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The Effect of Zingiber officinale and Allium sativum on the Microbial and Physiochemical Quality of Commercially Produced Tiger Nut Drink

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Abstract

This study investigated the effect of different spices and storage temperatures on the microbial dynamics and physiochemical characteristics of commercially produced tiger nut drinks over a 9-day period. Standard microbiological and physiochemical procedures were adopted. The total heterotrophic bacteria count of tiger nut drink ranged from 1.02 x 10^4 to 9.8 x 10^6 cfu/ml, the total coliform count ranged from 6.3 x10^3 to 6.9 x10^4 cfu/ml, and the total staphylococcus count ranged from 2.9 x 10^3 to 5.8 x10^4 cfu/ml. The effectiveness of refrigeration in preventing bacterial growth was demonstrated by the generally lower THBC and TC levels found in refrigerated samples. Higher THBC was observed in ambient Tiger nut juice treated with ginger and garlic (ATTGG), indicating possible effects of these additions on microbial development. The Tiger Nut Drink's Staphylococcus level varied depending on the treatment; samples that were refrigerated consistently had lower numbers. Staphylococcus levels were generally reduced after garlic treatment (ATTGA), indicating possible antibacterial qualities. The prevalence of *Bacillus, Staphylococcus, Micrococcus, Proteus, Lactobacillus,* and *Escherichia coli* varied. Temperature variations had a major impact on the spread of microorganisms, highlighting the importance of ideal storage conditions. Throughout the investigation, physiochemical factors changed. Tiger nut juice showed pH decreases, TTA fluctuations, ascorbic acid content changes, and TSS alterations. This study provides valuable insights into the microbial dynamics and physiochemical stability of commercially made Tiger nut drinks, emphasizing the importance of appropriate storage conditions for maintaining product quality and safety. The findings contribute to understanding the shelf life of these popular beverages, paving the way for improved preservation strategies and enhanced consumer safety, and the study revealed the synergistic effect of ginger and garlic in the tiger nut drink sample can be used to minimize bacterial load to an acceptable limit for at least six to nine days after production.

Keywords: *Tiger-Nut, Storage Temperature, Zingiber officinale, Allium sativum, Physiochemical and Microbial quality*

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I. INTRODUCTION

Known by its scientific name, Cyperus esculentus, tiger nut is a hardy perennial plant that is a member of the Cyperaceae family. It produces spherical tubers and rhizomes and has tough, upright fibrous roots. It can reach a height of one to three feet and a depth of roughly six inches in the ground. The plant reproduces via seeds, with pollination facilitated by wind. Tiger nut milk, a popular beverage during the dry season, is a sweet, non-dairy, nutritious, energizing, and diuretic liquid extract derived from tiger nut tubers (Ibrahim and Jumare, 2023).

It is well known that this type of milk is rich in nutrient and vitamin C as well as E, phosphorus, magnesium, potassium, calcium, iron and carbohydrates, unsaturated fats, proteins, and digestive enzymes. In comparison, the tiger nut drink has a bigger amount of iron, magnesium, and carbohydrates than cow's milk (Naeem and Youssef 2022). *Cyperus esculentus* L. is a type of perennial herb that produces edible tubers which is a good source of carbohydrates, lipids, dietary fibers, proteins, minerals, ascorbic acids, and alpha tocopherols (Ekeanyanwu and Ononogbu, 2010). Fiber has been reported to increase in gluten-free bread and biscuits when these nuts

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are included (Zahra and Ahmed, 2014; Aguilar *et al*., 2015). Despite their nutritional richness, tiger nut drinks have a limited shelf life due to potential microbial contamination during preparation, packaging, storage, and distribution. The drink contains nutrients conducive to microbial growth and spoilage. Various factors influence microbial growth in beverages, including the introduction of microbes from the soil during growth, harvest, packaging, storage, and handling (Nester *et al.,* 2022). The absence of effective antimicrobial treatments throughout the production process implies that pathogens introduced at any stage may persist in the final product (Maduka and Ire, 2019).

Some spices, especially ginger, garlic, etc., have also been reported to be preservative (Wood and Pittler, 2000; Ben-Nwadibia, 2005; Nwobosi *et al.,* 2013; Kayode *et al.,* 2017; Eke-Ejiorfor and Awaji, 2019). They have antioxidant properties and improve the antioxidant capacity of foods; their efficiency is matrix-dependent (Kayode *et al.,* 2017). Other hypolipidaemic effect of garlic (Allium sativum) include the inhibition of enzymes involved in lipid synthesis and platelet aggregation, the prevention of lipid peroxidation of oxidised erythrocyte and low-density lipoprotein (LDL), enhancement of the antioxidant status and inhibition of angiotensin-converting enzyme (Eke-Ejiorfor and Awaji, 2019). Garlic consumption is associated with reduced cholesterol and blood pressure and increased antioxidant status (Khalid, 2015). The study is aimed at determining the effect of natural spices on the microbial and physiochemical quality of commercially produced tiger nut drink (*Cyperus esculentus lativum*).

II. Materials And Methods

A. Tiger-Nut drink

Tiger-nut milk is prepared using the following recipes: Tiger nuts, Date seeds, Coconut and Water.

B. The procedures

One kilogramme full of tiger nuts was bathed in distilled water for 8 hours. The tiger nuts were washed and then blanched at 70 °C for 5 minutes, mainly to deactivate enzymes responsible for the agglomeration of the extract. Fresh tiger nuts were then blended at least three times into a slurry with water (approximately 6L made up in total) in an auto-clean blender. The milk was extracted from the slurry, which was pressed with muslin cloth. The extract was pasteurized at 72°C for 5 seconds, homogenized and cooled quickly. Extraction of tiger-nut milk was done as described by Maxwell *et al.,* (2019).

