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Effects Of Preservation Methods On Ph, Proximate Composition And Microbial Quality Of Laboratory Scale Tiger Nut Milk Beverage

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Abstract

Tiger nut (*Cyperus esculentus*) milk beverage is a nutritive, energetic and popular drink mostly produced and consumed in Northern Nigeria. However, the beverage has poor shelf-life which hampered its large-scale production and profitability. Big yellow tiger nut was soaked in 3 L of tap water at 60°C for 6 hours, additives such as coconut, date and ginger were added and blended. The extracted milk was divided into nine portions and subjected to the following treatments pasteurization, sterilization, ultraviolet light, freezing and sodium benzoate. Proximate composition and microbial quality of both the fresh and treated samples were investigated over 9 day storage period. The pH of the milk samples significantly (p<0.05) decreased over the storage period due to microbial activity. Preservation by freezing maintained most of the nutrient content of tiger nut milk whereas all ambient temperature preserved (28 to 32°C) samples had a significant (p<0.05) decrease in crude fiber and total carbohydrates and a significant increase in crude lipid. The less acidic the products are the higher their bacterial load. The bacterial load for the preserved milk ranged from 5.19±0.06 to 6.84±0.03 $\log_{10}$cfu/ml. The organisms isolated from the samples were *Staphylococcus species*, *Bacillus* species, *Clostridium species*, *Saccharomyces species* and *Rhizopus oryzae.* The findings indicate that the excellent keeping quality of tiger nut milk is due in great part to pH.

***Key words**: Microbial quality, milk, preservation methods, proximate, tiger nut beverage*

Introduction

Tiger nut (*Cyperus esculentus*) (also called chufa sedge, nut grass, yellow nut sedge, tiger nut sedge or earth almond) is a crop of the sedge family widespread across much of the world (Abaejoh *et al*., 2006). Tiger nut can be eaten fresh, dry or process to produce varieties of product including tiger nut milk, the tuber is known by various names in Nigeria, as "Aya" in Hausa, "Imumu" in Yoruba and "Aki Hausa" in Igbo. Tiger nut milk popularly known in the Northern part of Nigeria as "Kunu aya" is one of the indigenous, locally fermented, non-alcoholic beverage drinks that is widely consumed for its thirst-quenching property and nutritional content. Its extensive consumption occurs during the dry season (Okafor and Nwachukwu, 2003). Significant variations exist in the methods for production of the milk, depending on the desired taste that leads to differences in quality. While some prefer the milk with different fruit flavours, others prefer it with no sugar. Tiger nut milk has been tried as an alternative source of milk in fermented products, such as yogurt production, and other fermented products common in some African countries and can thus be useful replacing milk in the diet of people intolerant to lactose to a certain extent (Sánchez-Zapata *et al*., 2012). Tiger nut milk has a poor shelf life (Akoma *et al*., 2006) with significant microbial contamination, including bacteria and moulds (Onovo and Ogaraku, 2007; Nutso, 2014). This study was aimed at determining the effects of some preservation methods on the pH, proximate composition and microbial quality of laboratory produced tiger nut milk beverage.

Materials and Methods

Sample Collection

Big yellow tiger nut was obtained from Maggi Market in Sokoto, Sokoto State, Nigeria. The tubers 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, 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 nut tubers were sorted out to remove unwanted materials, it was then rinsed in water to remove adhering soils. Ingredients such as coconut, date, cinnamon and ginger were processed, thoroughly washed in warm water and added to tiger nut for the milk production.

Tiger Nut Milk Preparation

One kilogramme (1 kg) of tiger nuts was soaked in 3 litres of boiled water at 60ºC 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. The milk was divided into nine portions and packaged into sterile cork bottle as 300 mL portions and subjected to the various treatments after 15 to 20 minutes of preparation.

