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Research Article Sensory and Microbiological Analysis of Tiger Nut (Cyperus esculentus) Beverage

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Abstract

Background and Objective: Tiger nut (Cyperus esculentus) is an edible perennial grass-like plant of the Sedge family. It is widely used for human and animal consumption as a nutritious food and feed in Africa, Europe and America. Tiger nut can be eaten raw, roasted, dried, baked or made into a refreshing beverage which is very nutritive and healthy both for the young and old. There were many attempts to industrialize the locally prepared tiger nut beverage, but the inability to preserve the drink for a long time without spoilage has been a major drawback. Therefore, understanding the cause of the spoilage and promoting the process of production of the beverage from local home consumption to wider commercial consumption are the major objectives of this research study. Materials and Methods: Tiger nut tubers were processed into 4 major groups through different processing treatments, water soaked tiger nut beverage (WSTB), roasted tiger nut beverage (RTB), methanol soaked tiger nut beverage (MSTB) and boiled tiger nut beverage (BTB). Each of these mixtures was divided into two portions, one treated with Nisin (an antibiotic preservative) and the other portion left untreated. The two portions were further divided into two treatment, one stored at refrigerating temperature and the other one at room temperature, making 4 different treatments per mixture and a total of 16 samples. The samples were subjected to sensory and microbial analyses. Results: The results of the sensory analysis, colour, flavour, taste, mouth feel and overall acceptability showed that the water soaked tiger nut beverage samples were significantly different (p<0.05) than the other treatments. WSTB samples had the highest overall acceptability while BTB had the least. Microbiological analysis was carried out over a 3 week storage period. The products were microbiologically stable during the first week, but a rapid growth of microorganisms was observed after the 1st week which was high enough to cause spoilage. Conclusion: Results of the study indicated that processing treatments has effect on the stability and acceptability of tiger nut beverage products.

Key words: Nisin, water soaked tiger nut beverage (WSTB), roasted tiger nut beverage (RTB), methanol soaked tiger nut beverage (MSTB), boiled tiger nut beverage (BTB)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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INTRODUCTION

Research efforts have been concentrated on the development of beverages from local raw materials which has led to the consumption of both locally produced alcoholic and non-alcoholic beverages in the country. Among the developed beverages in Nigeria are 'fura da nono', 'kunun zaki', 'burukutu', 'zobo' (roselle plant extract) and soy milk. Fura da nono is a non-alcoholic beverage produced from nono (skimmed sour milk) and fura (millet powder). It is popularly consumed in the northern part of Nigeria. Kunun zaki is a non-alcoholic, cereal based beverage, widely consumed in most parts of Northern Nigeria, especially during the dry season. The cereals utilized in its production are millet (Pennisetum typoidum), sorghum (Sorghum bicolor) and maize (Zea mays) in decreasing order of preference¹. Burukutu is an indigenous alcoholic beverage produced mainly from the grains of guinea corn. Its production process involves malting, mashing, fermentation and maturation². Zobo which is rich in anthocyanins, belongs to the group of flavonoids that have been reported to have substantial antioxidant capacity, makes significant contribution to the diet and may reduce the risk of cardiovascular disease³. Among the oil seeds and tubers, only soybean has been extensively investigated while little research attention has been given to others like peanut and tiger nut⁴, as sources of vegetable milk/beverage. Soy milk has been reported to lower blood cholesterol and the risk of heart disease⁵.

Tiger nut (*Cyperus esculentus*) belongs to the Division-*Magnoliophyta*, Class-*Liliopsida*, Order-*cyperales* and Family-*Cyperaceae* and was found to be a cosmopolitan, perennial crop of the same genus as the papyrus plant. Tiger nut has been cultivated since early times (chiefly in South Europe and West Africa) for its small tuberous rhizomes which are eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage. Non-drying oil (usually called chufa) is equally obtained from the rhizome. The tubers are abundantly produced in Nigeria, where the Hausas call it "Aya", Yorubas "imumu", the Igbos "aki Hausa" and "ofio" in Southern Nigeria⁶. It has many other names like Zulu nut, yellow nut grass, ground almond and chufa, edible rush and rush nut⁶.