C. Sample collection and procedure

Three hundred fifty milliliters (350 ml) of commercially made tiger nut drinks were procured locally using a simple random sampling technique. A total of 26 samples were collected and organized into three sets of 8 samples each. The first set of 8 samples was treated with garlic, the second set with ginger, and the third set with a combination of ginger and garlic as seen in Table 1. Additionally, 2 samples were used as controls without any treatment as described by Kayode *et al.,* (2017) with slight modification.

For each treatment, 4 samples were kept at refrigeration temperature, while the other 4 samples were stored at ambient temperature, along with the control samples for each storage condition as presented in table 1. The samples were analyzed on days 1, 3, 5, 7, and 9.

Table 1**.** Experimental setup showing the different storage conditions and treatments of the tigernut drinks.

S/N	Ambient temperature	Refrigerated temperature
1	1 set of 350ml of tiger nut without treatments(control)	1 set of 350ml of tiger nut without treatments(control)
2	4 set of 350ml of tiger nut +1g of ginger	4 set of 350ml of tiger nut + 1g of ginger
3	4 set of 350ml of tiger nut + 1g of garlic	4 set of 350ml of tiger nut + 1g of garlic
4	4 set of 350ml of tiger nut + 0.5g of ginger & garlic	4 set of 350ml of tiger nut + 0.5g of ginger & garlic

D. Media preparation

Plate count agar (PCA), MacConkey agar, and Mannitol salt agar were prepared according to manufacturer's recommendations and autoclaved at 121 °C for 15min, and medium was allowed to cool to 45- 50 °C.

E. Microbial analysis: Enumeration of total heterotrophic bacterial count, total staphylococcus count, total coliform count and total fungal count.

One milliliter (1 ml) of each sample was serially transferred into nine milliliters (9 ml) of the sterile diluent (peptone water) with a sterile pipette and shaken vigorously. Serial dilution was continued until 10^6 dilutions were obtained . Aliquot portion (0.1 ml) of the 10^3 to 10^6 dilutions were inoculated onto freshly prepared, surface-dried nutrient agar (NA) and MacConkey agar (MCA) and Mannitol salt agar (MSA) respectively. As described by Kayode *et al.* (2017) with slight modification.

Total heterotrophic bacterial count was obtained using Plate count agar (PCA), Total Staphylococci count was on mannitol salt agar, and Total coliform count was on MacConkey agar. Counts were recorded after 18- 24hrs of incubation on Plate count agar, Mannitol salt agar and MacConkey agar. Counts from the incubated agar plates were enumerated after 24 hours for bacteria.

The cfu/ml was determined using Cfu/ml = Number of colonies x Dilution factor/ number of culture plate.

F. Identification of isolates

Bacterial isolates were characterized and identified using cultural, morphological and microscopic examinations. The macroscopic examination of the colonies was differentiated based on size, color, pigmentation, elevation surface texture and margin. Different biochemical tests such as Gram staining, Catalase, Coagulase, Methyl-red, Oxidase, Voges-Proskauer and sugar fermentation test were employed to differentiate the bacterial isolates according to the standard microbiological methods as described by Cheesbrough (2005).

G. Physicochemical parameters

The pH and titratable acidity were determined according to the AOAC method (1990). The total soluble solid was done as described by AOAC. method (2012) and the determination of ascorbic acid content was described by Vasant *et al*., (2013).

H. Statistical analyses

Analysis of variance (ANOVA) was used to compare means at $p < 0.05$. This analysis was performed to visualize the association between the microbial loads of tiger nut drinks treated with natural spices and the different storage conditions. Using SPSS (Statistical Package for the Social Sciences), also known as IBM SPSS Statistics, is a software package used for the analysis of statistical data.

III. Results

A. Total Heterotrophic bacteria count of Tiger nut drink for the different days and storage conditions of study

The total heterotrophic bacteria count (THBC) of the Tiger nut drink is seen in figure 1. Day 0 of the study showed that sample TC (Tiger nut drink without treatment) had a count of 9.6 x 10^4 CFU/ml while samples TGG (Tiger nut drink treated with Ginger and Garlic), TGI (Tiger nut drink treated with Ginger) and TGA (Tiger nut drink treated with Garlic) had counts of 8.2 x 10^4, 9.0 x 10^4, and 9.0 x 10^4 CFU/g.

On day 3,5,7,9 the respective treatments of Tiger nut drink stored at ambient temperature had different counts ranging from 1.02 x 10^4 to 7.2 x 10^6 cfu/ml for ATTO (Tiger nut drink stored at ambient temperature without treatment); 9.4 x 10^4 to 8.7 x 10^6 cfu/ml for ATTGG (Tiger nut drink stored at ambient temperature treated with ginger and garlic); 8.4 x 10^6 to 9.2 x 10^4 cfu/ml for ATTGI (Tiger nut drink stored at ambient temperature treated with ginger) and 8.8 x 10^4 to 9.8 x 10^6cfu/ml for ATTGA (Tiger nut drink stored at ambient temperature treated with garlic). Likewise, The respective treatments stored at refrigeration temperature on Day 3,5,7,9 had varying THB counts including the 9.1 x 10^4 to 4.6 x 10^5 cfu/ml recorded for RTTC (Tiger nut juice stored at refrigerated temperature without treatment); 7.3 x 10^4 to 4.6 x 10^5 cfu/ml recorded for RTTGG (Tiger nut drink stored at refrigeration temperature treated with ginger and garlic); 6.9 x 10^4 to 3.5 x 10^5 CFU/ml recorded for RTTGI (Tiger nut drink stored at refrigerated temperature treated with ginger) and 8.4 x 10^4 to 3.2 x 10^5 cfu/ml for RTTGA (Tiger nut juice stored at refrigerated temperature treated with garlic).

