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Research article

MICROBIOLOGICAL AND PHYSICOCHEMICAL CHANGES DURING TIGER NUT (*CYPERUS ESCULENTUS*) SOAKING AND THEIR IMPLICATIONS FOR FOOD SAFETY

Ginikachi Peace Onyenze[1], Kelechi Stanley Dike[2]✉

[1]*Microbiology, Biological Sciences, Imo State University, Owerri, Nigeria*

✉*Corresponding author: Kelechi Stanley Dike: 0000-0002-1723-2436 E-mail: kelechidike@imsuonline.edu.ng*

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Abstract

Tiger nut (*Cyperus esculentus*) soaking is an important processing step in beverage production that may influence microbial quality and food safety. This study evaluated microbiological and physicochemical changes during tiger nut soaking at 8, 16, and 24 h under ambient conditions using culture-based microbiology, physicochemical analysis, and exploratory full-length 16S rRNA gene sequencing. Total aerobic counts increased significantly ($p < 0.05$) from 6.07 $\log_{10}$ CFU/g at 8 h to 6.48 $\log_{10}$ CFU/g at 16 h, followed by a slight decline at 24 h. Lactic acid bacteria also increased significantly during soaking, whereas coliform counts fluctuated between 2.71 and 3.40 $\log_{10}$ CFU/g. pH decreased significantly from 6.65 to 5.50, while titratable acidity increased progressively throughout soaking. Sequencing analysis indicated increased representation of Firmicutes during soaking, particularly *Weissella* and *Leuconostoc*. *Weissella cibaria* emerged as a prominent taxon at later soaking stages, whereas *Lactococcus* persisted throughout soaking. The observed microbiological and physicochemical changes indicate incipient fermentation-associated microbial activity during soaking; however, the final pH of 5.5 may be insufficient to inhibit foodborne pathogens. These findings identify soaking as a microbiologically active processing stage requiring appropriate hygiene and process control to improve food safety in tiger nut beverage production.

1. Introduction

Tiger nut (*Cyperus esculentus* L.) is a tuberous crop belonging to the family Cyperaceae, cultivated across tropical, subtropical, and Mediterranean regions and widely consumed as a food and beverage ingredient. In West Africa, particularly Nigeria, tiger nuts are processed into non-alcoholic beverages through soaking, grinding, and filtration, producing a product commonly known as tiger nut milk or kunun aya (Adejuyitan, 2011). Globally, the crop is attracting increasing interest as a plant-based dairy alternative and functional food because of its nutritional composition, which is rich in carbohydrates, oleic acid, dietary fibre, and bioactive phenolics (Yu et al., 2022; Sánchez-Zapata et al., 2012).

Soaking is the first and most critical processing step in tiger nut beverage production.

It rehydrates tubers, softens fibrous tissue to facilitate milling, and promotes the release of soluble nutrients into the aqueous phase, thereby improving extraction efficiency (Djomdi et al., 2007). However, soaking also increases water activity and nutrient availability, creating conditions that support rapid microbial growth (Abadias et al., 2008). Previous studies on tiger nut beverages have reported high microbial loads, coliform-associated contamination, and occurrence of potential pathogens in informally processed products (Ire et al., 2020; Ukwuru & Ogbodo, 2011). These observations indicate that soaking represents a critical stage at which microbiological risks may be introduced or amplified.

Despite its importance, soaking is often treated primarily as a hydration step in tiger nut processing studies. Most existing microbiological investigations rely on culture-dependent methods, which provide useful information on microbial loads but may not fully capture broader bacterial changes during processing (Tamang et al., 2016; Ercolini, 2013). Culture-independent approaches such as 16S rRNA gene sequencing can provide complementary insight into dominant bacterial groups associated with food processing systems (Dike et al., 2022; Tigrero-Vaca et al., 2025). However, microbiological changes during tiger nut soaking remain poorly characterised using these approaches.