Tiger nuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate and are rich in sucrose (17.4-20.0%), fat (25.5%), protein (8.0%)⁷. Tiger nut is also rich in mineral elements such as sodium, calcium, potassium, magnesium, zinc and traces of copper⁷. Tiger nut had been reported to be a "health" food, since its consumption can help

prevent heart disease and thrombosis and is said to activate blood circulation⁸. It was also found to assist in reducing the risk of colon cancer⁹. The distinctive functional properties of tiger nut has made it a unique food¹⁰, like in beverage, flour¹¹, edible oil^{12,13} and a feed source¹⁴.

These nutritional contents of tiger nut stimulates it's utilization in the preparation of tiger nut beverage so as to provide protein-rich drink at affordable price in place of animal protein which is scarce and expensive.

MATERIALS AND METHODS

Fresh tiger nuts (*Cyperus esculentus*) and spices were obtained from Rimi market in Kano State, Nigeria. The tiger nut was identified by Bahauddeen Said Adam, a plant taxonomist in the Department of Plant Biology, Bayero University, Kano. A herbarium accession number BUKHAN 0367 was deposited at the department's herbarium. The spices used include ginger (*Zingiber officinale*) and cloves (*Evgenia coryphée*). The chemical preservative used was Nisin, then vanilla flavor and sugar.

The research was carried out between October-November, 2015 in the Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano.

Preparation of the drink: A total of 8 kg dried tiger nut was used for the research and divided into 4 portions of 2 kg each. The tiger nut was sorted to remove foreign materials, bad nuts and seeds which may affect the taste and the storage quality of the drink. The tiger nut (2 kg) was washed thoroughly in water so as to remove any adhering soil and then soaked in distilled water (3 L) at a temperature of 30°C for 2 h. After then, it was ground to a paste in a ratio of 3:1 (i.e., 3 L of water per kg of the nut) using a Marlex kitchen blender (IS 4250. Marlex Appliances Private Limited, 41/42, Fourth Floor, Mid Town co-op Hsg Society Ltd., S. V. Road, Borivali West Mumbai Mumbai City MH 400092, India) at the maximum speed for 5 min to almost smooth slurry. The homogenous slurry was filtered using a muslin cloth by squeezing until virtually no extract was recovered. Sugar and flavour were added. The method of preparation above is applied to all the portions.

First portion: In this portion, the fresh tiger nut was directly soaked in water for 2 h. The filtrate was divided into two portions, one treated with Nisin preservative (2 g to 2 L of the filtrate) and the other portion was not treated with any preservative. Each of these 2 portions was again divided into 2, one stored at refrigerating temperature and the other one at room temperature, making 4 different treatments:

- Water soaked tiger nut beverage+preservative (2 g to 2 L of the filtrate) at refrigeration temperature (WSTB+P_{RefT})
- Water soaked tiger nut beverage+preservative (2 g to 2 L of the filtrate) at room temperature (WSTB+P_{RT})
- Water soaked tiger nut beverage stored at refrigeration temperature (WSTB_{RefT})
- Water soaked tiger nut beverage stored at room temperature (WSTB_{RT})

Second portion: Here, the fresh tiger nut was roasted at 110 ± 5 °C for 30 min in an open pan. It was then blended and mixed and divided into 4 portions as done for the 1st portion.

- Roasted tiger nut beverage+preservative (2 g to 2 L of the filtrate) at refrigeration temperature (RTB+ P_{Ref.T})
- Roasted tiger nut beverage+preservative (2 g to 2 L of the filtrate) at room temperature (RTB+P_{RT})
- Roasted tiger nut beverage stored at refrigeration temperature (RTB_{RefT})
- Roasted tiger nut beverage stored at room temperature (RTB_{RT})

Third portion: In this portion, the tiger nut was partly ground and soaked in methanol solution for an hour. It was then removed, dried and soaked in water (water was changed 3 times). It was also divided into 4 portions as above:

- Methanol soaked tiger nut beverage+preservative
 (2 g to 2 L of the filtrate) at refrigeration temperature
 (MSTB+P_{Ref.T})
- Methanol soaked tiger nut beverage+preservative
 (2 g to 2 L of the filtrate) at room temperature (MSTB+P_{RT})
- Methanol soaked tiger nut beverage stored at refrigeration temperature (MSTB_{RefT})
- Methanol soaked tiger nut beverage stored at room temperature (MSTB_{RT})

Fourth portion: In the last portion, the tiger nut mixture was boiled.