B. Total coliform count of Tiger nut drink for the different days and storage conditions of study

The total coliform count (TC) of the Tiger nut drink is illustrated in figure 2. Day 0 of the study showed that sample TC (Tiger nut drink without treatment) had a count of 6.6 x 10^3CFU/ml while samples TGG (Tiger nut drink treated with Ginger and Garlic), TGI (Tiger nut drink treated with Ginger) and TGA (Tiger nut juice drink with Garlic) had counts of 6.2 x 10^3, 5.9 x 10^3, and 6.0 x 10^3 CFU/g respectively.

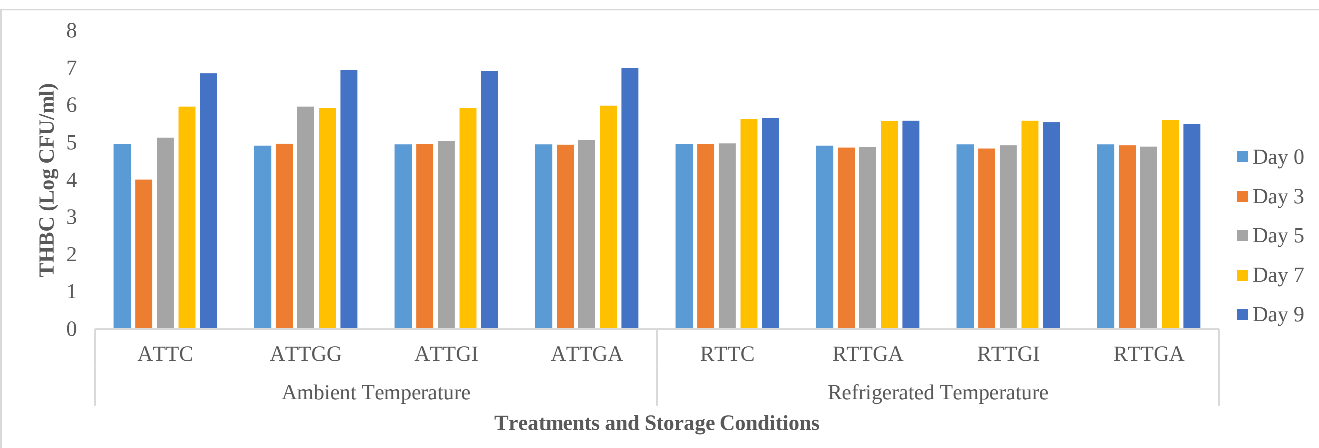

Figure 1. Total Heterotrophic bacteria count of the different Tiger nut drinks stored at different storage conditions.
Key: ATTC: Ambient Temperature Tiger nut drink Control; ATTGG: Ambient Temperature Tiger nut drink Garlic and Ginger; ATTGI: Ambient Temperature Tiger nut drink Ginger; ATZGA: ambient Temperature Tiger nut drink Garlic; RTTC = Refrigeration Temperature Tiger nut drink Control; RTZGG: Refrigeration Temperature Tiger nut drink Garlic and Ginger; RTTGI: Refrigeration Temperature Tiger nut drink Ginger; RTTGA: Refrigeration Temperature Tiger nut drink Garlic.

On day 3,5,7,9 the respective treatments of Tiger nut drink stored at ambient temperature had different counts ranging from 1.02 x 10^4 CFU/ml to 4.2x 10^4 for ATTO (Tiger nut juice stored at ambient temperature without treatment); 9.4 x 10^4 to 4.0x10^4 CFU/ml for ATTGG (Tigernut juice stored at ambient temperature treated with ginger and garlic) there was a reduction over the day studied; 9.2 x 10^4 to 3.3 x10^4cfu/ml for ATTGI (Tigernut juice stored at ambient temperature treated with ginger) and 8.8 x 10^4 to 3.5 x10 ^{4}cfu/ml for ATTGA (Tigernut juice stored at ambient temperature treated with garlic).

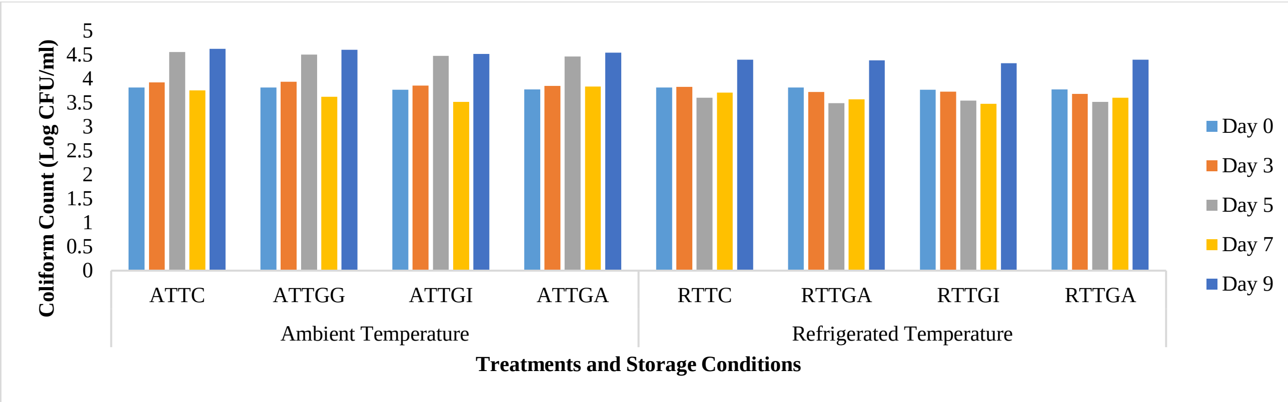

Figure 2. Total coliform count of the different Tiger nut drink stored at different storage conditions
Key: ATTC: Ambient Temperature Tiger nut drink Control; ATTGG: Ambient Temperature Tiger nut drink Garlic and Ginger; ATTGI: Ambient Temperature Tiger nut drink Ginger; ATZGA: ambient Temperature Tiger nut drink Garlic; RTTC = Refrigeration Temperature Tiger nut drink Control; RTZGG: Refrigeration Temperature Tiger nut drink Garlic and Ginger; RTTGI: Refrigerated Temperature Tiger nut drink Ginger; RTTGA: Refrigerated Temperature Tiger nut drink Garlic.