Experimental Design

The milk was divided into twenty five portions and packaged into sterile cork bottle as 100 mL portions (each group has three representative samples except UTTM with four samples) and subjected to the respective treatments after 15 to 20 minutes of preparation as follows:
UTTM: Untreated tiger nut milk.
FRTM: Tiger nut milk stored at 0ºC.
TMS: Tiger nut milk treated with 0.05% Sodium benzoate and stored at 28 to 32ºC.
FSTM: Tiger nut milk treated with 0.05% Sodium benzoate and stored at 0ºC.
UVTM: Tiger nut milk irradiated with ultraviolet light and stored at 28 to 32ºC.
LLPTM: Tiger nut milk Pasteurized at 70 to 75ºC for 30 minutes and stored at 28 to 32ºC.
HSPTM: Tiger nut milk Pasteurized at 90 to 95ºC for 15 to 30 seconds and stored at 28 to 32ºC.
STM: Tiger nut milk Sterilized at 121ºC for 15 minutes and stored at 28 to 30ºC.

Analysis of Sample

Proximate composition and pH of the milk samples were analysed at day 0 for UTTM and after 2, 6 and 9 days for the remaining portions of UTTM and the preserved samples according to standard methods of Association of Official Analytical Chemists (AOAC, 1995).

Microbiological Analysis

Media Preparation

The media: Nutrient Agar and Sabouraud Dextrose Agar were prepared according to the manufacturer's (Titan Biotech Ltd) instruction.

Bacterial Count

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).

Bacterial Identification

Bacteria were identified based on microscopy and biochemical tests as described by Cheesbrough (2002) and characterization was done by method of Holt *et al*. (2000).

Isolation and Identification of Fungi

A sterile syringe was used to transferred 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 37ºC for 72 hours. Colonies were identified by 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 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 $p<0.05$.

Results

Effect of Preservation on pH

Table1.0 presents the pH of preserved and unpreserved 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 unpreserved tiger nut milk at day 2 to 9 (2.53±0.01 to 2.67±0.01) but lower than that of UTTM 0 (6.75±0.02). FSTM after day 2 has the highest pH value (6.16±0.01) while TMA 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. 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 32ºC), have pH ranging from 2.58±0.01 to 4.36±0.01 (in the acidic range).

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Table 1. Effect of Storage Time (Day) on the pH of Fresh and Preserved Tiger Nut Milk

DAY	UTTM	FRTM	TMS	FSTM	UVTM	HSPTM	LLPTM	STM
0	6.75±0.02							
2	2.67±0.01 [a]	6.15±0.02 [ab]	2.69±0.02 [a]	6.16±0.01[a]	3.34±0.01[ab]	3.97±0.01[ab]	3.61±0.01[ab]	4.36±0.01[ab]
6	2.61±0.01 [a]	6.13±0.01 [ac]	2.63±0.01 [a]	6.10±0.01 [ac]	3.27±0.01 [ac]	3.30±0.01 [ac]	3.30±0.02 [ac]	4.19±0.02 [ac]
9	2.53±0.01 [a]	6.12±0.01 [ad]	2.58±0.01 [a]	6.08±0.01 [ad]	3.05±0.01 [ad]	3.09±0.01 [ad]	3.14±0.02 [ad]	3.74±0.02 [ad]

Key: UTTM= Unpreserved Tiger Nut Milk (control), FRTM= Tiger Nut Milk at 0ºC, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0ºC, 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. Values are means ± standard error of 3 determinations. ([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).

Effect of Preservation on Proximate Composition of Tiger Nut Milk

Table 2.0 shows the effect of storage time (day) on the proximate composition of preserved and unpreserved tiger nut milk. The moisture content of the preserved samples varied from 65.38±0.01 to 73.43±0.03%. FRTM 9 has the highest moisture value (73.43±0.03%) while STM 9 has the least value (64.43±0.02%). The moisture content of FRTM and FSTM increased with storage time (day). While the moisture content of preserved and unpreserved tiger nut milk stored at ambient temperature decreased with storage time (day). The ash content of the preserved samples ranged from 1.33±0.22 to 1.98±0.32%. STM 2 has the highest ash value (1.98±0.32%) while TMS 9 has the least value (1.33±0.22%). There are no significant ($p<0.05$) differences in the ash content of the samples compared to UTTM throughout the storage days.