It was hypothesised that soaking under ambient conditions would promote measurable microbiological and physicochemical changes associated with early fermentation-related activity and increased contamination risk. The present study therefore evaluated physicochemical and microbiological changes during tiger nut soaking. Physicochemical parameters and culture-based microbiology were monitored at 0, 8, 16, and 24 h, while exploratory full-length 16S rRNA gene sequencing was performed on samples collected at 8, 16, and 24 h. Specifically, the study: (i) determined total aerobic, coliform, and lactic acid bacteria counts during soaking; (ii) assessed changes in dominant bacterial groups across soaking time points; and (iii) examined microbiological changes alongside physicochemical conditions, with emphasis on implications for food safety.

2. Materials and methods

2.1. Sample Collection and Soaking

Tiger nuts (*Cyperus esculentus*) were procured from a local market in Owerri, Imo State, Nigeria. The nuts were washed thoroughly with clean water to remove adhering debris and soil particles, then soaked in sterile distilled water at a ratio of 1:3 (w/v) in covered containers under ambient laboratory conditions (25–30°C) following typical local processing practice. Baseline physicochemical and microbiological measurements were obtained prior to soaking (0 h). Samples (50 g) were collected aseptically for microbiological analyses at 0, 8, 16, and 24 h, and for sequencing analyses at 8, 16, and 24 h. Microbiological and physicochemical analyses were performed immediately following sample collection at each time point. A separate portion of each sample was stored at −20°C for less than 24 h prior to DNA extraction and sequencing.

2.2. Physicochemical Analysis

Physicochemical parameters were measured at 0, 8, 16, and 24 h. pH was determined using a calibrated digital pH meter (Hanna Instruments, USA) after homogenising 10 g of sample in 20 mL of sterile distilled water. Titratable acidity (TA), expressed as % lactic acid equivalent, was determined by titrating 10 mL of sample filtrate against 0.1 M NaOH using phenolphthalein as indicator. Temperature was measured in situ using a digital thermometer inserted into the soaking medium. Physicochemical analyses were performed in triplicate and results expressed as mean ± standard deviation.

2.3. Microbiological Analysis

Total viable counts (TVC) were determined by serial dilution of homogenised samples and plating onto nutrient agar (Oxoid, UK), followed by incubation at 37°C for 24 h. Coliform counts were determined on MacConkey agar (Oxoid, UK) incubated at 37°C for 24–48 h. Lactic acid bacteria (LAB) were enumerated on de Man,

Rogosa, and Sharpe (MRS) agar (Oxoid, UK) incubated anaerobically at 37°C for 48–72 h. All microbiological procedures were conducted in accordance with standard methods (APHA, 2001). Microbiological analyses were performed in triplicate and microbial counts were expressed as $\log_{10}$ CFU/g.

2.4. DNA Extraction and 16S rRNA Gene Sequencing

To provide exploratory molecular insight into dominant bacterial groups during soaking, total community DNA was extracted from samples collected at 8, 16, and 24 h using the Zymo Research Quick-DNA Faecal/Soil Microbe Kit (D6010, Zymo Research, Irvine, USA) according to the manufacturer's instructions. DNA concentration was quantified using a Qubit 2.0 Fluorimeter (Life Technologies, USA), and quality was assessed using an Agilent 2200 TapeStation system.

Sequencing was performed on a single sample collected at each time point (8, 16, and 24 h), without biological or technical replication, consistent with the exploratory scope of this molecular component of the study. Accordingly, taxonomic and diversity comparisons across time points are presented as descriptive/indicative rather than statistically inferential. See Section 3.5 for further discussion of this limitation.

The full-length 16S rRNA gene (V1–V9) was amplified using universal primers 27F and 1492R, an approach suitable for improved taxonomic resolution using long-read sequencing (Callahan et al., 2019). Amplicons were barcoded using PacBio M13 barcodes via limited-cycle PCR, quantified, pooled in equimolar concentrations, and purified using AMPure PB beads. SMRTbell libraries were prepared following the manufacturer's protocol, and sequencing was performed on the PacBio Sequel IIe platform Inqaba Biotechnical Industries (Pretoria, South Africa)

2.5. Bioinformatics and Data Analysis

Raw sequencing reads were processed using standard quality control procedures, including primer removal, quality trimming, denoising, and contaminant filtering in R using the DADA2 package (Callahan et al., 2016). Reads were quality-filtered using DADA2's filterAndTrim function, with a maximum expected error of 2, minimum quality score of 3, minimum and maximum read lengths of 1000 and 1500 bp respectively, no ambiguous bases permitted, and removal of PhiX reads. Error learning and denoising were performed using a PacBio-specific error model (BAND_SIZE = 32).

