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Influence of Natural Spices and Storage Condition on Microbial and Physicochemical Quality Commercially Prepared Hibiscus Drink (Zobo Drink)

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Abstract

This study examined the effects of various spices and storage temperatures on the physicochemical properties and microbial dynamics of commercially made Zobo drinks over a nine-day period. The use of standard physiochemical and microbiological techniques was implemented. The Total heterotrophic bacteria count (THBC) showed that counts ranging from 8.2x104 to 9.4x104 CFU/ml on day 0 and 2.2 x.10^6 CFU/ml on day 9 for zobo. The total coliform count (TC) of Zobo samples varied with spice treatments, storage conditions, and duration. Zobo without treatment (ZC) displayed uniform TC on Day 0, but ambient-stored samples generally had higher TC than refrigerated ones as the storage duration increased. Ginger and Garlic treatments (ZGG) consistently exhibited lower TC, indicating their potential antimicrobial impact. Staphylococcus count in Zobo varied among treatments, with refrigerated samples generally displaying lower counts. Storage temperature played a vital role in influencing Staphylococcus counts, emphasizing the importance of temperature control in preserving microbial quality. The microbial composition analysis revealed a diverse array of microorganisms in Zobo drink with varying prevalence of Bacillus, Staphylococcus, Micrococcus, Proteus, Lactobacillus and Escherichia coli. Temperature fluctuations significantly influenced microbial distribution, emphasizing the need for optimal storage conditions. Physiochemical parameters exhibited variations during the study. Zobo juice displayed pH stability, minimal TTA changes, consistent ascorbic acid content, and TSS stability. Zobo drink exhibited great stability in physiochemical parameters indicating differences in susceptibility to environmental factors This study provides valuable insights into the microbial dynamics and physicochemical stability of commercially made Zobo drinks, emphasizing the importance of appropriate storage conditions for maintaining product quality and safety .This study found that the combination of ginger and garlic in Zobo drink has a synergistic effect and can be used to reduce the bacterial load to a level that is acceptable for at least six to nine days following production. Increased consumer safety and better preservation techniques are made possible by the findings, which advance knowledge of the shelf life of common beverages.

Keywords: Hibiscus drink; Storage Temperature, Spices; Physicochemical and Microbial quality.

Introduction

Local indigenous drinks are gaining popularity in both urban and rural areas of Nigeria due to their affordability compared to imported alternatives. These beverages are widely accepted among Nigerians (Aworh, 2023; Chukwu & Dogbe, 2023). As a category distinct from other food items, beverages are typically in a liquid state and possess lower nutritional values than solid foods (Shkembi & Huppertz, 2023; Moraes et al., 2023). Despite their lower food value, beverages contribute essential nutrients, phytonutrients, phenolic acids, and flavonoids to our diets (Rao & Poonia, 2023).

Research indicates that street foods and drinks, which are commercially prepared and sold by vendors, are increasingly popular among consumers due to their affordability and constant availability (Negassa et al., 2023; Suraini et al., 2023). However, these foods and beverages may suffer from poor personal hygiene practices by the vendors and have limited shelf life. Additionally, the production process often lacks quality assurance and control measures (Effiong et al., 2023). Consuming these street foods can potentially increase the risk of foodborne illnesses caused by various pathogens. Consequently, making cautious beverage choices is essential for maintaining a healthy dietary pattern (Ferruzzi et al., 2020; Snetselaar et al., 2020).

Zobo leaf, known as Hibiscus Sabdariffa is a small (2-8m) tall vegetable plant in the family Malvaceae, widely grown in the tropical and semi-tropical regions of the world mainly in Africa and the East Indians (Nwachukwu et al., 2007; Umeh et al., 2015; Izah et al., 2015). Zobo juice is a reddish, non-alcoholic local beverage produced from the dried succulent calyces of the Hibiscus Sabdariffa flower by boiling and filtration (Ogiehor et al., 2008). The calyces have been found to be rich in vitamins, natural carbohydrates, protein, and other antioxidants (Wong et al., 2002). One important vegetable, potent in naturally occurring antioxidant and antiviral compounds is *Hibiscus sabdariffa (Roselle*) called 'Isapa' in the Yoruba language (Tabe *et al.*, 2015; Khalil *et al.*, 2020). *Hibiscus sabdariffa* belongs to the superorder Malvaceae and it is believed to originate from East Africa (Ugwu *et al.*, 2020).