- Boiled tiger nut beverage+preservative (2 g to 2L of the filtrate) at refrigeration temperature (BTB+P_{Ref.T})
- Boiled tiger nut beverage+preservative (2 g to 2 L of the filtrate) at room temperature (BTB+P_{RT})
- Boiled tiger nut beverage stored at refrigeration temperature (BTB_{RefT})
- Boiled tiger nut beverage stored at room temperature (BTB_{RT})

The sensory analysis was carried out on the day of production, while total microbial count of the sample was analyzed at an interval of 1 week for 3 weeks consecutively.

Analysis of products

Sensory evaluation: Sixteen samples of tiger nut beverage were subjected to sensory evaluation by 30 trained panelists selected from among 500 level students of Food Science and Technology (Kano University of Science and Technology Wudil, Kano State) who were familiar with beverages. Samples of the tiger nut beverage were coded and presented to the panelist using white transparent disposable cups. Water was provided for mouth wash in between evaluations. Panelists were asked to evaluate the samples for colour, flavour, taste, mouth feel and overall acceptability using a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely)¹⁵.

Microbial analysis: Plate counts of all samples were carried out every week for a period of three weeks. Aliquots from serially diluted samples were mixed with plate count agar for bacteria and incubated at 37°C for 24 h and on potato dextrose agar for moulds and incubated at 25°C for 3 days. Total plate counts for the nutrient and potato dextrose agar were done by counting colonies at the reverse side of the culture plates. Total colony count was expressed in colony forming units per millilitre (CFU mL⁻¹)¹⁶.

Statistical analysis: Data were subjected to one-way analysis of variance (ANOVA) and means were compared using Turkey's test. Statistical significance was considered at p<0.05.

RESULTS AND DISCUSSION

The result of the sensory evaluation of the tiger nut beverages was presented in Table 1. The WSTB samples had the highest score for all the sensory attributes evaluated. While the BTB samples had the least score. And the results of the bacterial and fungal counts (in CFU mL⁻¹) of the various tiger nut beverage products were presented in Table 2 and 3, respectively.

Effect of processing on the sensory quality of tiger nut beverage: Various treatments differed in most of the quality attributes evaluated indicating the effect of processing on the organoleptic properties of the beverage. There was a significant difference observed between the various processing treatments in terms of colour and flavor, where the WSTB+P_{RefT} had the highest mean score for colour (8.0 ± 0.9) and WSTB+P_{RT} had the highest mean score for

Table 1: Sensory attributes of tiger nut beverage products obtained from various processing treatments

Treatment samples	Colour/Appearance	Flavour/Aroma	Taste	Mouth feel	Overall acceptability
WSTB+P _{Ref.T}	8.0±0.9	7.1±1.6	7.9±1.1	7.4±1.9	8.1±0.8
WSTB+P _{RT}	7.5 ± 1.1	7.3 ± 1.4	7.9 ± 1.0	7.0 ± 1.5	7.6±1.1
$WSTB_{Ref.T}$	7.4 ± 1.5	7.2 ± 1.4	7.1 ± 1.9	7.0 ± 1.9	7.3 ± 1.6
WSTB _{RT}	7.2 ± 1.7	7.2 ± 1.6	7.4 ± 1.7	7.0 ± 1.7	7.4 ± 1.7
$RTB+P_{Ref.T}$	5.6±2.1	6.1 ± 2.0	6.6±1.9	6.3 ± 2.0	6.6 ± 1.8
RTB+P _{RT}	6.5 ± 1.8	6.1 ± 2.2	6.7 ± 1.5	5.8±2.3	6.3 ± 2.1
$RTB_{Ref.T}$	5.6±2.1	5.9±1.9	5.8±1.8	6.0 ± 1.8	6.0 ± 2.0
RTB _{RT}	5.7±2.2	5.7±2.0	6.0±2.1	5.8 ± 1.9	6.1 ± 1.9
MSTB+P _{Ref.T}	7.3 ± 1.6	5.7±1.9	6.4 ± 1.7	5.6±2.2	6.4 ± 1.8
MSTB+P _{RT}	7.5 ± 1.5	6.0 ± 2.2	6.1 ± 1.9	6.0 ± 2.6	6.2±2.2
$MSTB_{Ref,T}$	7.0 ± 2.1	5.5±1.7	5.4±1.8	5.2±2.2	6.2 ± 1.8
MSTB _{RT}	7.1 ± 1.8	5.8 ± 1.7	5.8±2.0	5.7±2.1	6.1 ± 2.1
BTB+P _{Ref.T}	5.4±2.3	5.3 ± 2.4	6.6±2.3	5.2±2.3	6.1 ± 2.3
BTB+P _{RT}	5.4±2.2	5.8±2.2	6.3 ± 2.3	5.3±2.3	5.8±2.4
$BTB_{Ref.T}$	5.9±2.1	5.3±2.4	6.2±2.3	5.4±2.4	5.9±2.3
BTB_{RT}	6.9±1.8	5.8±2.1	6.4 ± 1.5	6.1 ± 2.0	6.3 ± 1.8