Chloroplast-derived sequences were removed prior to downstream analysis. Amplicon sequence variants (ASVs) were generated using DADA2 (Callahan et al., 2016), and chimeric sequences were identified using DADA2's de novo detection method (isBimeraDenovo, minFoldParentOverAbundance = 3.5). Taxonomic assignment was performed against the SILVA reference database (Quast et al., 2013), using SILVA v138.1 at genus level and SILVA v138 for species-level assignment (minBoot = 0).

Sequencing data were used to provide indicative information on dominant bacterial groups across soaking time points. Alpha diversity was assessed using observed ASVs and Chao1 richness estimators. Beta diversity was evaluated using Bray–Curtis dissimilarity and visualised through principal coordinates analysis (PCoA). Following chloroplast removal, samples were rarefied to 90% of the minimum post-filtering read count per sample (rngseed = 1, without replacement) prior to alpha and beta diversity analysis. Descriptive statistical analyses and data visualisation were performed in R (R Core Team, 2024).

Given the single sample analysed per time point, alpha and beta diversity results are reported descriptively; no inferential statistical testing (e.g. PERMANOVA) was applied to these metrics.

3. Results and Discussion

3.1. Physicochemical Changes During Soaking

Progressive changes in physicochemical parameters were observed during tiger nut soaking (Table 1). pH decreased significantly (p

< 0.05) from 6.65 at 0 h to 6.20 at 8 h, 5.80 at 16 h, and 5.50 at 24 h, indicating gradual acidification during soaking. Titratable acidity increased significantly from 0.035% lactic acid equivalent at 0 h to 0.55% at 24 h. Temperature also increased from 25.9°C at 0 h to 30.0°C at 24 h, remaining within the mesophilic range throughout soaking.

The observed decline in pH and corresponding increase in titratable acidity indicate progressive microbial metabolic activity during soaking. The temperature range recorded remained favourable for growth of mesophilic microorganisms, including lactic acid bacteria and other food-associated bacterial groups. Similar physicochemical trends have been reported in plant-derived food systems undergoing spontaneous microbial activity under ambient processing conditions (Abadias et al., 2008; Jay et al., 2005). The gradual acidification observed in this study suggests development of early fermentation-associated activity during soaking, although the final pH remained within a range capable of supporting growth of several food-associated microorganisms.

Table 1. Physicochemical changes during tiger nut soaking under ambient conditions

Time point	Temperature (°C)	pH	Titratable acidity (% lactic acid)
0 h	25.9 ± 0.4^{c}	6.65 ± 0.02^{a}	0.035 ± 0.003^{d}
8 h	25.1 ± 0.4^{c}	6.20 ± 0.03^{b}	0.20 ± 0.02^{c}
16 h	28.0 ± 0.2^{b}	5.80 ± 0.02^{c}	0.40 ± 0.02^{b}
24 h	30.0 ± 0.5^{a}	5.50 ± 0.03^{d}	0.55 ± 0.03^{a}

Values are expressed as mean ± standard deviation of triplicate determinations. Means within a column bearing different superscript letters differ significantly ($p < 0.05$).

3.2. Microbiological Changes During Soaking

Total aerobic counts increased significantly ($p < 0.05$) from 6.08 $\log_{10}$ CFU/g at 8 h to 6.48 $\log_{10}$ CFU/g at 16 h, followed by a slight decline to 6.32 $\log_{10}$ CFU/g at 24 h (Table 2). Coliform counts fluctuated significantly during soaking, decreasing from 3.40 $\log_{10}$ CFU/g at 8 h to 2.72 $\log_{10}$ CFU/g at 16 h before increasing to 3.00 $\log_{10}$ CFU/g at 24 h. Lactic acid bacteria increased significantly from 2.54 $\log_{10}$ CFU/g at 8 h to 3.26 $\log_{10}$ CFU/g at 16 h and remained comparatively elevated at 24 h (3.18 $\log_{10}$ CFU/g). The increase in lactic acid bacteria broadly corresponded with declining pH and increasing titratable acidity observed during soaking.