The crude lipid content of the preserved samples ranged from 13.44±0.29 to 22.32±0.10%. The values within this range are significantly ($p<0.05$) lower than 21.18±0.11 to 24.31±0.19% for UTTM 2, UTTM 6 and UTTM 9 whereas, the crude lipid value for UTTM 0 (14.44±0.06%) is within the range. UVTM 9 has the highest crude lipid value (22.32±0.10%) and FSTM 9 has the least value (13.44±0.29%). The crude lipid content of FRTM and FSTM decreased with increased in storage time (day) while that of the preserved and unpreserved samples (kept at 28 to 32ºC) increased with increased in storage time (day). The crude protein content of the preserved samples ranged from 6.47±0.08 to 11.54±0.21%. STM 9 has the highest value (11.54±0.21%) while FSTM 2 has the least (6.47±0.08%). There are significant ($p<0.05$) differences in the crude protein of the samples as compared with UTTM 0 except with FRTM 6, FRTM 9, FSTM 2, FSTM 6 and FSTM 9 which are not significantly ($p>0.05$) different. There are significant ($p<0.05$) differences in the crude protein content of the milk samples as compared with UTTM 2, 6 and 9 at the respective days except TMS 2, UVTM 2, UVTM 6, HSPTM 6, HSPTM 9, LLPTM 6, LLPTM 9 and STM 2 ($p>0.05$).

The crude fiber content of the preserved samples ranged from trace to 2.13±0.01%. The values in this range are significantly ($p<0.05$) higher than that of UTTM at day 2, 6 and 9. FSTM 2 has the highest crude fiber content (2.13±0.01%). The total carbohydrate content of the preserved samples followed the same trend with the crude fiber, the values varied (trace to 5.67±0.73%). The values within this range are lower than that of UTTM 0 (7.54±0.64%). FSTM 9 has the highest carbohydrate content (5.67±0.73%) followed by FSTM 2 (5.15±0.62%) and the least carbohydrate content is in samples UTTM 2, 6, 9 and HSPTM throughout the storage period. There are significant ($p<0.05$) decrease in the total carbohydrate content of the samples as compared with UTTM 0 except FSTM 9. There are no significant ($p>0.05$) differences in the total carbohydrate content of most of the preserved tiger nut milk compared to UTTM 2, UTTM 6 and UTTM 9 except FRTM and FSTM which are significantly ($p<0.05$) higher throughout the storage period.

Effect of Preservation on Bacterial Count

Table 3.0 presents the effect of storage time (day) on the total bacterial count of preserved and unpreserved tiger nut milk. 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 UTTM 0 (6.58±0.05$\log_{10}$cfu/ml) and significantly higher ($p<0.05$) than UTTM 2 to 9 (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).

Discussion

The importance of nutrient content of food to the growth and welfare of microorganisms cannot be overemphasized. With respect to nutrient content of food, moulds have the lowest requirement, followed by yeasts, gram-negative bacteria, and gram-positive bacteria (James, 2000; Norman, 2012). All the samples had high moisture contents. This could affect the stability and safety of food with respect to microbial growth and proliferation. Microorganisms need water in an available form to grow in food products (James, 2000). The range of moisture content obtained in this study does not corroborate previous studies; Belewu and Belewu (2007) reported 92.27% moisture content, Ukwuru and Ogbodo (2011) reported 77.0 to 80.7%, Musa and Hamza (2013) reported 62.80 to 82.50%, Adedokun *et al*. (2014) reported 79.29% and Sherifah *et al*. (2014) reported 70 to 80% moisture content for milk extracted from tiger nut.