Values are Mean \pm SD of 30 determinations, WSTB+P_{Ref.T}: Soaked tiger nut beverage drink+preservative (at refrigerating temperature). WSTB+P_{RT}: Soaked tiger nut beverage drink+preservative (at room temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB+P_{RT}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB+P_{RT}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB+P_{RT}: Soaked tiger nut beverage drink (at refrigerating temperature).

flavor(7.3 \pm 1.4), while BTB+P_{RT} and BTB_{RefT} had the least mean score for colour (5.4 \pm 2.2) and flavor (5.3 \pm 2.4), respectively. The WSTB+P_{RT} had the highest mean score for taste (7.9 ± 1.1) and WSTB+P_{Reft} had the highest mean score for mouth feel (7.4 ± 1.9) , while MSTB_{RefT} had the least mean score for both taste and mouth feel. The highest mean score for overall acceptability was observed in WSTB+P_{RefT}, while the least score was observed in BTB+ P_{RT} . The WSTB samples had the highest score for colour, flavour, mouth feel, taste and overall acceptability compared to the other treatments. The high mean score of WSTB_{RT} might be attributed to its high fat content (6.5%). This is because fat is known to promote good mouth feel¹⁷. The MSTB samples also had a high score for colour as the samples appeared to be creamier like dairy milk. Boiled tiger nut beverage samples had the least mean score for most of the sensory attributes especially colour, flavour and mouth feel. This was attributed to the heating process (boiling) of the beverage samples after mixture. However, despite the significant differences that existed, the panelists commented that all the tiger nut beverage products were good but WSTB samples were the best and the BTB samples were least preferred.

Tiger nut beverage drink has no beany flavour and throat-catching sensations like other phytomilk (e.g., Soybean milk). Similar processing treatment carried out on tiger nut^{4,18} and other similar vegetable milk recorded high overall acceptability¹⁹.

Effect of processing on the microbial load of tiger nut beverage drink: The microbial quality of tiger nut beverage products from various processing treatments are shown in Table 2 and 3. There was no growth of microorganisms on

the freshly prepared samples. A rapid growth of microorganisms was observed after the 1st week of storage in both the refrigerated and room temperature samples. The total bacterial count was presented in Table 2 which revealed that bacterial growth was highest in WSTB_{RefT} (8.5 × 105 CFU mL⁻¹) and lowest in WSTB+P_{RT} (6.3 × 105 CFU mL⁻¹) for all the samples at 1st week and was highest in BTB_{RT} (8.7 × 105 CFU mL⁻¹) and lowest in BTB+P_{RT} (7.0 × 105 CFU mL⁻¹) in all samples at 2nd week. In the 3rd week, bacterial growth was highest in WSTB_{RefT} (8.1 × 10⁵ CFU mL⁻¹) and lowest in WSTB+P_{RT} (6.9 × 10⁵ CFU mL⁻¹).

The total fungal count was presented in Table 3 which revealed that the mould and yeast contaminants in the samples ranged from 7.6×10^5 CFU mL $^{-1}$ (WSTB+P $_{RefT}$) to 8.7×10^5 CFU mL $^{-1}$ (WSTB $_{RefT}$,MSTB $_{RefT}$ and BTB $_{RefT}$), 7.7×10^5 CFU mL $^{-1}$ (MSTB+P $_{RT}$ and BTB+P $_{RT}$) to 8.5×10^5 CFU mL $^{-1}$ (RTB $_{RefT}$, RTB $_{RT}$ and BTB $_{RefT}$) and 7.8×10^5 CFU mL $^{-1}$ (WSTB+P $_{RT}$ and MSTB+P $_{RefT}$) to 9.0×10^5 CFU mL $^{-1}$ (MSTB $_{RefT}$) for the 1st, 2nd and 3rd week, respectively. It was observed that all the samples that had least bacterial and fungal growth are the preservative treated samples (both at room and refrigerated temperature).