Likewise, The respective treatments stored at refrigerated temperature on day 3,5,7,9 had varying THB counts ranged from 9.1 x 10^4 to 2.5 x 10^4 cfu/ml recorded for RTTC (Tiger nut drink stored at refrigerated temperature without treatment); 7.3 x 10^4 to 2.4 x 10^4 cfu/ml recorded for RTTGG (Tiger nut drink stored at refrigeration temperature treated with ginger and garlic); 6.9 x 10^4 to 2.1 x 10^4 cfu/ml recorded for RTTGI (Tiger nut drink stored at refrigerated temperature treated with ginger) and 8.4 x 10^4 to 2.5 x10^4 cfu/ml for RTTGA (Tiger nut drink stored at refrigerated temperature treated with garlic). There was a notable reduction in the count over the period studied.

C. Staphylococcus count of Tiger nut drink for the different days and storage conditions of the study

The staphylococcus counts of Tiger nut drink as presented in figure 3. Day 0 of the study showed that sample TC (Tigernut drink without treatment) had a count of 3.6 x 10^3 CFU/ml. In contrast, samples TGG (Tiger nut drink treated with Ginger and Garlic), TGI (Tiger nut drink treated with Ginger), and TGA (Tiger nut drink treated with Garlic) had counts of 3.2 x 10^3, 3.0 x 10^3, and 2.9 x 10^3 CFU/ml, respectively.

On day 3,5,7,9, the respective treatments of Tiger nut drink stored at ambient temperature had different counts ranged from 7.8 x 10^3 to 3.6 x 10^4 cfu/ml for ATTO (Tiger nut drink stored at ambient temperature without treatment); 6.6 x 10^3 to 5.8 x 10^4 cfu/ml for ATTGG (Tiger nut drink stored at ambient temperature treated with ginger and garlic); 6.0 x 10^3 to 3.0 x 10^4 cfu/ml for ATTGI (Tiger nut juice stored at ambient temperature treated with ginger) and 5.8 x 10^3 to 4.4 x 10^4 cfu/ml ATTGA (Tiger nut juice stored at ambient temperature treated with garlic). Likewise, The respective treatments stored at refrigeration temperature on day 3,5,7,9 had varying THB counts ranging from 3.6 x 10^3 to 2.6 x 10^4 cfu/ml recorded for RTTC (Tiger nut drink stored at refrigeration temperature without treatment); 3.2 x 10^3 to 2.3 x 10^4 cfu/ml recorded for RTTGG (Tiger nut drink stored refrigeration temperature treated with ginger and garlic) 2.9 x 10^3 to 2.3 x 10^4 CFU/ml recorded for RTTGI (Tiger nut drink stored at refrigeration temperature treated with ginger) and 3.1 x 10^3 to 2.1 x 10^4 CFU/ml for RTTGA (Tiger nut drink stored at refrigeration temperature treated with garlic).

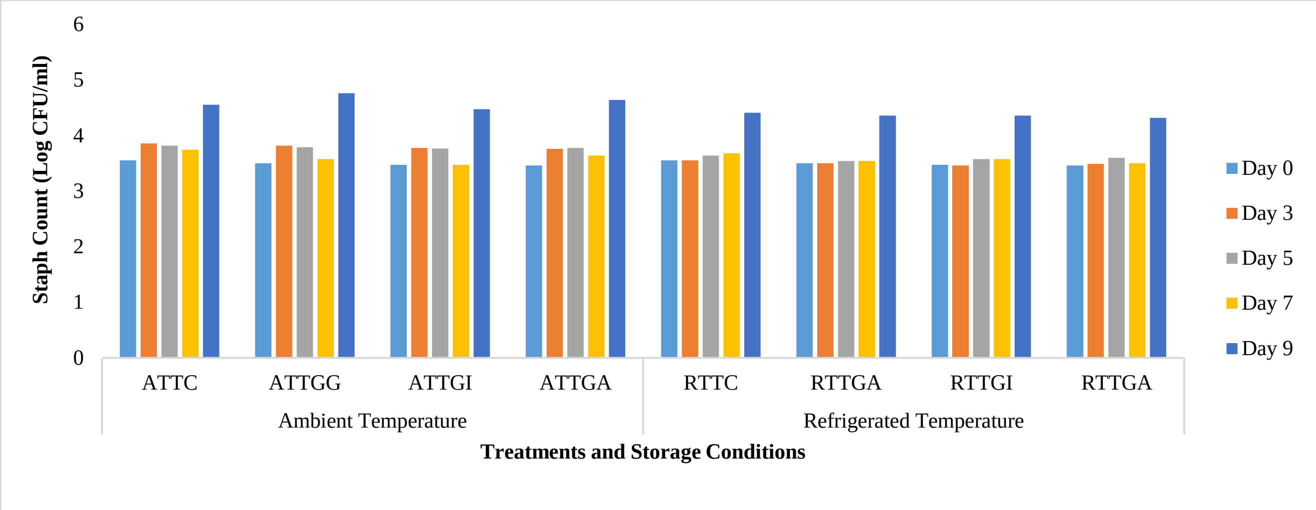

Figure 3: Total Staphylococcus count of different Tiger nut drinks stored at different storage conditions.
Key: ATTC: Ambient Temperature Tiger nut drink Control; ATTGG: Ambient Temperature Tiger nut drink Garlic and Ginger; ATTGI: Ambient Temperature Tiger nut drink Ginger; ATZGA: Ambient Temperature Tiger nut Juice Garlic; RTTC = Refrigeration Temperature Tiger nut drink Control; RTZGG: Refrigeration Temperature Tiger nut drink Garlic and Ginger; RTTGI: Refrigeration Temperature Tiger nut drink Ginger; RTTGA: Refrigeration Temperature Tiger nut drink Garlic.