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Table 2. Effect of Storage Time (Day) on the Proximate Composition of Preserved and Unpreserved Tiger Nut Milk

Samples	% Moisture	% Ash	% Crude lipid	% Crude Protein	% Fiber	% TC
UTTM	76.43±0.02	2.48±0.34	14.44±0.06	1.61±0.10	1.25±0.02	5.04±0.51
UTTM 2	72.70±0.01^{a}	1.67±0.34	23.18±0.11^{a}	2.45±0.10^{a}	Trace ^{a}	Trace ^{a}
UTTM 6	71.54±0.88^{a}	1.33±0.34	25.55±0.23^{a}	1.96±0.06	Trace ^{a}	Trace ^{a}
UTTM 9	70.48±0.01ad	1.33±0.34	26.23±0.19^{a}	1.96±0.06	Trace a	Trace ^{a}
FRTM 2	78.38±0.01^{ab}	2.67±0.34	14.58±0.11^{b}	1.09±0.08^{ab}	1.01±0.02^{ab}	3.28±0.53^{b}
FRTM 6	79.18±0.01^{ac}	2.33±0.66	13.33±0.19^{ac}	1.85±0.10	1.22±0.02^{c}	3.31±0.97^{c}
FRTM 9	79.68±0.03^{ad}	2.32±0.66	13.08±0.19^{ad}	1.68±0.09	1.22±0.02^{d}	3.24±0.98^{d}
TMS 2	73.14±0.01^{ab}	1.67±0.66	21.44±0.12^{ab}	2.28±0.14^{a}	0.50±0.01^{ab}	1.47±0.60^{a}
TMS 6	72.32±0.01^{a}	1.67±0.34	23.15±0.11^{ac}	1.95±0.06	0.20±0.02^{ac}	0.91±0.51^{a}
TMS 9	71.15±0.01^{ad}	1.67±0.34	24.56±0.12^{ad}	2.21±0.08^{a}	Trace ^{a}	0.41±0.54^{a}
FSTM 2	78.44±0.01^{ab}	2.33±0.58	14.68±0.88^{b}	1.97±0.06^{a}	1.51±0.03^{ab}	2.58±0.71^{ab}
FSTM 6	79.95±0.01^{ac}	1.33±0.34	13.89±0.29^{c}	1.96±0.06	1.98±0.06^{ac}	2.87±0.71^{c}
FSTM 9	80.11±0.02^{ad}	1.33±0.34	11.56±0.29^{ad}	2.03±0.16^{a}	2.38±0.09^{ad}	4.97±0.80^{d}
UVTM 2	72.83±0.01^{ab}	1.50±0.09	23.22±0.19^{a}	1.95±0.07^{b}	0.50±0.01^{ab}	0.50±0.36^{a}
UVTM 6	72.48±0.01^{a}	1.50±0.14	23.86±0.19^{ac}	1.71±0.06	0.45±0.01^{ac}	0.45±0.40^{a}
UVTM 9	71.19±0.01^{ad}	1.52±0.10	25.11±0.20^{ad}	1.68±0.06	0.50±0.01^{ad}	0.50±0.37^{a}
HSPTM 2	72.85±0.02^{ab}	2.00±0.31	23.33±0.10^{a}	1.82±0.08^{b}	Trace ^{a}	Trace ^{a}
HSPTM 6	72.18±0.02^{a}	1.97±0.28	24.08±0.12^{ac}	1.77±0.08	Trace ^{a}	Trace ^{a}
HSPTM 9	71.56±0.01^{ad}	1.95±0.29	24.89±0.12^{ad}	1.60±0.06	Trace ^{a}	Trace ^{a}
LLPTM 2	73.08±0.03^{ab}	2.00±0.32	22.92±0.24^{a}	1.67±0.03^{b}	0.33±0.02^{ab}	0.33±0.62^{a}
LLPTM 6	72.78±0.02^{ac}	1.72±0.32	23.85±0.18^{ac}	1.43±0.06^{c}	0.22±0.01^{ac}	0.22±0.58^{a}
LLPTM 9	72.44±0.02^{ad}	1.69±0.09	24.45±0.21^{ad}	1.42±0.06^{d}	Trace ^{a}	Trace ^{a}
STM 2	70.68±0.02^{ab}	2.41±0.34	24.11±0.07^{a}	2.30±0.19^{a}	0.50±0.01^{ab}	0.50±0.62^{a}
STM 6	69.72±0.02^{ac}	2.32±0.44	24.85±0.08^{a}	2.61±0.16^{ac}	0.50±0.01^{ac}	0.50±0.70^{a}
STM 9	69.18±0.01^{ad}	2.31±0.34	25.55±0.06^{ad}	2.45±0.13^{ad}	0.50±0.01^{ad}	0.51±0.54^{a}