Credible documentation attests to the culinary and medicinal qualities of extracts from both the leaves and calyces of Hibiscus sabdariffa. These extracts are rich in polyphenolic compounds and anthocyanins, offering significant health benefits (Hapsari & Setyaningsih, 2021). The green leaves of the H. sabdariffa plant are traditionally used in soup making by various tribes worldwide,

such as the Yoruba tribe in Nigeria. Conversely, the dried calyces of red cultivars are predominantly utilized in crafting a refreshing beverage known as Zobo or Soborodo. While originating from the northern region, this beverage has gained popularity across all tribes in Nigeria (Mohammed et al., 2017).

The inadequate hygienic practices during the production, packaging, and distribution of zobo juice in nylon or plastic containers, along with the absence of proper facilities such as clean water, sanitation, storage, and waste disposal at preparation and service points, have resulted in unsanitary conditions. This exposes the beverage to potential contaminants, posing a heightened risk to public health (Rocha-Melogno et al., 2022; Näsänen-Gilmore et al., 2023). Various microorganisms have been identified as contributors to the deterioration and spoilage of zobo drink, including *S. faecalis, Proteus spp, E. coli, Bacillus spp, S. aureus, Enterobacter spp, Klebsiella spp, Micrococcus spp, Aspergillus spp, Penicillium citrinum, Fusarium oxysporum, Rhizopus spp,* and *Mucor spp* (Yinusa et al., 2022).The objective of the study is to investigate the impact of ginger, garlic, and a combination of ginger and garlic on commercially produced zobo made from Hibiscus sabdariffa. Under different storage conditions.

MATERIALS AND METHODS

Preparation of Zobo Drink

Zobo drink was prepared, using the following recipes, Zobo leaves (hibiscus flower), Pineapple Flavoring.

Method of preparation

The dried calyx of Hibiscus sabdariffa, known as zobo leaves, were carefully sorted and thoroughly rinsed with cold water. Subsequently, the water was heated in a pot, and the zobo leaves were introduced into the boiling liquid, where they were allowed to boil for a duration of 5 minutes. Following this, pineapple flavoring was incorporated into the mixture and permitted to boil for an additional minute. The pot was then taken off the heat, allowing the zobo drink to cool down. Finally, the drink was filtered through a muslin cloth and stored in a transparent bottle.as described by (Linda *et al.*, 2018).

Sample collection and Procedure

A total of three hundred fifty milliliters (350 ml) of Zobo drink were obtained locally through a straightforward random sampling method. In total, 26 samples were gathered and categorized into three groups of 8 samples each. The initial group of 8 samples was subjected to garlic treatment, the second group to ginger treatment, and the third group to a mixture of both ginger and garlic. Furthermore, 2 samples were designated as a control group without any treatment.

For each treatment category, 4 samples were maintained at refrigeration temperatures, while the remaining 4 samples were stored at room temperature. The analysis of the samples occurred on days 1, 3, 5, 7, and 9, as illustrated in Table 1 of the experimental setup.

Table 1 Experimental set up.

The table below outlines the treatment combinations and storage conditions applied to Zobo samples during the study. Each treatment was replicated in four sets, except for the control.

S/N	Ambient Temperature	Refrigerated Temperature
1	350 ml of Zobo (control)	350 ml of Zobo (control)
2	4 sets of 350 ml Zobo + 1 g of ginger	4 sets of 350 ml Zobo + 1 g of ginger
3	4 sets of 350 ml Zobo + 1 g of garlic	4 sets of 350 ml Zobo + 1 g of garlic
4	4 sets of 350 ml Zobo + 0.5 g of ginger and garlic	4 sets of 350 ml Zobo + 0.5 g of ginger and garlic

Media Preparation

Plate Count Agar (PCA), MacConkey Agar, and Mannitol Salt Agar were prepared following the manufacturer's instructions. The media were sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, the media were allowed to cool to a temperature range of 45–50 °C before being poured into sterile Petri dishes for use.

Microbial analysis:

Enumeration of total heterotrophic bacterial count, total staphylococcus count, total coliform count.

Serial dilution of each sample was achieved by transferring 1 ml of each sample into 9 ml of sterile peptone water, utilizing a sterile pipette and vigorous agitation. This process was repeated until a dilution factor of 10^6 was obtained. Thereafter, 0.1 ml aliquots from dilutions 10^3 to 10^6 were inoculated onto previously prepared, surface-dried agar plates (Plate count agar (PCA), mannitol salt agar and Mac conkey agar) specifically designed for enumerating total heterotrophic bacteria, Staphylococci, and total coliform populations. Counts were recorded after 18- 24hrs of incubation on plate count agar, mannitol salt agar and Mac conkey agar Subsequently, these plates were incubated for 18-24 hours, and the resulting microbial counts were recorded. The cfu/ml was determined using Cfu/ml = Number of colonies x Dilution factor/ number of culture plate .