Table 2. Frequency of occurrence of bacteria genera isolated from Tiger nut drink.

	Tiger nut drink Ambient n (%)	Tiger nut Drink Refrigeration n (%)	Total N (%)
Bacillus	1(20.0)	1(20.0)	2(20)
Staphylococcus	1(20.0)	1(20.0)	2(20)
Micrococcus	1(20.0)	1(20.0)	2(20)
Proteus	1(20.0)	1(20.0)	2(20)
Lactobacillus	0	1(20.0)	1(10)
E. coli	1(20.0)	1(20.0)	2(20)
Total	5	6	11(100)

D. Physiochemical parameters of Tiger nut drink stored at different temperatures

Physiochemical parameters of Tiger nut drink stored at different temperatures is presented in Table 3. The physiochemical parameter on day 0 for the different tiger nut drinks showed that sample Tiger nut control (TC) had pH, TTA, Ascorbic acid, and TSS of 5.1, 0.90 33.50 and 9.20 respectively at ambient temperature while the samples stored at refrigeration temperature had values of 6.5, 0.130, 8.98 and 11.26 respectively for pH, TTA, Ascorbic acid and TSS. On day 0, the sample Tiger nut drink treated with garlic (TGA) had a pH of 5.4, TTA of 0.84, ascorbic acid of 33.6 and TSS of 8.80 for ambient temperature while that stored at refrigeration temperature was 6.6 for pH, 0.110 for TTA, 8.60 for ascorbic acid and 10.26 for TSS. The sample Tigernut drink treated with ginger (TGI) had a pH of 5.2, TTA of 0.86, ascorbic acid of 38.5 and TSS of 8.86 for ambient temperature, while that stored at refrigeration temperature had a pH of 6.8, TTA of 0.10, ascorbic acid of 8.52 and TSS of 10.43. The Sample Tigernut drink treated with ginger and garlic(TGG) had a pH of 5.5, TTA of 0.82, ascorbic acid of 36.6 and TSS of 8.80 for ambient temperature, while pH of 6.6, TTA of 0.104, ascorbic acid of 8.64 and TSs of 10.67 on day 0.

The physiochemical parameter on day 9 for the different tiger nut drink treatments showed that sample TC had pH, TTA, Ascorbic acid, and TSS of 3.0, 1.06, 33.51 and 9.1 at ambient temperature while one stored at refrigeration temperature had values of 4.8, 0.166, 8.98 and 11.74 respectively for pH, TTA, Ascorbic acid and TSS. On day 9, Sample TGA had a pH of 5.0, TTA of 0.80, ascorbic acid of 36.50 and TSS of 8.61 for ambient temperature while that stored at refrigeration temperature was 6.0 for pH, 0.138 for TTA, 8.60 for ascorbic acid and 11.16 for TSS. Sample TGI had a pH of 4.7, TTA of 0.84, ascorbic acid of 33.5 and TSS of 8.4 for ambient temperature while that stored at refrigeration temperature had pH of 6.1, TTA of 0.139, ascorbic acid of 8.52 and TSS of 10.65. Sample TGG had a pH of 6.1, TTA of 0.94, ascorbic acid of 38.21 and TSS of 8.46 for ambient temperature while pH of 5.8, TTA of 0.134, ascorbic acid of 8.64 and TSs of 10.80- on day.

Table 3. Physiochemical parameters Tiger nut juice stored at different temperatures.

Tiger nut drink								
	Ambient Temperature				Refrigeration Temperature			
	pH	TTA	Ascorbic	TSS	pH	TTA	Ascorbic	TSS
TC	5.1	0.90	33.50	9.20	6.5	0.130	8.98	11.26
TGA	5.4	0.84	36.50	8.80	6.6	0.110	8.60	10.26
TGI	5.2	0.86	38.50	8.86	6.8	0.106	8.52	10.43
TGG	5.5	0.82	38.60	8.80	6.6	0.104	8.64	10.67
Day 3								
TC	4.4	1.02	33.55	9.22	6.0	0.133	8.98	11.34

GA	5.0	0.80	36.50	8.66	6.4	0.115	8.60	10.38
TGI	5.1	0.84	38.50	8.60	6.7	0.112	8.52	10.51
TGG	6.1	0.80	38.30	8.60	6.5	0.109	8.64	10.73
Day 5								
TC	4.3	1.02	33.55	9.23	5.6	0.143	8.98	11.41
GA	5.0	0.80	36.50	8.60	6.2	0.126	8.60	10.39
TGI	5.1	0.83	36.28	8.66	6.5	0.124	8.52	10.53
TGG	5.1	0.82	38.20	8.45	6.1	0.117	8.64	10.79
Day 7								
TC	4.3	1.06	33.55	9.2	5.3	0.153	8.98	11.52
GA	4.0	0.80	36.57	8.61	6.2	0.127	8.60	10.99
TGI	4.9	0.91	38.53	8.68	6.5	0.124	8.52	10.55
TGG	5.0	0.80	38.30	8.56	6.1	0.117	8.64	10.72
Day 9								
TC	3.0	1.06	33.51	9.1	4.8	0.166	8.98	11.74
TGA	5.0	0.80	36.50	8.61	6.0	0.138	8.60	11.16
TGI	4.7	0.84	33.50	8.4	6.1	0.139	8.52	10.65
TGG	6.1	0.94	38.21	8.46	5.8	0.134	8.64	10.80

TC –Tiger nut control, TGA -Tiger nut garlic, Tiger nut Ginger -TGI, Tiger nut Ginger/Garlic-TGG.