Values are means ± standard error of 3 determinations. (^{a} denotes $p<0.05$ compared to control at day 0 (UTTM 0); ^{b,c,d} denote $p<0.05$ compared to control (UTTM) at day 2, 6 and 9, respectively).
Key: UTTM= Untreated Tiger Nut Milk (control), FRTM= Tiger Nut Milk at 0ºC, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0ºC, 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.

Table 3. Effect of Storage Time (Day) on the Total Bacterial Count of Fresh and Preserved Tiger Nut Milk

DAY	UTTM	FRTM	TMS	FSTM	UVTM	HSPTM	LLPTM	STM
	(Log_{10}cfu/ml)							
0	6.58±0.05							
2	5.85±0.06 ^{a}	6.22±0.01 ^{ab}	5.70±0.03 ^{a}	6.44±0.08 ^{b}	6.01±0.04 ^{a}	5.52±0.10^{ab}	5.93±0.01 ^{a}	6.15±0.01^{ab}
6	4.44±0.02 ^{a}	6.57±0.12 ^{c}	5.43±0.27 ^{ac}	6.84±0.03 ^{c}	5.27±0.04 ^{ac}	5.28±0.01 ^{ac}	5.19±0.06 ^{ac}	5.47±0.04 ^{ac}
9	4.98±0.15^{a}	6.30±0.05^{ad}	5.56±0.04^{ad}	6.44±0.05^{ad}	5.53±0.08^{ad}	5.56±0.06^{ad}	5.47±0.04^{ad}	5.93±0.03^{ad}

Key: UTTM= Unpreserved Tiger Nut Milk (control), FRTM= Tiger Nut Milk at 0ºC, TMS= Tiger Nut Milk Treated with 0.05% Sodium Benzoate, FSTM= Tiger Nut Milk Treated with 0.05% Sodium Benzoate and Stored at 0ºC, 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. Values are means ± standard error of 3 determinations. (^{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 variation in the moisture content reported in earlier research works and present research could be due to the type of tiger nut used, environment where the nut was cultivated, different methods of preparation employed and varied ratios of ingredients used for the milk production. The addition of 0.05% sodium benzoate to tiger nut milk and the use of freezer for preservation of tiger nut milk might be a direct consequence of binding of moisture, without which microorganisms do not grow (James, 2000). The influence of freezing on microbial growth and proliferation might be bacteriostatic in nature. During de-freezing of the preserved milk binding water might be made available which might have rapidly led to high proliferation of inherent microorganisms as the total bacterial count of these samples were high. The fact that preservation by freezing maintained most of the nutrient content of tiger nut milk and as the milk de-froze the moisture content increased.
Morris (1962) stated that the presence of nutrients increases the range of water activity over which organisms can survive. This might support the result of total bacterial load of FRTM and FSTM.