Identification of isolates

Microbial isolates were taxonomically identified via a battery of characterization techniques encompassing morphological, microscopic, and macroscopic examinations. Macroscopic assessment of colonies distinguished based on dimensions, pigmentation, r morphology, surface texture, and periphery were performed. Biochemical differentiations, encompassing Gram staining, catalase activity, coagulase production, methyl red, oxidase, Voges-Proskauer, and sugar fermentation testing, were conducted according to established microbiological protocols as outlined by Cheesbrough (2006).

Physicochemical parameters

Determination of pH

The pH of the drinks was determined directly using a Pye Unikam pH meter at ambient and refrigeration temperature after using standard buffers 4.0 and 7.0 pH to calibrate the pH meter Determination of Ascorbic acid

Analysis of the sample was conducted via titrimetric method, whereby a homogenized sample, obtained by mixing a weighed quantity with a 6% EDTA/TCA solution and subsequently filtering, was subjected to further analysis. Following this treatment, 20 ml of a 30% potassium iodide solution was added, followed by the introduction of 100 ml of distilled water. A 1% starch solution was then introduced, and the resulting homogenate was titrated against a 0.1 M copper(II) sulfate solution, thereby facilitating the detection of a distinct visual end point characterized by a darkened colouration following the reaction with excess copper (II) ion.

Estimation of total titratable acidity

Utilizing phenolphthalein as an indicator, the pH of the sample was assessed by titrating it with 0.1N sodium hydroxide. A volume of 10 millilitres of the sample was mixed with 5 drops of phenolphthalein in a beaker, and subsequently, 0.1N sodium hydroxide was incrementally added until a pinkish hue emerged. The volume of sodium hydroxide employed at the point of colouration was then utilised to calculate the total titratable acidity (TTA) of the samples by multiplying it by a correlation factor of 0.15 and adjusting according to the formula: TTA = (volume of 0.1N NaOH × 100.

Total suspended solid (TSS)

The refractometer was calibrated using distilled water, with the cross-hair positioned at the interface between the dark and clear regions of the instrument. This established a reference point at 0°Brix. Subsequently, a minute quantity of each sample was placed on the prism, and the cross-hair was repositioned to coincide with the boundaries separating the dark and clear areas. The scale reading obtained in this configuration was taken to denote the total soluble solids content of the concentrate, expressed in degrees Brix**.**

Statistical analyses

A one-way analysis of variance (ANOVA) was employed to investigate differences in mean microbial loads among samples of zobo treated with natural spices, subjected to varying storage conditions, at a significance threshold of $p < 0.05$. The analysis was conducted utilizing the SPSS software package.

Result

Total Heterotrophic Bacteria count of Zobo drink for the different days of study.

The total heterotrophic bacterial count (THBC) of Zobo from Day 0 to Day 9, as illustrated in Figure 1, indicated that the untreated sample (ZC) as well as those treated with ginger (ZGI), garlic (ZGA), and a combination of ginger and garlic (ZGG) exhibited microbial loads ranging from 8.2×10^4 to 9.9×10^4 CFU/ml at the initial stage. Between Day 3 and Day 9, Zobo samples stored at ambient temperature showed progressive increases in THBC, ranging from 9.8×10^4 to 1.72×10^7 CFU/ml. Among these, the untreated ambient-stored Zobo (ATZC) recorded the highest bacterial load, whereas the garlic-treated sample (ATZGA) had the lowest count. Similarly, for samples stored under refrigeration between Day 3 and Day 9, THBC values varied from 6.2×10^4 to 3.3×10^5 CFU/ml. The refrigerated untreated sample (RTZC) exhibited the highest microbial count, while the sample treated with both ginger and garlic under refrigeration (RTZGG) recorded the lowest bacterial load.