Table 2. Microbiological changes during tiger nut soaking under ambient conditions.

Time point	Total aerobic count ($\log_{10}$ CFU/g)	Coliform count ($\log_{10}$ CFU/g)	LAB count ($\log_{10}$ CFU/g)
0 h	5.00 ± 0.02^{c}	3.00 ± 0.09^{b}	1.78 ± 0.14^{c}
8 h	6.07 ± 0.04^{b}	3.40 ± 0.03^{a}	2.54 ± 0.06^{b}
16 h	6.48 ± 0.03^{a}	2.71 ± 0.03^{c}	3.26 ± 0.05^{a}
24 h	6.32 ± 0.04^{a}	3.00 ± 0.05^{b}	3.18 ± 0.06^{a}

Values are expressed as mean ± standard deviation of triplicate determinations. Means within a column bearing different superscript letters differ significantly ($p < 0.05$).

The increase in total aerobic counts and lactic acid bacteria indicates that soaking created favourable conditions for microbial proliferation through increased moisture availability and

nutrient release. The observed increase in lactic acid bacteria during soaking is consistent with reports from plant-based processing systems in which increased water availability and ambient temperatures support growth of acid-tolerant bacterial groups (Abadias et al., 2008; Jay et al., 2005). The persistence of coliforms throughout soaking suggests continued contamination risk despite progressive acidification. These findings indicate that spontaneous soaking alone may not provide sufficient microbiological control under ambient processing conditions.

