



Extending the Shelf Life of Tiger-nut Milk Drinks Using *Amaranthus Caudatus* (L) (african Spinach) and *Syzygium Aromaticum* (L) Merr. & I.m. Perry (clove) as Natural Preservatives

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ABSTRACT

Tiger-nut (*Cyperus esculentus* L.) is a perennial *monocotyledonous* plant with a tough, erect fibrous root system. Tiger-nut tubers possess a slightly sweet flavour, and their texture is comparable to coconut. Fresh, semi-dried, or dried tubers are commonly consumed as snacks due to their nutty taste. However, many consumers are not well informed about the wide range of products that can be derived from tiger-nut and its nutritional benefits. This contributes to its continued status as an underutilised crop despite widespread consumption in regions where it is cultivated. The high cost of animal milk in developing countries has increased the demand for plant-based milk alternatives. An inexpensive substitute in the form of beverages produced from locally available plant sources, rich in nutrients, can help reduce protein-energy malnutrition. This study evaluates microorganisms associated with tiger-nut milk spoilage, the methods of preparation, and their effects on nutritional composition and shelf stability. It also assesses the effectiveness of *Amaranthus caudatus* and *Syzygium aromaticum* as natural preservatives in extending the shelf life of tiger-nut milk.

Keywords:

Tiger-nut,
Milk,
Processing,
Spoilage,
Shelf-life.

INTRODUCTION

Tigernut (*Cyperus esculentus* L.) belongs to the plant division Magnoliophyta, class Liliopsida, order Cyperales, and family Cyperaceae. Schippers et al. (2014) collected *Cyperus esculentus* from different continents where it grows to carry out an infraspecific taxonomy of the plant. There are three main varieties of tigernut tubers, namely yellow, brown, and black varieties. The black variety is not common in Nigeria but is readily available in Ghana. The yellow and brown varieties are commonly sold in local markets across many states in Nigeria, with the yellow variety generally preferred due to its sweeter taste. Tigernut is also known by various names such as 'Zulu nut', 'chufa', 'yellow nut sedge', 'flat sedge', and 'edible rush nut'. In Nigeria, it is called 'Aya' (Hausa), 'Ofio' (Yoruba), and 'Imumu' (Igbo). It is widely referred to as 'Aki Hausa' in Southern Nigeria, reflecting its major cultivation and trade by people from Northern Nigeria. A large proportion of tigernut consumed as snacks in Nigeria is cultivated in several Northern states. Tigernut tubers have a slightly sweet flavour, and their taste is comparable to coconut. They are consumed fresh, semi-dried, or dried as snacks due to their nutty flavour.

However, many consumers are not aware of the wide range of products and nutritional benefits derived from tigernut, which contributes to its status as an underutilised crop.

GLOBAL DISTRIBUTION AND UTILIZATION

Historically, tigernut cultivation and use is believed to have begun in Egypt around 5000 BC and has since spread to other parts of the world. It is one of the wild edible plants that contributes to human nutrition and supports the economy and cultural practices of rural communities in Sub-Saharan Africa.

Tigernut belongs to the family Cyperaceae, and although many species exist within the genus, *Cyperus esculentus* is the most widely consumed due to its edible tubers. In some countries, it is regarded as a wild plant and used as animal feed, while in others it is directly consumed by humans.

NUTRITIONAL COMPOSITION AND HEALTH BENEFITS

Tigernut is rich in nutrients such as vitamins, amino acids, fats, minerals, proteins, and sugars. It has been reported to possess good-quality plant protein that can help reduce protein-energy malnutrition. Its fatty acid profile,

vitamin content, and mineral composition also contribute to its nutritional and therapeutic value. Tigernut oil has been reported to have nutritional quality comparable to olive oil and may serve as an alternative edible oil source. Products derived from tigernut include milk, flour, and oil, and it can also be used in confectioneries such as biscuits, cookies, chocolate, and traditional beverages like kunu. Phytochemical studies have revealed the presence of alkaloids, tannins, saponins, sterols, resin, and cyanogenic glycosides in tigernut. No confirmed hypersensitivity or allergic reactions have been widely associated with its consumption.

Tigernut milk has been reported to reduce low-density lipoprotein (LDL) cholesterol while increasing high-density lipoprotein (HDL), thereby supporting cardiovascular health. It also contains vitamin E, which acts as an antioxidant against lipid oxidation and reduces the risk of coronary heart disease.