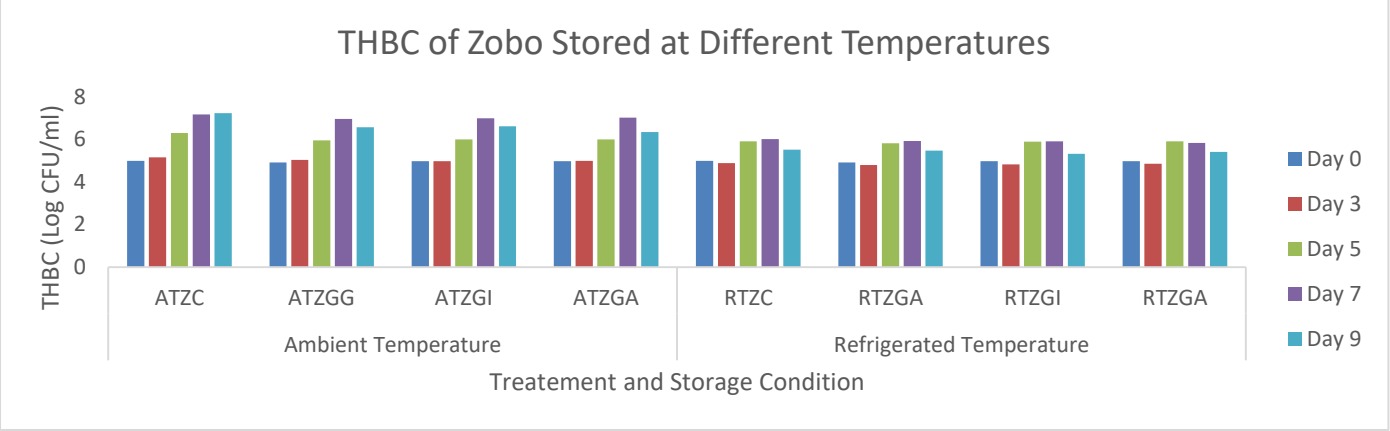

Fig 1 Total Heterotrophic Bacteria Count of Zobo Treated with Garlic and Ginger stored at Different Conditions

Key: ATZC: Ambient Temperature Zobo Control; ATZGG: Ambient Temperature Zobo Garlic and Ginger; ATZGI: Ambient Temperature Zobo Ginger; ATZGA: ambient Temperture Zobo Garlic; RTZC = Refrigerated Temperature Zobo Control; RTZGG: Refrigerated Temperature Zobo Garlic and Ginger; RTZGI: Refrigerated Temperature Zobo Ginger; RTZGA: Refrigerated Temperture Zobo Garlic

Total coliform count of Zobo for the different days of study.

The total coliform (TC) count of Zobo from Day 0 to Day 9, as illustrated in Figure 2, revealed that all samples—including ZC, ZGG, ZGI, and ZGA—initially exhibited comparable microbial loads, ranging from 2.2×10^4 CFU/ml to 5.6×10^5 CFU/ml.

Between Days 3 and 9, samples stored at ambient temperature demonstrated varying TC levels depending on the treatment applied. The untreated ambient sample (ATZC) recorded counts from 3.8×10^4 CFU/ml to 5.4×10^5 CFU/ml. For samples treated with ginger and garlic (ATZGG), counts ranged from 3.6×10^4 CFU/ml to 4.5×10^4 CFU/ml, while those treated with only ginger (ATZGI) showed values between 2.8×10^4 CFU/ml and 4.8×10^4 CFU/ml. Similarly, samples treated with garlic (ATZGA) recorded microbial loads from 2.4×10^4 CFU/ml to 4.6×10^4 CFU/ml.

In contrast, samples stored under refrigeration on Day 3 exhibited lower TC counts. The untreated refrigerated sample (RTZC) ranged from 2.8 × 10^4 CFU/ml to 5.0 × 10^4 CFU/ml. Between Days 3 and 9, Zobo treated with ginger and garlic (RTZGG) had counts between 2.3 × 10^4 CFU/ml and 3.7 × 10^4 CFU/ml. The sample treated with ginger (RTZGI) showed a range of 2.3 × 10^4 CFU/ml to 3.8 × 10^4 CFU/ml, while that treated with garlic (RTZGA) ranged from 2.4 × 10^4 CFU/ml to 3.6 × 10^4 CFU/ml.