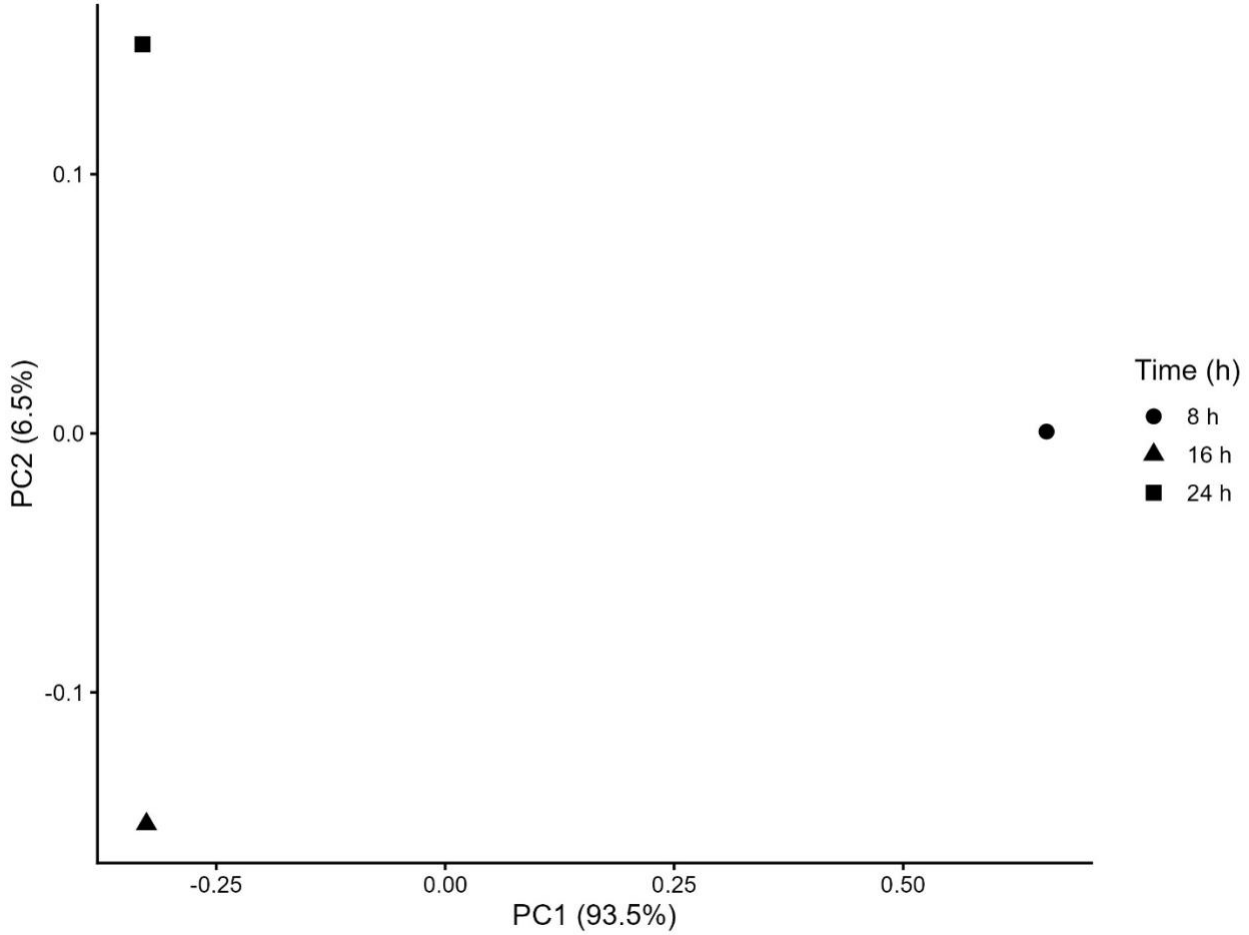

Figure 1. Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity showing temporal separation of bacterial community profiles during tiger nut soaking at 8, 16, and 24 h. n = 1 sample per time point; no replicate error bars apply.

3.3. Sequencing output and diversity patterns

Sequencing outputs across bioinformatic processing stages are summarised in Supplementary Table S1. The 8 h sample yielded the highest number of reads across processing stages (81,403 raw reads; 5,820 reads after chloroplast removal). The 16 h and 24 h samples produced 34,938 and 43,315 raw reads, respectively, declining to 1,670 and 1,323 reads after filtering and chloroplast removal. A total of 70 ASVs were initially generated across all samples, of which 28 bacterial ASVs were retained for downstream analysis following removal of chloroplast-derived sequences.

Alpha diversity showed limited variation across soaking stages. Observed ASVs were 11 at 8 h, 13 at 16 h, and 12 at 24 h. Chao1 estimates were identical to the observed values. Beta diversity analysis based on Bray–Curtis dissimilarity revealed temporal separation of bacterial profiles across soaking stages.

Principal coordinates analysis (PCoA) showed that the 8 h sample clustered separately from the 16 h and 24 h samples, which grouped more closely together, indicating greater similarity in bacterial composition at later soaking stages (Fig. 1).

The low sequencing depth after chloroplast removal likely reflects the high proportion of plant-derived sequences. Reads retained after filtering and chloroplast removal were 7.2%, 4.8% and 3.1% of raw reads at 8, 16 and 24 h, respectively. Nevertheless, the clustering pattern suggests shifts in dominant bacterial groups during soaking.

PC1 and PC2 explained 93.5% and 6.5% of total variance, respectively. The 16 h and 24 h samples were closer to each other than to the 8 h sample. With one sample per time point, this pattern is indicative only.