The WSTBD and MSTBD recorded more fungal counts than bacterial counts. Comparatively, 8.7×10^5 and 9.0×10^5 of bacterial and fungal counts, respectively were above 1.2×10^3 and 0.2×10^3 CFU mL⁻¹ microbial load for exposed and unexposed tiger nut beverage, respectively as indicated by Onovo and Ogaraku²⁰. This implies that the microbial counts (Table 2, 3) were above the limit of acceptance which is 2.0×10^5 CFU mL⁻¹ for dairy milk by Codex Alimentarius Commission²¹.

Table 2: Bacterial count (CFU mL^{−1}) of various tiger nut beverage products stored under freezing and room temperature

	Storage period (weeks)					
Treatment samples	0	1	2	3		
WSTB+P _{Ref.T}	NG	7.2×10 ⁵	7.8×10 ⁵	7.9×10 ⁵		
WSTB+P _{RT}	NG	6.3×10⁵	7.7×10 ⁵	6.9×10 ⁵		
$WSTB_{Ref,T}$	NG	8.5×10⁵	8.5×10 ⁵	8.1×10 ⁵		
WSTB _{RT}	NG	8.1×10⁵	8.4×10 ⁵	7.8×10 ⁵		
$RTB+P_{Ref,T}$	NG	7.5×10⁵	7.8×10 ⁵	7.3×10 ⁵		
RTB+P _{RT}	NG	8.0×10 ⁵	7.5×10 ⁵	7.1×10 ⁵		
$RTB_{Ref,T}$	NG	8.3×10⁵	8.4×10 ⁵	7.5×10 ⁵		
RTB _{RT}	NG	8.4×10 ⁵	7.7×10 ⁵	7.2×10 ⁵		
$MSTB+P_{Ref,T}$	NG	6.4×10⁵	8.0×10 ⁵	7.0×10 ⁵		
MSTB+P _{RT}	NG	6.6×10⁵	7.9×10⁵	7.1×10 ⁵		
$MSTB_{Ref,T}$	NG	8.2×10 ⁵	8.6×10 ⁵	8.0×10 ⁵		
MSTB _{RT}	NG	8.3×10⁵	8.1×10 ⁵	7.4×10 ⁵		
$BTB+P_{Ref,T}$	NG	7.4×10⁵	7.3×10 ⁵	7.3×10 ⁵		
BTB+P _{RT}	NG	7.0×10⁵	7.0×10 ⁵	7.0×10 ⁵		
$BTB_{Ref,T}$	NG	7.3×10⁵	8.4×10 ⁵	8.0×10 ⁵		
BTB _{RT}	NG	8.5×10⁵	8.7×10 ⁵	7.6×10 ⁵		

Values are means of triplicate determinations, Key: WSTB+ $P_{Ref.T}$: Soaked tiger nut beverage drink+preservative (at refrigerating temperature). WSTB+ $P_{Rf.T}$: Soaked tiger nut beverage drink + preservative (at room temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB_{Rf.T}: Soaked tiger nut beverage drink (at room temperature), RTB: Roasted tiger nut beverage drink, MSTB: Methanol soaked tiger nut beverage drink, BTB: Boiled tiger nut beverage drink and NG: No growth

Table 3: Fungal count (CFU mL⁻¹) of various tiger nut beverage products stored under freezing and room temperature

	Storage period (weeks)					
Treatment samples	0	1	2	3		
WSTB+P _{Ref.T}	NG	7.6×10 ⁵	7.9×10 ⁵	8.4×10 ⁵		
WSTB+P _{RT}	NG	8.0×10 ⁵	8.1×10 ⁵	7.8×10 ⁵		
$WSTB_{Ref,T}$	NG	8.7×10 ⁵	8.1×10 ⁵	8.2×10 ⁵		
WSTB _{RT}	NG	8.2×10 ⁵	8.2×10 ⁵	8.6×10 ⁵		
RTB+P _{Ref.T}	NG	7.8×10 ⁵	8.1×10 ⁵	8.5×10 ⁵		
RTB+P _{RT}	NG	7.7×10 ⁵	7.9×10⁵	8.5×10 ⁵		
$RTB_{Ref,T}$	NG	8.6×10 ⁵	8.5×10⁵	8.6×10 ⁵		
RTB _{RT}	NG	8.3×10 ⁵	8.5×10 ⁵	8.2×10 ⁵		
MSTB+P _{Ref.T}	NG	8.1×10 ⁵	8.1×10 ⁵	7.8×10 ⁵		
MSTB+P _{RT}	NG	7.9×10⁵	7.7×10⁵	8.6×10 ⁵		
MSTB _{Ref.T}	NG	8.7×10 ⁵	8.2×10 ⁵	9.0×10 ⁵		
MSTB _{RT}	NG	8.5×10⁵	8.4×10 ⁵	8.7×10 ⁵		
BTB+P _{Ref.T}	NG	7.8×10 ⁵	7.8×10 ⁵	8.2×10 ⁵		
BTB+P _{RT}	NG	7.7×10 ⁵	7.7×10 ⁵	8.2×10 ⁵		
BTB _{Ref.T}	NG	8.7×10 ⁵	8.5×10 ⁵	8.6×10 ⁵		
BTB _{RT}	NG	8.3×10 ⁵	8.3×10 ⁵	8.9×10 ⁵		