It has been associated with health benefits such as improved digestion, prevention of constipation, support for urinary tract health, and possible reduction in colon cancer risk. It is also suitable for individuals with lactose intolerance and gluten sensitivity.

PROCESSING AND SHELF-LIFE LIMITATION

Tigernut milk is a widely accepted beverage consumed as a refreshing drink and as a plant-based alternative to dairy milk, especially during hot seasons. It is traditionally produced using simple processing methods; however, it has a very short shelf-life due to microbial spoilage and lack of preservation methods.

The limited storage stability of tigernut milk remains a major challenge, leading to underutilization despite its nutritional and economic potential.

MATERIALS AND METHODS

Tiger-nut (*Cyperus esculentus L.*), *Amaranthus caudatus* leaves, and *Syzygium aromaticum* (clove) were used in this study. Other materials included crystal violet, Lugol's iodine, acetone, hydrogen peroxide, sterile deionized water, hydrochloric acid, ethanol-acetone, normal saline, lactophenol cotton blue stain, and sodium benzoate (chemical preservative).

Tiger-nut tubers were obtained from Kuje Market, Federal Capital Territory, Abuja, Nigeria, and were authenticated herbarium at the Department of Botany, Nigerian Defence Academy Kaduna, Kaduna State, Nigeria, where a voucher specimen number VSN: TN-2024-001

PREPARATION OF TIGER-NUT MILK DRINK

Tiger-nut milk was prepared using a soaking and wet milling method. Soaking is a unit operation used to soften plant materials and reduce anti-nutritional factors such as

tannins, oxalates, phytic acid, and polyphenols, which may be leached into the soaking water and discarded.

Cleaned and sorted tiger-nut tubers were soaked in clean water at approximately 60°C for 12 hours to allow water absorption and softening (Ukwuru and Ogbodo, 2011).

After soaking, the softened tubers were wet-milled and filtered using muslin cloth to obtain tiger-nut milk. The filtrate obtained was pasteurized at 72°C for 15 minutes and allowed to cool before further treatments.

SAMPLE PREPARATION AND EXPERIMENTAL DESIGN

The cooled tiger-nut milk was divided into four treatment groups:

Sample A: Control (tiger-nut milk only)

Sample B: Tiger-nut milk + *Syzygium aromaticum* (clove)

Sample C: Tiger-nut milk + *Amaranthus caudatus*

Sample D: Tiger-nut milk + combination of clove and *Amaranthus caudatus*

The preservatives were incorporated during preparation as follows:

Clove was added at 1 g during soaking

Amaranthus caudatus was blended with extracted milk prior to filtration

STORAGE CONDITIONS

The prepared samples were subdivided and stored under four conditions:

1. Ambient temperature

TNA (control)

TNCA (clove)

TNAA (*Amaranthus*)

TNCAA (combination)

2. Refrigeration temperature (4°C)

TNR, TNCR, TNAR, TNCAR

3. Accelerated temperature (35°C)

TNAc, TNCAc, TNAAc, TNCAAc

4. Chemical preservative (sodium benzoate)

TNBn, TNCBn, TNABn, TNCAbn

Each treatment was stored in sterile containers for microbial and physicochemical analysis over a defined storage period.

STORAGE STABILITY ANALYSIS

Changes in pH and crude protein content were monitored during storage under different temperature conditions.

A progressive increase in crude protein content and a corresponding decrease in pH were observed across storage days, indicating increased acidity during storage, especially in untreated samples.

MICROBIOLOGICAL ANALYSIS

Microbial analysis was conducted at regular intervals (0, 7, 14, and 21 days) using standard microbiological methods.

Samples were cultured and examined for bacterial growth, and isolates were identified based on morphological and biochemical characteristics.

TIGER-NUT PRESERVATION

Tiger-nut milk is highly perishable due to its rich nutritional composition (Nutso, 2014). It has a very short shelf-life, often less than 24 hours depending on storage conditions (Akoma et al., 2006). High temperature and humidity further reduce its shelf stability. The rapid spoilage is mainly due to microbial activity, which limits its wider consumption and commercial utilization (Ejoh et al., 2006).

NATURAL PRESERVATIVES FOR TIGER-NUT MILK DRINKS

Preservatives can improve the stability and shelf-life of tiger-nut milk by inhibiting microbial growth (Akoma et al., 2006). In this study, two natural preservatives were investigated: *Amaranthus caudatus* (African spinach) and *Syzygium aromaticum* (clove).