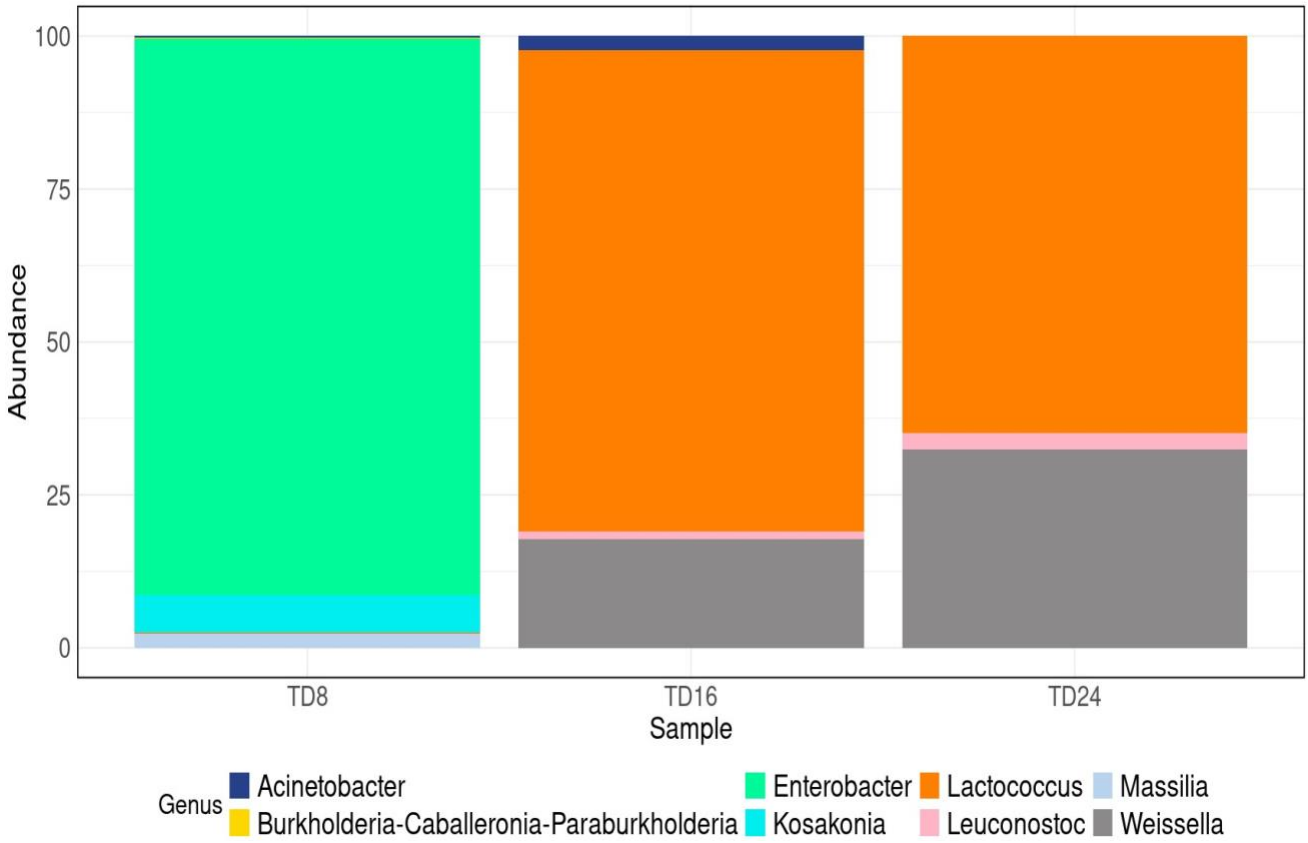

Figure 2. Relative abundance of dominant bacterial genera during tiger nut soaking at 8, 16, and 24 h, based on taxonomic assignment against the SILVA reference database (v138.1, genus level). n = 1 sample per time point; no replicate error bars apply.

3.4. Taxonomic composition during soaking

At phylum level, Firmicutes dominated the bacterial profile across all soaking stages, whereas Proteobacteria contributed more prominently at 8 h and declined thereafter. At genus level, *Weissella*, *Leuconostoc*, and *Lactococcus* were the dominant taxa. The 8 h sample exhibited a more heterogeneous bacterial profile, with lower relative abundance of these genera. As soaking progressed, *Weissella* and *Leuconostoc* increased in representation, with *Weissella* showing the most pronounced increase across soaking stages. *Weissella cibaria* was identified among dominant taxa at 16 h and 24 h. *Leuconostoc* increased progressively but remained less abundant than *Weissella*, whereas *Lactococcus* remained relatively stable throughout soaking (Fig. 2).

The increased representation of *Weissella* and *Leuconostoc* at later soaking stages is consistent with the progressive acidification observed during soaking. Previous studies have associated these bacterial groups with plant-derived fermentative environments and mildly acidic processing conditions (Fusco et al., 2015; Bintsis, 2018). The detection of *Weissella cibaria* among dominant taxa at later soaking stages is also consistent with reports describing its occurrence in spontaneous plant-associated fermentative environments. The persistence of *Lactococcus* across soaking stages further suggests that multiple lactic acid bacteria-associated taxa contributed to microbial changes during soaking, consistent with observations from traditional plant-based fermentation systems (Holzapfel, 2002).