IV. Discussion

The tiger nut drink is a refreshing high-nutritive energy drink produced mainly from tiger nut, a good source of energy, fat, starch, fibre, glucose and protein (Oke *et al*., 2019).

The current study is aimed at evaluating the impact of different spices and storage temperatures on the microbial and physiochemical. quality over a 9-day period. The 9-day period is chosen as a timeframe to study microbial and physiochemical changes that occur within a typical short-term shelf life while also examining the effectiveness of natural preservatives under varying storage conditions.

The results revealed significant variations in microbial counts as well as physicochemical characteristics for Tiger nut drink subjected to different spices and stored at ambient and refrigeration temperatures.

As presented in figure 1, On day 0, Tiger nut juice without treatment (TC) displayed a THBC of 9.6×10^4 CFU/ml, while samples treated with Ginger and Garlic (TGG), Ginger (TGI), and Garlic (TGA) had counts of 8.2×10^4, 9.0×10^4, and 9.0×10^4 CFU/g, respectively. These initial counts indicated differences in the baseline microbial load introduced by the various spices.

The effects of various spices on the THBC were noticeable during the nine-day testing period. Tiger nut juice without treatment (ATTO) had a THBC of 1.02 x 104 CFU/ml on day 3 at room temperature, but refrigerated Tiger nut juice without treatment (RTTC) had a count of 9.1 x 104 CFU/ml. On the days that followed, this tendency continued, with ambient-stored samples typically showing lower THBC than refrigerated samples. Tiger nut juice samples' THBC was significantly impacted by storage temperature.

The THBC of ambient-stored Tiger nut juice without treatment (ATTO) was 9.2 x 105 CFU/ml on day 7, but the count of chilled Tiger nut juice without treatment (RTTC) was 4.3 x 105 CFU/ml. The importance of temperature control in maintaining microbiological purity was underscored by the steady trend of higher THBC in ambient-stored samples as opposed to refrigerated ones as storage duration increased. Tiger nut juice samples treated with ginger and garlic (ATTGG) typically showed more THBC than other treatments during the course of the investigation, indicating possible effects of these additions on microbial development. Furthermore, samples that were chilled consistently showed lower THBC than samples that were maintained at room temperature, highlighting the effectiveness of refrigeration in delaying the growth of total heterotrophic bacteria.

The counts obtained in this study were lower than the counts reported by Maduka *et al*. (2022), which was $1.35\pm0.026 \times 10^9$ - $6.50\pm0.040 \times 10^9$ CFU/ml. Bashir *et al*. (2014), Okwelle (2020), Opeyemi and Obuneme (2020), and Badua *et al*. (2018) reported that bacterial population within the range 2.2×10^4 - 1.4×10^6, 1.11- 2.40×10^5, 1.2 - 12.0×10^4 and 9.92×10^4 - 3.13×10^4 CFU/ml were present in samples of tiger nut milk sold within the campuses of selected tertiary institutions in Nigeria, respectively, The ranges are quite similar to this present study. Microbiological Guidelines for Ready-to-Eat Food (2014) stipulate that aerobic colony count (ACC) of non-fermented dairy products below 10^5 CFU/ml is satisfactory for human consumption; 10^5 - $\leq 10^7$ CFU/ml is borderline; $\geq 10^7$ CFU/g is unacceptable for human consumption. Based on the results obtained from this study, the samples of tiger nut drink though a non-dairy product is microbiologically safe for human consumption.

As seen in figure 2, On day 0 for total coliform count, the Tiger nut drink without treatment (TC) exhibited an initial TC of 6.6×10^3 CFU/ml, while samples treated with Ginger and Garlic (TGG), Ginger (TGI), and Garlic (TGA) had counts of 6.2×10^3, 5.9×10^3, and 6.0×10^3 CFU/g, respectively. These initial counts provided a baseline for the subsequent assessment of the effects of different treatments and storage conditions.

Throughout the nine-day study period, it became clear how various spices affected the total coliform count. Tiger nut drink without treatment that was kept at room temperature (ATTO) on Day 3 had a TC of 1.02 x 104 CFU/ml, whereas the RTTC, or chilled Tiger nut drink without treatment, had a count of 9.1 x 104 CFU/ml. In general, ambient-stored samples showed higher TC than refrigerated samples, according to this trend, which continued on subsequent days. On day 7, ambient-stored Tiger nut drink without treatment (ATTO) reached a TC of 5.7×10^3 CFU/ml, while refrigerated Tiger nut drink without treatment (RTTC) had a count of 5.1×10^3 CFU/ml. The steady trend of lower TC in refrigerated samples as opposed to samples kept at ambient temperature implies that refrigeration aids in limiting the development of total coliforms. The total coliform counts of Tiger Nut drink samples treated with ginger and garlic (ATTGG) were generally higher than those of other treatments during the course of the study, suggesting that these spices may have an impact on microbial growth. The effectiveness of refrigeration in regulating or inhibiting microbial growth was further demonstrated by the consistently lower total coliform counts in refrigerated samples when compared to ambient-stored samples.