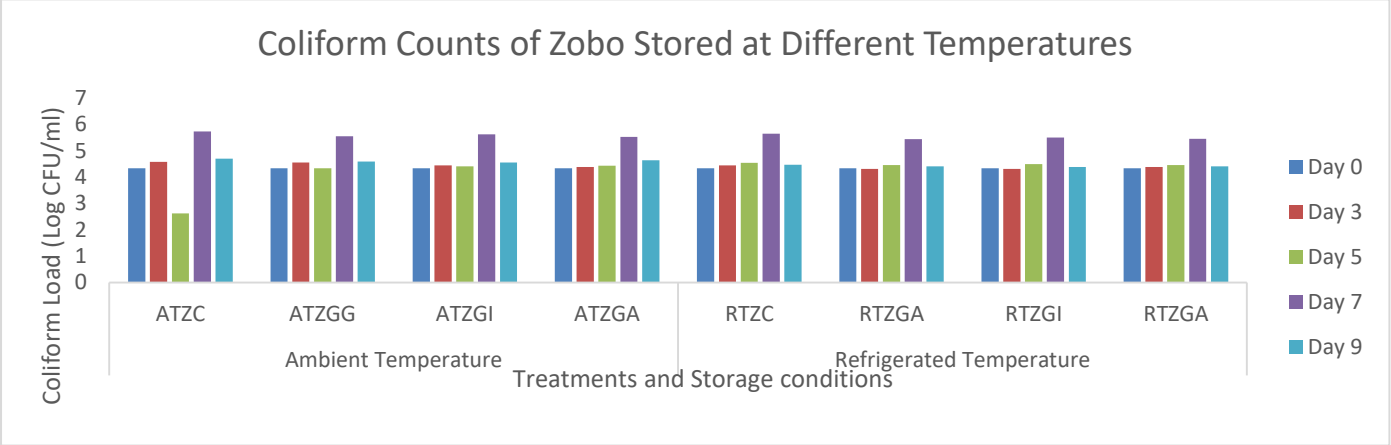

Fig 2 Total Coliform Count of Zobo Treated with Garlic and Ginger stored at Different Conditions

Key: ATZC: Ambient Temperature Zobo Control; ATZGG: Ambient Temperature Zobo Garlic and Ginger; ATZGI: Ambient Temperature Zobo Ginger; ATZGA: ambient Temperture Zobo Garlic; RTZC = Refrigerated Temperature Zobo Control; RTZGG: Refrigerated Temperature Zobo Garlic and Ginger; RTZGI: Refrigerated Temperature Zobo Ginger; RTZGA: Refrigerated Temperture Zobo Garlic

Total Staphylococcus count Zobo for the different days of study day

The Staphylococcus count of Zobo on Day 0, as illustrated in Figure 3, indicated that the untreated sample (ZC) recorded a microbial load of 4.2 × 10^3 CFU/ml. In comparison, samples treated with ginger and garlic (ZGG), ginger alone (ZGI), and garlic alone (ZGA) exhibited initial counts of 3.1 × 10^3, 3.8 × 10^3, and 3.6 × 10^3 CFU/ml, respectively.

From Day 3 to Day 9, the Staphylococcus counts of Zobo stored at ambient temperature varied across treatments. The untreated ambient sample (ATZC) showed a microbial range from 4.2 × 10^3 to 6.7 × 10^4 CFU/ml. For ATZGG (treated with ginger and garlic), counts ranged between 3.1 × 10^3 and 4.1 × 10^4 CFU/ml. The ginger-treated sample (ATZGI) had counts from 3.8 × 10^3 to 4.8 × 10^4 CFU/ml, while the garlic-treated sample (ATZGA) ranged from 3.6 × 10^3 to 5.6 × 10^4 CFU/ml.

In contrast, refrigerated samples showed lower Staphylococcus counts between Day 3 and Day 9. The untreated refrigerated sample (RTZC) recorded values from 2.7 × 10^4 to 4.1 × 10^4 CFU/ml. The sample treated with ginger and garlic (RTZGG) showed counts between 2.5 × 10^4 and 2.8 × 10^4 CFU/ml, while RTZGI (ginger-treated) ranged from 2.6 × 10^4 to 2.8 × 10^4 CFU/ml. The lowest counts were observed in RTZGA (garlic-treated), ranging from 2.1 × 10^4 to 2.3 × 10^4 CFU/ml.