Amaranthus caudatus (African spinach)

Amaranthus caudatus is widely used as a leafy vegetable and has been reported to possess medicinal properties. It is traditionally used as a sudorific and febrifuge and is recommended for eruptive fevers. The leaves are also considered emollient and lactogenic, and are used in the treatment of colic (Ayethan et al., 1996). Externally, the leaves may be applied to treat eczema.

The plant is cultivated in different regions of the world, including Africa, Asia, and South America, and thrives in a wide range of soil conditions (Faccila, 1990). In Nigeria, the leaves are commonly used in the preparation of vegetable soups (Oke, 1983).

Several studies have reported that medicinal plants possess antimicrobial and phytochemical constituents that can inhibit microbial growth. These bioactive compounds include tannins, alkaloids, flavonoids, saponins, terpenes, and glycosides, which contribute to antimicrobial activity (Akinpelu and Onakoya, 2006; Evarando et al., 2005).

Plant-derived compounds have been widely investigated as alternatives to synthetic antimicrobials due to increasing microbial resistance. These compounds exhibit different structures and mechanisms of action compared to conventional antimicrobial agents (Nascimento et al., 2000).

Syzygium aromaticum (Clove)

Syzygium aromaticum (clove) is a spice widely used for centuries as a food preservative and medicinal plant due to its strong antioxidant and antimicrobial properties (Shan et al., 2005). It contains a high concentration of phenolic compounds, particularly gallic acid, tannins, flavonoids, and other bioactive substances.

Clove essential oil contains eugenol as its major component, along with eugenol acetate and β -caryophyllene (Jirovetz et al., 2006). These compounds are

responsible for its antimicrobial activity against a wide range of bacteria and fungi.

Studies have shown that clove extracts exhibit strong inhibitory effects against food-borne pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, confirming its potential as a natural preservative (Sofia et al., 2011).

RESULTS AND DISCUSSION

MICROBIAL COMPOSITION OF TIGER-NUT MILK STORED AT DIFFERENT TEMPERATURES

Refrigeration Temperature

The microbial composition of tiger-nut milk drink samples stored at refrigeration temperature with different preservatives showed varying microbial loads over 21 days of storage.

The total bacterial count, total coliform count, and total fungal count were highest on day 21 for the tiger-nut milk drink without preservatives. No coliform or fungal counts were detected on day 1 in the control sample (without preservative).

For tiger-nut milk drink preserved with cloves, all microbial counts (total bacterial, coliform, and fungal counts) increased progressively and were highest on day 21 (were all highest at day 21 at refrigeration temperature).

For samples preserved with *Amaranthus caudatus*, the total bacterial count was highest on day 7, while the total fungal count peaked on day 21. The total coliform count peaked on days 14 and 21.

For tiger-nut milk drink preserved with *Amaranthus caudatus* and *Syzygium aromaticum*, the total bacterial count was highest on day 14, while total coliform and fungal counts were highest on day 21.

Ambient Temperature

At ambient temperature, microbial counts increased with storage duration across all samples.

For tiger-nut milk drink without preservatives, the total bacterial count peaked on day 14, while total coliform and fungal counts peaked on day 21.

For samples preserved with cloves, total bacterial, coliform, and fungal counts were all highest on day 21.

For samples preserved with *Amaranthus caudatus*, the total bacterial and fungal counts were highest on day 21, while the total coliform count peaked on day 7

For samples preserved with *Syzygium aromaticum* and *Amaranthus caudatus*, total bacterial and fungal counts peaked on day 21, while total coliform count peaked on day 14.

Elevated Temperature (35°C)

At elevated temperature (35°C), microbial growth was generally accelerated across all samples.

For tiger-nut milk drink without preservatives, total bacterial, coliform, and fungal counts were highest on day 21 and lowest on day 1.

A similar pattern was observed in samples preserved with cloves, where all microbial counts peaked on day 21.

For samples preserved with *Amaranthus caudatus*, total bacterial and coliform counts peaked on day 21, while fungal count peaked on day 14. All microbial counts were lowest on day 1.

For samples preserved with *Syzygium aromaticum* and *Amaranthus caudatus*, total bacterial and fungal counts peaked on day 21, while total coliform count peaked on day 14. No microbial counts were recorded on day 1.

Chemical Preservative (Sodium Benzoate)

The microbial composition of tiger-nut milk preserved with sodium benzoate is presented in Table 4. The control and clove-treated samples showed highest microbial counts on day 21 and lowest on day 1 across all microbial groups.