As shown in figure 3, On day 0, Tiger nut juice without treatment (TC) exhibited an initial Staphylococcus count of 3.6 x 10^3 CFU/ml. In comparison, samples treated with Ginger and Garlic (TGG), Ginger (TGI), and Garlic (TGA) had counts of 3.2 x 10^3, 3.0 x 10^3, and 2.9 x 10^3 CFU/g, respectively. These initial counts serve as the baseline for evaluating the effects of treatments and storage temperatures on Staphylococcus growth. Throughout the 9-day study, differences in Staphylococcus counts were observed among the treatments. On Day 3, Tiger nut juice without treatment stored at ambient temperature (ATTO) displayed a Staphylococcus count of 7.8 x 10^3 CFU/ml, while refrigerated Tiger nut juice without treatment (RTTC) showed a count of 3.6 x 10^3 CFU/ml. This trend persisted on subsequent days, suggesting that ambient-stored samples generally exhibited higher Staphylococcus counts compared to refrigerated samples. Storage temperature emerged as a significant factor influencing Staphylococcus counts in Tiger nut juice samples. On day 7, ambient-stored Tiger nut juice without treatment (ATTO) reached a Staphylococcus count of 5.6 x 10^3 CFU/ml, while refrigerated Tiger nut juice without treatment (RTTC) had a count of 4.8 x 10^3 CFU/ml. Refrigeration may help inhibit the growth of Staphylococcus bacteria, as evidenced by the consistent pattern of lower Staphylococcus counts in refrigerated samples when compared to ambient-stored samples. Staphylococcus counts were generally lower in Tiger nut juice samples treated with garlic (ATTGA) than in samples treated with other treatments during the study, suggesting that garlic may have antimicrobial qualities. Additionally, samples that were refrigerated consistently showed lower Staphylococcus counts than samples that were stored at room temperature, highlighting the significance of refrigeration in regulating microbial count.

Throughout the whole storage period, it was noted that microbial contamination was rising, particularly when the product was stored at ambient temperature. Some of these microorganisms might be the result of the product being stored at air temperature, which can cause spoiling. Additionally, the samples contain bacterial growth because of the raw plant contamination, non-aseptic handling during the process, and the use of water and equipment during production and packaging.

The bacterial count of the samples generally increases with the storage period. The combination of ginger and garlic in tiger nut drink lower the bacterial load as compared to the control drink. Although the count of microbial populations in samples maintained at low temperatures has decreased, it might be due to the synergist effect of boiling, adding some spices, and the low temperature on the microorganisms present. This phenomenon led to the reduction of microorganisms by forming obstructions which the microbes were not able to get through (Ogiehor *et al.,* 2008). Refrigeration along with preservative compounds helps the drink to preserve its functional, nutritive quality (Ogiehor *et al*., 2008) and lengthen the shelf time of the product. The same outcomes have been noted for the palm wine, apku, and zobo, items that are both indigenous and novel by Ogiehor (1998), Ogiehor (2004), and Ogiehor *et al*., (2008).

The comprehensive examination of the cultural and biochemical characteristics of isolates obtained from tiger nut drinks subjected to various spices and storage temperatures provides valuable insights into microbial composition and potential spoilage organisms. The predominant microorganisms were identified across treatments and storage conditions, revealing percentages for *Bacillus, Staphylococcus, Micrococcus, Proteus, Lactobacillus,* and *Escherichia coli.* Similar organisms have been reported by Nwafor and Ikenebomeh, 2009; Egbere *et al.,* 2007, Aboh and Oladosine, (2014), and Ibrahim *et al*. (2016). The study identified a diverse array of microorganisms within tigernut drinks. *Bacillus, Staphylococcus*, and *Micrococcus* were the most prevalent genera, constituting 20.8%, 20.8%, and 25.0%, respectively, of the isolated strains. *Proteus, Lactobacillus,* and *Escherichia coli* were also present, contributing to 12.5%, 8.3%, and 12.5% of the isolates, respectively.

Staphylococcus are recognized as being intoxication risk (Ameh and Abubakar, 2002) which is why their presence in the tiger nut drink analyzed in this study should be of concern. The natural flora of humans includes Staphylococcus aureus on the skin but in a situation where there is relaxation in hygienic practices, the high load on products which are handled by man become not unusual (Jablonski and Bohach, 1997) observed that transfer of Staphylococcus aureus from human to food may be directly or indirectly by skin flakes or through droplet nuclei from respiratory tract. *E. coli* is indicative of fecal and environmental contamination and serves as a sentinel organism for the occurrence of other enteric pathogens that may have been introduced via contaminated water, and utensils Oranusi *et al*. (2003). This acidity of Tiger nut drink has been reported by a few scholars like (Efiuvwevwere and Akoma (2013), and Akoma *et al*. (2013) also correlate this phenomenon with the presence of specific species of lactic acid bacteria (LAB), including Lactobacillus during fermentation. High acidic pH values have also been recorded for zobo and orange juice products Bolarinwa et. However, as regards the value and manner of consumption of alcoholic beverages in Nigeria, the traditional alcoholic drinks such as zobo (Adesokan *et al*., 2013) and burukutu and pito (Kolawole and Afonja, 2007).

This microbial diversity underscores the complexity of the microbial community in these beverages. The impact of different spices on the microbial composition of tiger nut drinks is evident from the prevalence of specific genera in each treatment group. Notably, the proportions of the detected bacteria were affected by the kinds of spices used. The mechanisms by which these treatments specifically impact particular microorganisms may be further investigated in future studies, which would help with the creation of focused preservation techniques. The distribution of microbial species in tiger nut drinks was found to be significantly influenced by storage temperature. The sensitivity of these microorganisms to temperature changes was highlighted by the variation in the prevalence of particular genera under various storage conditions.

It is noteworthy that the types of spices administered influenced the proportions of the identified bacteria. Future research may delve deeper into the mechanisms by which these treatments selectively affect certain microorganisms, aiding in the development of targeted preservation strategies. Storage temperature emerged as a critical factor influencing the distribution of microbial species in tiger nut drinks. The prevalence of specific genera varied with different storage conditions, highlighting the sensitivity of these microorganisms to temperature fluctuations.