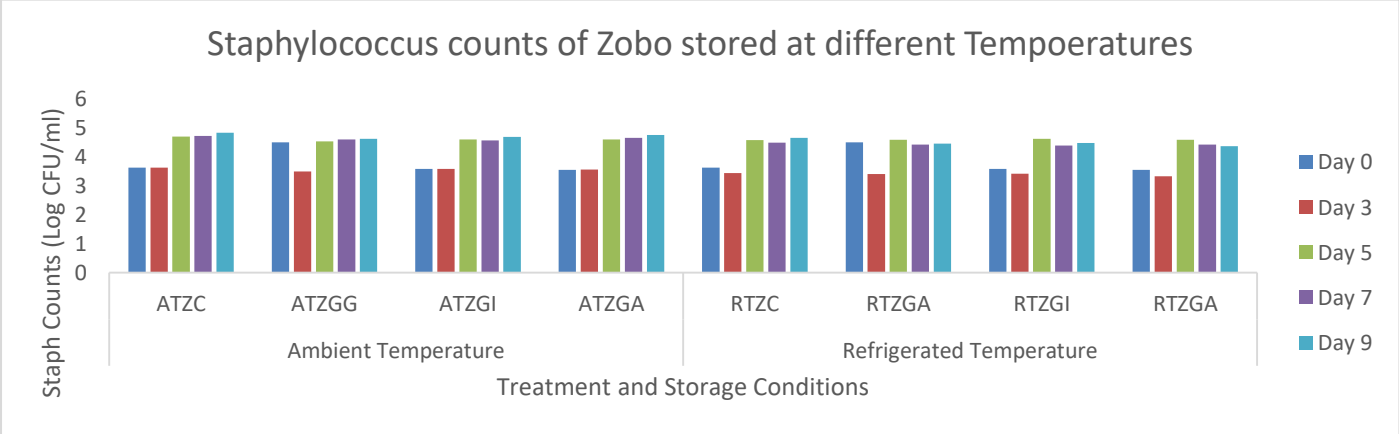

Fig 3 Staphylococcus Count of Zobo Treated with Garlic and Ginger stored at Different Conditions

Key: ATZC: Ambient Temperature Zobo Control; ATZGG: Ambient Temperature Zobo Garlic and Ginger; ATZGI: Ambient Temperature Zobo Ginger; ATZGA: ambient Temperture Zobo Garlic; RTZC = Refrigerated Temperature Zobo Control; RTZGG: Refrigerated Temperature Zobo Garlic and Ginger; RTZGI: Refrigerated Temperature Zobo Ginger; RTZGA: Refrigerated Temperture Zobo Garlic

Table w: Frequency of Occurrence of Bacteria Isolated from Zobo drink

	Zobo at Ambient temp n (%)	**Zobo at Refrigeration n (%)**	**Total N (%)**
Bacillus	2 (20.0)	1(25.0)	3(21.4)

Staphylococcus	2 (20.0)	1(25.0)	3(21.4)
Micrococcus	3 (30.0)	1(25.0)	4(28.57)
Proteus	1 (10.0)	0	1(7.14)
Lactobacillus	2 (20.0)	0	2(14.2)
E. coli	0	1(25.0)	1(7.14)
Total	**10**	**4**	**14(100)**

Physicochemical Parameters Zobo Juice Stored at different Temperature

The physicochemical characteristics of Zobo juice stored under varying temperature conditions, as presented in Table 3, showed notable differences across treatment types and storage durations. On Day 0, the control Zobo sample stored at both ambient and refrigerated temperatures exhibited identical values for pH (4.9), titratable acidity (TTA, 0.90%), ascorbic acid content (33.50 mg/100ml), and total soluble solids (TSS, 9.20 °Brix).

For Zobo treated with garlic, the initial measurements at ambient temperature were pH 5.4, TTA 0.84%, ascorbic acid 33.60 mg/100ml, and TSS 8.80 °Brix. The same sample stored at refrigerated temperature recorded pH 5.4, TTA 0.84%, a slightly higher ascorbic acid content of 36.50 mg/100ml, and the same TSS value of 8.80 °Brix.

The sample treated with ginger had a pH of 5.2, TTA of 0.86%, ascorbic acid content of 38.50 mg/100ml, and TSS of 8.86 °Brix at both ambient and refrigerated conditions on Day 0.

Similarly, Zobo treated with a combination of ginger and garlic exhibited a pH of 5.5, TTA of 0.82%, ascorbic acid of 36.60 mg/100ml, and TSS of 8.80 °Brix at ambient temperature. When refrigerated, this sample showed a slightly lower pH of 5.2, with the same TTA (0.82%), increased ascorbic acid (38.60 mg/100ml), and unchanged TSS (8.80 °Brix).

By Day 9, significant changes in the physicochemical properties were observed. The control sample stored at ambient temperature showed a drop in pH to 3.0 and an increase in TTA to 1.06%, while ascorbic acid remained relatively stable at 33.51 mg/100ml and TSS rose to 9.40 °Brix. In contrast, the same control sample stored under refrigeration had a higher pH (4.0), slightly lower TTA (0.98%), marginally increased ascorbic acid (33.55 mg/100ml), and elevated TSS (9.94 °Brix).

For the garlic-treated Zobo stored at ambient temperature, Day 9 readings were pH 5.0, TTA 0.80%, ascorbic acid 36.50 mg/100ml, and TSS 8.61 °Brix. The corresponding refrigerated sample maintained similar values of ascorbic acid content (35.50"), .