For samples preserved with *Amaranthus caudatus*, bacterial and coliform counts peaked on day 14, while fungal counts peaked on day 7. In the combined treatment (clove + *Amaranthus caudatus*), bacterial and coliform counts were highest on day 21, while fungal counts peaked on day 14.

Microbial Quality Of Untreated Tiger-nut Milk Drink

The total bacterial species isolated from tiger-nut milk drink without any preservative were monitored at 7-day intervals up to day 21. The isolates identified included *Bacillus pumilus*, *Staphylococcus xylosus*, *Staphylococcus aureus*, and *Bacillus spp.*, which varied across the storage period.

Bacillus cereus was present during the early storage period (day 1–14) but was not detected on day 21, indicating possible environmental or competitive microbial changes. In contrast, *Lactobacillus spp.*, *Bacillus brevis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Bacillus subtilis* were not detected in the early storage days (day 1–7) but appeared from day 14 onward, suggesting microbial succession during storage.

Effect of *Amaranthus Caudatus* on Microbial Load

In the tiger-nut milk drink preserved with *Amaranthus caudatus*, *Bacillus pumilus* was consistently isolated throughout the storage period. However, *Bacillus cereus* was not detected by day 21. *Staphylococcus xylosus* was absent at day 14, while *Bacillus brevis* was not detected during the early storage period (day 1–7).

These findings suggest that *Amaranthus caudatus* extract exhibits moderate antimicrobial activity, particularly against *Bacillus cereus* and *Staphylococcus xylosus*, thereby reducing microbial persistence during storage.

Combined Effect of *Syzygium aromaticum* and *Amaranthus caudatus*

The sample preserved with *Syzygium aromaticum* (clove) and *Amaranthus caudatus* showed improved microbial

control compared to the control. *Bacillus cereus* and *Bacillus pumilus* were present during days 1, 7, and 14 but were absent by day 21.

Staphylococcus xylosus was detected only on days 7 and 21, while *Bacillus brevis* appeared at days 14 and 21. The reduction in microbial diversity over time suggests synergistic antimicrobial effects between clove and African spinach extracts.

Effect of Sodium Benzoate (Chemical Preservative)

In the sodium benzoate-treated sample, *Bacillus pumilus* and *Staphylococcus xylosus* were detected throughout all storage days. However, *Bacillus brevis* was only present on days 14 and 21, while *Staphylococcus aureus* was not detected on day 21.

Lactobacillus plantarum was absent on day 1 but appeared from day 7 onward, indicating gradual microbial adaptation in chemically preserved samples.

The presence and persistence of bacterial species in untreated tiger-nut milk confirm its high perishability due to nutrient-rich composition that supports microbial growth. This agrees with earlier findings that tiger-nut milk deteriorates rapidly under ambient storage conditions.

The reduction in microbial load observed in samples treated with plant-based preservatives (*Amaranthus caudatus* and *Syzygium aromaticum*) indicates their antimicrobial potential, likely due to bioactive compounds such as flavonoids, tannins, and essential oils. The combined plant extract treatment showed better microbial suppression than single or untreated samples, suggesting possible synergistic antimicrobial action.

Table 1: Summary of Microbial Growth Pattern of Tiger-nut Milk at Refrigeration Temperature Treatment Total Bacterial Count

| | | |
|---------------------------------------|----|----------------|
| Control (No Preservative) | 21 | Highest on Day |
| Clove (<i>S. Aromaticum</i>) | 21 | Highest on Day |
| African spinach (A. <i>Caudatus</i>) | 7 | Highest on Day |
| Clove + African spinach | 14 | Highest on Day |

Table 2: Summary of Microbial Growth Pattern of Tiger-nut Milk at Ambient Temperature Treatment Total Bacterial Count

| | |
|--------------------------------|-------------------|
| Control (No Preservative) | Highest on Day 14 |
| Clove (<i>S. Aromaticum</i>) | Highest on Day 21 |

| | |
|---------------------------------------|-------------------|
| African spinach (A. <i>Caudatus</i>) | Highest on Day 21 |
| Clove + African spinach | Highest on Day 21 |

Table 3: Summary of Microbial Growth Pattern of Tiger-nut Milk at Elevated Temperature Treatment Total Bacterial Count

| | |
|---------------------------------------|---------------------------------------|
| Control (No Preservative) | Highest on Day 21; Lowest on Day 1 |
| Clove (<i>S. Aromaticum</i>) | Highest on Day 21; Lowest on Day 1 |
| African spinach (A. <i>Caudatus</i>) | Highest on Day 21; Lowest on Day 1 |
| Clove + African spinach | Highest on Day 21 |