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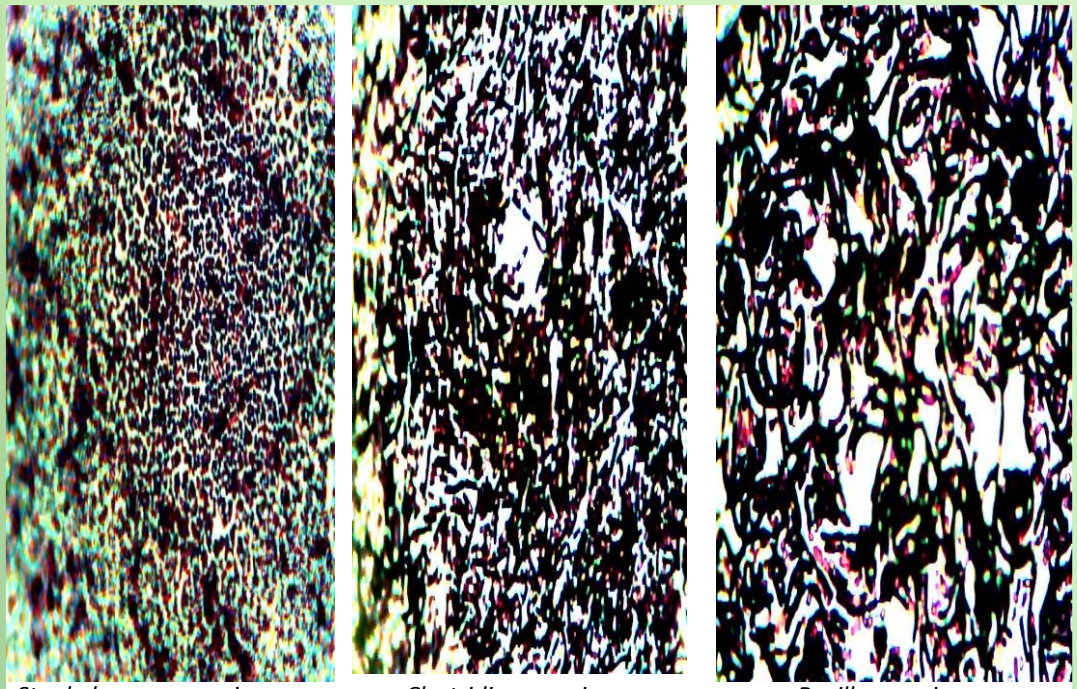

Figure 1. Light Microscopy Images of Isolated Dominant Bacteria in Tiger Nut Milk Beverage