The ginger-treated sample on Day 9 had a pH of 4.7, TTA of 0.84%, ascorbic acid of 38.50 mg/100ml, and TSS of 8.66 °Brix under ambient conditions. Under refrigeration, it recorded a slightly higher pH (4.9) and similar values for the other parameters.

Finally, the Zobo sample treated with both ginger and garlic on Day 9 showed a pH of 5.0, TTA of 0.80%, ascorbic acid content of 38.30 mg/100ml, and TSS of 8.58 °Brix at ambient temperature. The refrigerated counterpart exhibited slightly improved pH (5.1), unchanged TTA (0.80%), consistent ascorbic acid levels (38.30 mg/100ml), and the same TSS (8.58 °Brix).

Table 3 Physiochemical Parameters Zobo Juice Stored at different Temperature

Zobo Juice								
	Ambient Temperature				**Refrigerated Temperature**			
	pH	**TTA**	**Ascorbic**	**TSS**	**pH**	**TTA**	**Ascorbic**	**TSS**
ZC	4.9	0.90	33.50	9.20	4.9	0.90	33.50	9.20
ZGA	5.4	0.84	36.50	8.80	5.4	0.84	36.50	8.80
ZGI	5.2	0.86	38.50	8.86	5.2	0.86	38.50	8.86
ZGG	5.2	0.82	38.60	8.80	5.2	0.82	38.60	8.80
Day 3								
ZC	4.4	1.02	33.55	9.22	4.9	0.92	33.55	9.22
ZGA	5.0	0.80	36.50	8.66	5.4	0.84	36.50	8.66
ZGI	5.1	0.84	38.50	8.60	5.2	0.86	38.50	8.60
ZGG	5.1	0.80	38.30	8.60	5.2	0.80	38.30	8.60
Day 5								
ZC	4.3	1.02	33.55	9.23	4.8	0.92	33.55	9.55
ZA	5.0	0.80	36.50	8.60	5.0	0.80	36.50	9.00

ZGI	5.1	0.83	36.28	8.66	5.1	0.83	36.28	8.66
ZGG	5.1	0.82	38.20	8.49	5.1	0.80	38.20	8.49
Day 7								
ZC	4.3	1.06	33.55	9.2	4.3	0.95	33.55	9.86
ZGA	5.0	0.80	36.50	8.61	5.0	0.80	36.50	8.61
ZGI	4.9	0.88	38.50	8.66	4.9	0.84	38.50	8.66
ZGG	5.0	0.80	38.30	8.58	5.1	0.80	38.30	8.58
Day 9								
ZC	3.0	1.06	33.55	9.4	4.0	0.98	33.55	9.94
ZGA	5.0	0.80	36.50	8.61	5.0	0.80	35.50	8.61
ZGI	4.7	0.84	38.50	8.66	4.9	0.84	38.50	8.66
ZGG	5.0	0.80	38.30	8.58	5.1	0.80	38.30	8.58

ZC -Zobo control, Zobo garlic- ZGA , ZGI- Zobo Ginger ,ZGG-Zobo Ginger/Garlic

Discussion

Effect of natural spices on the microbial quality of Zobo drink stored at different storage temperatures

The study involved assessing commercially prepared Zobo drink and investigating the impact of adding ginger, garlic, a combination of both, and different storage temperatures on the microbial quality and physiochemical composition over a span of nine days, the total heterotrophic bacteria count (THBC), Staphylococcus count, coliform count, and various physiochemical parameters were measured. Results demonstrated significant differences in microbial counts and physiochemical properties among the Zobo drink samples subjected to different treatments and stored at either ambient or refrigeration temperatures. The bacterial count in the samples consistently increased from the initial counts. The THBC serves as an indirect measure of the hygiene level in the processing environment, encompassing factors such as processing procedures, personnel hygiene, cleanliness of contact surfaces and utensils, quality of packaging materials, and adherence to time-temperature control and storage conditions. THBC counts play a crucial role in predicting the shelf life or overall quality of food products. The unsatisfactory data obtained from the Zobo drink samples may be attributed to inadequate knowledge of food hygiene practices and the absence or improper implementation of standard operating procedures. Contamination of the samples could have occurred through various means, including utensils, water and other additives, and packaging materials.