Table 4: Summary of Microbial Growth Pattern of Tiger-nut Milk with Sodium Benzoate Treatment Total Bacterial Count

| | |
|---------------------------------------|---------------------------------------|
| Control (No Preservative) | Highest on Day 21; Lowest on Day 1 |
| Clove (<i>S. Aromaticum</i>) | Highest on Day 21; Lowest on Day 1 |
| African spinach (A. <i>Caudatus</i>) | Highest on Day 14; Lowest on Day 1 |
| Clove + African spinach | Highest on Day 21; Lowest on Day 1 |

This study evaluated the microbial composition of tiger-nut milk drinks stored under different temperature conditions (refrigeration, ambient, and elevated temperature) using various natural and chemical preservatives. It highlights the influence of storage conditions and preservatives on microbial growth dynamics over time.

The data indicate that microbial load (total bacterial count, total coliform count, and total fungal count) increased progressively with storage duration, reaching peak values on day 21 for most samples. This trend suggests that even under refrigeration, microbial proliferation still occurs, although at a reduced rate compared to higher temperatures (Santos et al., 2020; Duret et al., 2021).

At ambient temperature, microbial growth was more rapid and pronounced across all treatments. This confirms that room temperature provides favorable conditions for microbial activity, thereby accelerating spoilage of tiger-nut milk drinks. The observed increase in bacterial, coliform, and fungal populations over time indicates poor shelf stability in the absence of effective preservation. Recent studies in JOBASR have reported that tiger-nut-based extracts exhibit antimicrobial and bioactive

properties that support food preservation and microbial control (Mallo et al., 2022).

The microbial composition of tiger-nut milk drinks stored at an elevated temperature of 35°C revealed significant microbial growth patterns over time. Across all samples, microbial counts were lowest on day 1 and highest around day 21, indicating progressive microbial colonization during storage. This suggests that prolonged exposure to elevated temperatures enhances microbial multiplication and spoilage risk.

The observed variations in microbial peaks across different organisms and preservative types indicate that microbial adaptation and survival depend on both environmental conditions and preservative efficacy. Initial low counts observed on day 1 may be due to temporary microbial suppression or lag phase adaptation under stress conditions.

High temperature conditions initially may suppress certain microbial populations; however, over time, favorable nutrient availability in tiger-nut milk supports microbial proliferation (Ruan et al., 2024). This finding suggests that tiger-nut milk drinks are highly perishable under elevated temperatures, reinforcing the need for refrigeration and/or effective preservatives to extend shelf life.

Overall, the findings demonstrate that both natural preservatives (*Amaranthus caudatus* and *Syzygium aromaticum*) and chemical preservatives can influence microbial growth patterns, although their effectiveness varies with storage conditions and duration.

CONCLUSION

The microbial quality of tiger-nut drink stored under different conditions (refrigerated, ambient, and elevated temperatures) over a 21-day period was evaluated at 7-day intervals using different preservation treatments. Total bacterial count ranged from no detectable growth to 2.7×10^5 CFU/mL, total coliform count ranged from no detectable growth to 2.3×10^4 CFU/mL, and total fungal count ranged from no detectable growth to 3.9×10^3 CFU/mL. These findings indicate progressive microbial proliferation during storage, with variations depending on temperature and preservative type.

Sensory evaluation showed that tiger-nut drink preserved with *Syzygium aromaticum* (clove extract) recorded the highest acceptability scores, followed by samples treated with sodium benzoate and the unpreserved control. Samples preserved with a combination of *Syzygium aromaticum* and *Amaranthus caudatus* ranked intermediate in sensory quality, while those treated solely with *Amaranthus caudatus* had the lowest acceptability scores.

Microbial analysis revealed the presence of *Bacillus cereus*, *Lactobacillus spp.*, *Bacillus pumilus*, *Staphylococcus xylosum*, *Bacillus brevis* *Amaranthus*

caudatus), *Staphylococcus aureus*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Bacillus spp.*, and *Lactobacillus acidophilus*. The unpreserved tiger-nut drink exhibited a higher diversity and load of microbial isolates compared to the preserved samples, indicating the effectiveness of the applied preservatives in limiting microbial contamination.

Over the 21-day storage period, crude protein content generally increased, while pH values decreased across all storage conditions and preservative treatments. This trend suggests ongoing biochemical and microbial activities during storage that contributed to changes in the nutritional and physicochemical properties of the beverage.

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