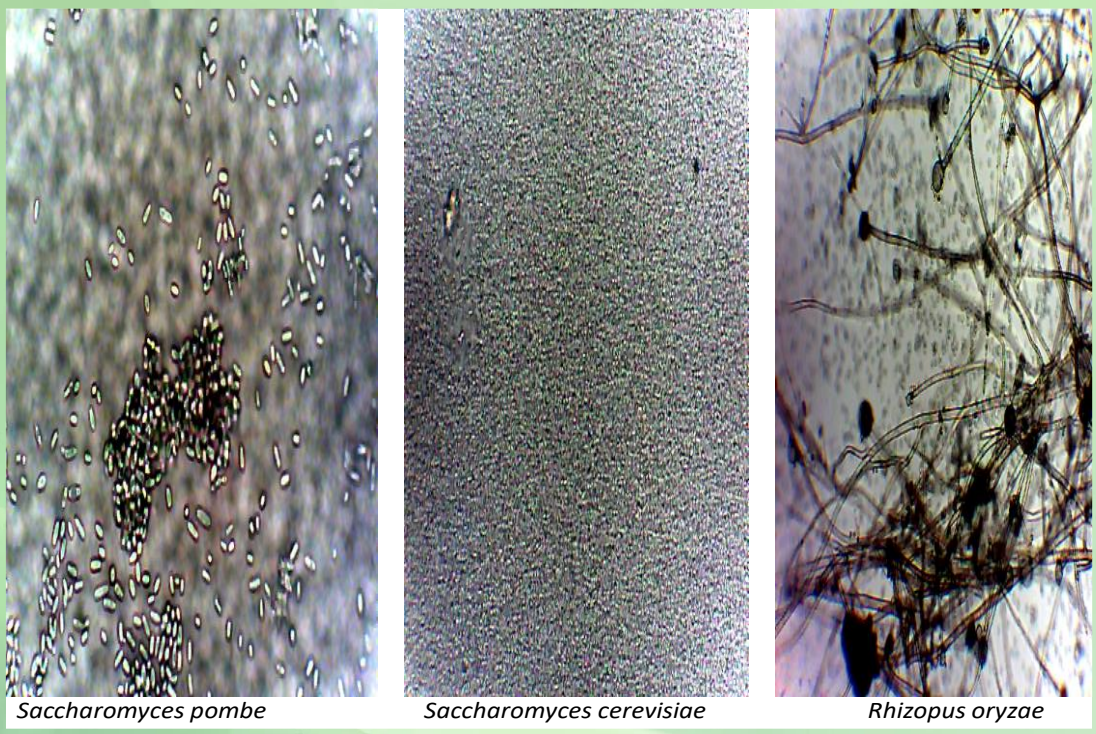

Figure 2. Light Microscopy Images of Isolated Fungi in Tiger Nut Milk Beverage

Microorganisms utilize carbohydrates as sources of energy (James, 2000) evidence from the result of proximate composition. The total carbohydrates of ambient temperature stored samples were low; this might have contributed to the low pH value of these samples, as anaerobic fermentation of sugar leads to increase in acidity and thus low pH which might cause a decrease in the total bacterial count. The significant increase (p<0.05) in the percentage total carbohydrates of FRTM and FSTM further support the assertion that preservation by freezing maintain most of the nutrient content of food (James, 2000; George, 2005). The low carbohydrates content of ambient temperature stored samples is an indication that most of the microorganisms in the tiger nut milk samples utilized carbohydrates as source of energy and as this component decreased, the milk become more acidic and the total viable bacterial load declined.

Fats are used also by microorganisms as sources of energy, but these compounds are attacked by a relatively small number of microbes in foods (James, 2000). The isolated organisms from the milk samples might not have utilize fats as source of energy. This may be attested to the fact that the percentage crude lipid content of ambient temperature stored tiger nut milk samples increased, this might be due to the types of spoilage microorganisms isolated as the organisms are oily in nature. Ukwuru and Ogbodo (2011) reported a decrease in percentage crude lipid content which differed from the finding of the present study. The range of percentage crude protein content obtained in this study agrees with previous studies. Belewu and Belewu (2007) reported 8.07%; Ukwuru and Ogbodo (2011) reported 6.40 to 8.20% from tiger nut milk. The high percentage crude protein content of TMA and all the preserved tiger nut milk that were stored at ambient temperature was not surprising as microorganisms are protein in nature and they will add to the protein content of the milk. The observed differences in the values of percentage ash content of all the samples were not statistically significant (p>0.05). This might be that activities of microorganisms have no effect on this component. The value of percentage ash content of previous study (Ukwuru and Ogbodo, 2011) was lower than what was reported in this study.

Most of the bacterial strains isolated were pathogenic (Figure 1) while the fungi were basically fermentative microorganism (Figure 2) which contributes to the acidity of the milk. 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). *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). *Bacillu*s species are food-borne pathogens associated with health hazards (FAO, 1979; Odu and Adeniji, 2013). 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 fungi isolated were *Saccharomyces pombe, Saccharomyces cerevisiae and Rhizopus oryzae* (Figure 2). Udeozor and Awonorin (2014) also, reported the isolation of these organisms from tiger nut-soya milk drink. *Saccharomyces cerevisiae* has also been reported to be isolated from tiger nut milk (Onovo and Ogaraku, 2007). They are major spoilage organisms of carbohydrate foods (Rhodes and Fletcher, 1966). *Saccharomyces pombe* and *Saccharomyces cerevisiae* are harmless; there are no pathologies associated with the organisms (Lindner, 1893). 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) also, used in fermented foods and alcoholic beverages.

Conclusion

Preservation methods have no significant effect on ash content of tiger nut milk beverage. The more acidic the milk beverages are, the lower the viable total bacterial load. The significant decreased in the total carbohydrates of tiger nut milk beverage over storage time suggest that, the harboured microorganisms utilized the macromolecule as source of energy. The dominant organism isolated from the milk beverage are *Staphylococcus* species, *Clostridium* species, *Bacillus* species, *Saccharomyces* species and *Rhizopus oryzae*. Preservation by sterilization and ultraviolet light were more effective at reducing the total viable bacterial load of the beverage, while preservation by freezing maintained the pH and nutrient quality of the tiger nut milk beverage than the other preservation methods.

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