3.5. Practical implications and study limitations

Despite progressive acidification, the final pH remained comparatively high (5.5) relative to levels generally considered inhibitory to many foodborne pathogens in acidic food systems (Beuchat & Ryu, 1997). This indicates that spontaneous soaking alone may not provide sufficient acidification to ensure microbiological safety. Coliform counts also persisted throughout soaking, indicating continued contamination risk under ambient processing conditions. The increase in coliform counts at 24 h suggests possible contamination from multiple sources, including the raw tubers, soaking water, processing containers, and handling practices (Doyle et al., 2013). Furthermore, the persistent coliform counts observed during soaking raise concerns about the microbiological safety of the process, particularly under uncontrolled conditions. These findings underscore the importance of appropriate hygiene and sanitation measures during soaking to minimize potential microbial contamination.

These findings indicate that tiger nut soaking should not be regarded solely as a hydration step but rather as a microbiologically active processing stage requiring appropriate hygiene and process control. Practical interventions including optimisation of soaking duration, use of potable water, temperature management, and improved sanitation of soaking containers may help reduce microbial risks during production.

The use of controlled starter cultures, rather than reliance on spontaneous microbial activity, could direct fermentation-associated changes toward a more predictable and food-safe microbial profile. Post-soaking pasteurisation would provide a defined lethality step prior to further processing, addressing the persistent coliform contamination observed here. Refrigerated soaking may also be considered as a lower-risk alternative to ambient soaking, since reduced temperature would be expected to slow both spoilage and pathogen proliferation, at the cost of a longer processing time

Similar concerns have been reported for informally processed plant-based beverages where inadequate hygiene and poorly controlled processing conditions contribute to microbiological safety risks (Nout, 2009). In contrast, industrial tiger nut beverage systems such as horchata production incorporate defined soaking conditions and post-processing treatments to improve microbiological stability and product safety (Codina-Torrella et al., 2018).

As noted in Section 2.4, a limitation of this study is that sequencing was performed on a single sample per time point and therefore provides an indicative rather than fully replicated representation of bacterial composition during soaking. In addition, sequencing depth after chloroplast removal was comparatively low at later soaking stages (1,670 and 1,323 reads at 16 and 24 h), likely reflecting the high proportion of plant-derived sequences. Nevertheless, the sequencing results complemented the culture-based observations and provided supportive insight into dominant bacterial groups associated with soaking progression.

4. Conclusions

Tiger nut soaking under ambient conditions promoted progressive microbiological and physicochemical changes associated with early

fermentation-related activity. Increases in total aerobic counts and lactic acid bacteria corresponded with declining pH and increasing titratable acidity, while sequencing analysis indicated increased representation of lactic acid bacteria-associated genera including *Weissella* and *Leuconostoc* during soaking. However, persistent coliform contamination and comparatively high final pH values indicated that spontaneous soaking alone may not provide sufficient microbiological safety. These findings identify soaking as a microbiologically active processing stage requiring improved hygiene and process control during tiger nut beverage production.

5. References

Abadias, M., Usall, J., Anguera, M., Solsona, C. and Vinas, I. (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. International Journal of Food Microbiology, 123(1-2), 121-129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013

Adejuyitan, J.A. (2011). Tigernut processing: its food uses and health benefits. American Journal of Food Technology, 6(3), 197-201. https://doi.org/10.3923/ajft.2011.197.201

APHA (2001). Compendium of Methods for the Microbiological Examination of Foods, 4th ed. American Public Health Association, Washington DC.