Values are means of triplicate determinations, Key: WSTB+ $P_{Ref.T}$: Soaked tiger nut beverage drink+preservative (at refrigerating temperature). WSTB+ $P_{Rf.T}$: Soaked tiger nut beverage drink+preservative (at room temperature), WSTB_{Ref.T}: Soaked tiger nut beverage drink (at refrigerating temperature), WSTB_{RT}: Soaked tiger nut beverage drink (at room temperature), RTB: Roasted tiger nut beverage drink, MSTB: Methanol soaked tiger nut beverage drink, BTB: Boiled tiger nut beverage drink and NG: No growth

The growth of these microorganisms may be attributed to the cells of lactic acid bacteria that might have survived through processing treatments. In a study conducted by Nyarko *et al.*²² on the assessment of microbiological safety of tiger nut in the cape coast metropolis of Ghana, it was observed that the most predominantly encountered species were *E. coli* and *Bacillus* spp. which had 18.9% each. *Bacillus* are spore-forming bacteria that are commonly found in soil, water (through soil-water contamination) and also on vegetables. The

presence of these bacteria in food samples in this study may be inevitable because the spores of some strains of these organisms are resistant to pasteurization temperature. Others include *Enterococcus* spp. (16.2%), *S. aureus* and *P. aeruginosa* (13.5% each) and *Streptococcus* spp. (10.8%). *Staphylococcus aureus* is a common environmental bacterium and could have been introduced after processing through cross-contamination. *Staphylococcus aureus* is known to produce an enterotoxin of importance in foodborne illness²³.

High moisture content typically allows microbial growth²⁴. The possible sources of these organisms in the samples could be from nose, hand, skin and clothing of handlers, coughing, talking and sneezing droplets which could settle on the food during storage^{25,26}. The fungal count of tiger nut beverage samples calls for concern. This has been linked to the spoilage of the storage microflora. The microbial spoilage of refrigerated tiger nut beverage is manifested primarily by development of off-flavours. A study conducted by Chukwu et al.²⁷ indicated that some fungus (Aspergillus niger, A. flavus and A. terreus) were associated with both fresh and dry tiger nuts and they can possibly endure processing treatments. Also, pH values of the various treatment samples of tiger nut beverage decreased with storage time. The treatments became more acidic with storage time, this could lead to further deterioration of the beverage as bacteria grow well and reproduce in an acidic medium.

From the results of this study, it was observed that the microbial counts (Table 2, 3) were above the limit of acceptance, which shows that the beverage was not suitable for consumption after the first few days of storage. And this goes in line with the findings of Abaejoh *et al.*²⁸, who indicated that the deterioration effect of microorganisms on tiger nut beverage drink hampered its production in Nigeria.

CONCLUSION AND RECOMMENDATIONS

The microbiological analysis of the samples revealed that the shelf life of tiger nut beverage was differently affected by the various treatments (the preservative and non-preservative treated samples, refrigerated and room temperature samples). Based on the above results, an acceptable Nigerian tiger nut beverage can be produced using a preservative but an effective control measure to minimize contamination during processing and higher moisture content of the tiger nut should be adopted, as the higher the moisture content, the higher the susceptibility to microbial growth.

Further studies should be directed towards using the processing technique of ultra high temperature sterilization in order to maximize the nutritional value and shelf life of the beverage.

SIGNIFICANCE STATEMENT

This study discovers the possible preserving effect of Nisin, an antibiotic preservative which can be beneficial in curtailing the activities of microorganisms in the beverage samples during storage. This study will help the researcher to discover the main problems leading to the spoilage of tiger

nut beverage that many researchers were not able to explore. Thus, a new processing treatment on the preparation of tiger nut beverage may be considered.

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