM samples throughout the storage period. This may be due to the ultraviolet light possibly breaking the bond within the DNA producing thymine-thymine dimer that can kill the organisms.

CONCLUSION

The results of this study revealed that the more acidic the tiger nut milk, the lower the viable bacterial load. Several pathogenic bacteria and fermentative fungi were implicated in the milk spoilage with *Staphylococcus* species and *Saccharomyces* species being the dominant organisms isolated in this study. Ultraviolet light and sterilization methods were more effective at eliminating most of the bacteria implicated in the milk spoilage. None of the preservation methods was effective at eliminating *Saccharomyces* species.

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