Beuchat, L.R. and Ryu, J.H. (1997). Produce handling and processing practices. Emerging Infectious Diseases, 3(4), 459-465. https://doi.org/10.3201/eid0304.970407

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: an update in their metabolism and genetics. AIMS Microbiology, 4(4), 665-684. https://doi.org/10.3934/microbiol.2018.4.665

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. and Holmes, S.P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581-583. https://doi.org/10.1038/nmeth.3869

Callahan, B.J., Wong, J., Heiner, C., Oh, S., Theriot, C.M., Gulati, A.S., McGill, S.K. and Dougherty, M.K. (2019). High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. Nucleic Acids Research, 47(18), e103. https://doi.org/10.1093/nar/gkz569

Codina-Torrella, I., Guamis, B., Zamora, A., Quevedo, J.M. and Trujillo, A.J. (2018). Microbiological stabilization of tiger nuts' milk beverage using ultra-high pressure homogenization. Food Microbiology, 69, 143-150. https://doi.org/10.1016/j.fm.2017.08.003

Dike, K.S., Okafor, C.P., Ohabughiro, B.N., Maduwuba, M.C., Ezeokoli, O.T., Ayeni, K.I., Okafor, C.M. and Ezekiel, C.N. (2022). Analysis of bacterial communities of three cassava-based traditionally fermented Nigerian foods. Letters in Applied Microbiology, 74(3), 452-461. https://doi.org/10.1111/lam.13621

Djomdi, E., Ejoh, R. and Ndjouenkeu, R. (2007). Soaking behaviour and milky extraction performance of tiger nut (Cyperus esculentus) tubers. Journal of Food Engineering, 78(2), 546-550. https://doi.org/10.1016/j.jfoodeng.2005.10.022

Doyle, M.P., Beuchat, L.R. and Montville, T.J. (2013). Food Microbiology: Fundamentals and Frontiers, 4th ed. ASM Press, Washington DC.

Ercolini, D. (2013). High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Applied and Environmental Microbiology, 79(10), 3148-3155. https://doi.org/10.1128/AEM.00256-13

Fusco, V., Quero, G.M., Cho, G.S., Kabisch, J., Meske, D., Neve, H., Bockelmann, W. and Franz, C.M. (2015). The genus Weissella: taxonomy, ecology and biotechnological potential. Frontiers in Microbiology, 6, 155. https://doi.org/10.3389/fmicb.2015.00155

Holzapfel, W.H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology, 75(3), 197-212. https://doi.org/10.1016/S0168-1605(01)00707-7

Ire, F.S., Vincent, E.S. and Elechi-Joshua, O. (2020). Microbiological assessment of locally produced tiger nut milk drinks sold in Port Harcourt metropolis, Nigeria. Asian Food Science Journal, 14(4), 33-42. https://doi.org/10.9734/afsj/2020/v14i430129

Jay, J.M., Loessner, M.J. and Golden, D.A. (2005). Modern Food Microbiology, 7th ed. Springer, New York.

Nout, M.J.R. (2009). Rich nutrition from the poorest cereal fermentations in Africa and Asia. Food Microbiology, 26(7), 685-692. https://doi.org/10.1016/j.fm.2009.07.002

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590-D596. https://doi.org/10.1093/nar/gks1219

R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sánchez-Zapata, E., Fernández-López, J. and Pérez-Álvarez, J.A. (2012). Tiger nut (Cyperus esculentus) commercialization: health aspects, composition, properties, and food applications. Comprehensive Reviews in Food Science and Food Safety, 11(4), 366-377. https://doi.org/10.1111/j.1541-4337.2012.00190.x

Tamang, J.P., Watanabe, K. and Holzapfel, W.H. (2016). Review: diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology, 7, 377. https://doi.org/10.3389/fmicb.2016.00377

Tigrero-Vaca, J., Díaz, B., Gu, G. and Cevallos-Cevallos, J.M. (2025). Next-generation sequencing applications in food science: fundamentals and recent advances. Frontiers in Bioengineering and Biotechnology, 13, 1638957. https://doi.org/10.3389/fbioe.2025.1638957

Ukwuru, M.U. and Ogbodo, A.C. (2011). Effect of processing treatment on the quality of tiger nut milk. Pakistan Journal of Nutrition, 10(1), 95-100. https://doi.org/10.3923/pjn.2011.95.100

Yu, Y., Lu, X., Zhang, T., Zhao, C., Guan, S., Pu, Y. and Gao, F. (2022). Tiger nut (Cyperus esculentus L.): nutrition, processing, function and applications. Foods, 11(4), 601. https://doi.org/10.3390/foods11040601

Data availability

Sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA1456651. These data are publicly accessible.

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