Nuanced insights into the design of ideal storage conditions for these beverages may be obtained from additional research into the temperature-dependent dynamics of microbial growth and survival. Determining the shelf life and general quality of commercially produced tiger nut drinks requires an understanding of the microbial composition. The existence of potentially harmful organisms, like *Escherichia coli,* highlights how crucial it is to follow the right handling, storage, and treatment protocols to protect consumers. Furthermore, the function of helpful microbes like Lactobacillus points to possible ways to improve these beverages' functional aspects through specific fermentation processes. The examination of physiochemical parameters on day 0 and day 9 provides crucial insights into the shelf life and stability of commercially made tiger nut drinks. The parameters analyzed include pH, Total Titratable Acidity (TTA), Ascorbic Acid content, and Total Soluble Solids (TSS).

On day 0, Tiger nut juice sample TC exhibited a pH of 5.1 at ambient temperature and 6.5 at refrigerated temperature. Over the storage period, the pH of TC decreased to 3.0 at ambient temperature and 4.8 at refrigerated temperature on day 9. Similar trends were observed in other treatments (TGA, TGI, and TGG). The pH in the study records a higher value compared to the pH value by Fasoyine *et al.* (2005) ranged from 2.19 to 3.62. Akoma *et al.,* (2006) also observed a similar result. These pH variations suggest acidity changes during storage, possibly linked to microbial activity, fermentation, or chemical reactions. The pH values of this study fall within the ranges obtained from other studies. The result does confirm that tiger nut drink is acidic in nature as declared by other authors (Doughari *et al*., 2008; Omemu *et al*., 2006; Ifie *et al.,* 2012) but contrasts with the values by Adesokan *et al*. (2013), which are 6.63 and 7.05. An equally low pH value of 3.0 has already been reported to be suitable for the preservation of zobo juice, orange juice has a pH of between 3.30 and 3.82, while pineapple juice ranges between 3.30 and 3.60 and grapefruit juice between 2.90 and 3.25. These fruit juices fall under the category of high-acid foods since they fall within limits provided for high-acid foods. Tiger nut drink falls within a group of food products referred to as high-acid foods. These generally do not support the growth of many pathogenic microorganisms due to low pH, but they survive mainly based on acidity.

TTA values reflect acidity levels, with higher values indicating increased acidity. Tiger nut drink treatments displayed fluctuations in TTA over the storage period. For instance, sample TC showed an increase from 0.90 on day 0 to 1.06 on day 9 at ambient temperature. Similar trends were observed in other treatments. This acidity evolution is indicative of ongoing biochemical transformations within the beverages. Values increased with time irrespective of the preservatives added; this may be due to the producing ability of spoilage bacteria. A similar increase was observed in the work carried out by Fasoyiro *et al.* (2005) and Gbadegesin and Odunlade, (2016).

Ascorbic acid, known for its antioxidant properties, showed notable changes. Tiger nut juice sample TC recorded a decrease from 33.50 on day 0 to 33.51 on day 9 at ambient temperature. Conversely, the refrigerated counterpart showed a decrease from 8.52 to 8.98. These variations may be attributed to environmental factors, such as temperature, impacting the stability of ascorbic acid. Tiger nut juice treatments exhibited alterations in TSS. For instance, sample TC had a decrease from 9.20 on day 0 to 9.1 on day 9 at ambient temperature, while refrigerated storage showed an increase from 11.26 to 11.74. These changes could result from solubility shifts or compositional modifications during storage. The vitamin C content in this study differs compared to the range of (17.2 to 30.7 mg/100 ml) reported by Bolade *et al.,* (2009). Besides, pH is the major factor influencing vitamin C stability, thus, high pH values promote vitamin C oxidation reactions (Leahu *et al.,* 2013). Although the antioxidants of Hibiscus sabdariffa have been documented (Oboh and Okhai, 2012), garlic and ginger also contributed to the antioxidant content.

Ascorbic acid, also known as vitamin C, is a vital nutrient that can degrade over time due to various factors, such as exposure to heat, light, and oxygen. Refrigeration helps to slow down this degradation process by maintaining a lower temperature, which reduces the metabolic activities of microorganisms and enzymes that contribute to the breakdown of ascorbic acid.

Tiger nut drink was stored at lower temperature which helps to limit the rate of chemical reactions that cause the ascorbic acid to break down. However, it is essential to note that this process cannot be entirely halted. The ascorbic acid content will still decrease over time but at a slower rate compared to storing the drink at room temperature. To further preserve the ascorbic acid content in your tiger nut drink, it is advisable to store it in an airtight container to minimize exposure to oxygen and light. Additionally, consuming the drink within a reasonable time frame after refrigeration will help ensure that you enjoy its maximum nutritional benefits, including the ascorbic acid content. Hence the preserved drinks stored at ambient temperature were able to withstand oxidation and prevent degradation of vitamin C during storage.

TSS for tiger nut drink for refrigeration temperature ranged from 10.26- 11.74 while the tiger nut stored at ambient temperature ranged from 8.4 and 9.3. Refrigeration can help maintain the TSS levels by slowing down the degradation of certain components, but it may not significantly affect the overall TSS content. Total soluble solids (TSS) are the total amount of dissolved substances in the liquid, which can include sugars, salts, and organic acids. The TSS content can vary depending on the preparation method and ingredients used. Storing a tiger nut drink at refrigeration temperature (typically around 2-4°C or 36-40°F) will slow down the degradation of its components, including soluble solids, compared to ambient temperature storage (usually around 20-30°C or 68-86°F). This is because lower temperatures reduce the rate of chemical reactions and microbial growth, preserving the drink's quality for a more extended period. Some spices may have antioxidant properties that can help preserve the drink's soluble solids, while others may contribute additional flavors, aromas, or nutritional benefits. The specific impact of each spice on the total soluble solids will depend on its properties and the concentration used in the drink.

V. Conclusion

The finding suggests that the combined effect of ginger and garlic has a positive effect on inhibiting microbial growth for a period. Overall, the study provided valuable insights into the microbial stability and shelf life of tiger nut drinks.

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