The total heterotrophic bacteria count (THBC) as presented in figure1 showed on Day 0, before any storage, Zobo samples displayed varying THBC depending on the treatment applied. Zobo without treatment (ZC) had a count of $9.9x10^4$ CFU/ml, while samples treated with Ginger and Garlic (ZGG), Ginger (ZGI), and Garlic (ZGA) had counts of $8.2x10^4$, $9.4x10^4$, and $9.4x10^4$ CFU/ml, respectively. This is in contrary to the findings of Olasunmbo et al., (2016) with a lower total heterotrophic bacteria count of 2.2 x 10^3 cfu/ ml and Ezearigo et al, (2014) having a total heterotrophic bacteria count of. $6.00x10^9$ and $2.50x10^9$ cfu/ml treated with ginger and garlic respectively. The results are slightly lower than the reports of Ayandele (2015) and Nwachukwu et al.,(2007) who obtained THBC in the range of 0.4-15.0 × 10^5 CFU/mL and 2.79 to 2.62 log CFU/mL, respectively for zobo samples. This result showed that there was a progressive increase in THBC with storage time. However, the zobo drink containing ginger or ginger, garlic and ginger in combination had lower THBC ($p < 0.05$) than zobo samples containing no spice or treatment. These initial counts suggest that the different treatments had varying impacts on the initial microbial load of the Zobo samples according to Adesokan et al., 2013. Over the 9-day storage period, the THBC exhibited dynamic changes for both ambient and refrigeration storage conditions. On Day 3, at ambient temperature, Zobo samples without treatment (ATZO) showed a THBC of $1.22x10^5$ CFU/ml, whereas refrigeration Zobo without treatment (RTZC) displayed a count of $7.6x10^4$ CFU/ml. This pattern continued on subsequent days, with ambient-stored samples generally having higher THBC compared to their refrigerated counterparts. As the storage duration increased, THBC showed a consistent trend of growth for both ambient and refrigerated samples. On Day 7 to 9. There was a substantial increase in microbial load over time, suggesting the need for careful consideration of the duration of storage for maintaining microbial quality. Throughout the study, Zobo samples treated with Ginger and Garlic (ZGG) consistently exhibited lower THBC compared to other treatments, indicating potential antimicrobial effects of these additives according to Braide et al., (2012). Additionally, refrigerated samples generally displayed lower THBC compared to ambient-stored samples, highlighting the effectiveness of refrigeration in retarding microbial growth.

Total coliform count as illustrated in figure 2 showed Day 0, before any storage, all Zobo samples (ZC, ZGG, ZGI, and ZGA) exhibited similar total coliform counts of $2.2x10^4$ CFU/g. This uniformity suggests that the initial microbial load in all samples, irrespective of treatment, was consistent at the start of the study. Throughout the 9-day study period, Zobo samples treated with different additives (Ginger, Garlic, or their combination) displayed varied total coliform counts. On Day 3, at ambient temperature, Zobo without treatment (ATZC) showed a TC of $3.8x10^4$ CFU/ml, while refrigerated Zobo without treatment (RTZC) displayed a count of $2.8x10^4$ CFU/ml. This pattern continued on subsequent days, with ambient-stored samples generally having significantly high total coliform counts compared to their refrigerated counterparts. ($p < 0.05$) The storage temperature played a crucial role in influencing the total coliform count of Zobo samples. On Day 7 to 9. There

was a consistent trend of higher total coliform counts in ambient-stored samples compared to refrigerated ones as the storage duration increased. Throughout the study, Zobo samples treated with Ginger and Garlic (ZGG) consistently exhibited lower total coliform counts compared to other treatments, suggesting potential antimicrobial effects of these additives Adeoye et al., (2018). The presence of coliform bacteria, particularly faecal coliforms such as E. coli, in zobo drinks is indicative of faecal or sewage contamination. This microbial contamination can be attributed to the use of contaminated water, unsanitary environmental conditions, or improper handling by food operators. E. coli, which inhabits the intestinal tracts of humans and animals, serves as a faecal indicator and its isolation in consumable beverages signifies a hazardous food product. As faecal indicators, these enteric bacteria are recognized vectors responsible for a substantial number of gastrointestinal disease cases and fatalities annually. Additionally, refrigerated samples generally displayed lower total coliform counts compared to ambient-stored samples, highlighting the efficacy of refrigeration in retarding the growth of coliform bacteria.

On Day 0, before any storage, as seen in figure 3 the Staphylococcus count varied among the Zobo samples. Zobo without treatment (ZC) had a count of 4.2 x 10^3 CFU/ml, while samples treated with Ginger and Garlic (ZGG), Ginger (ZGI), and Garlic (ZGA) had counts of 3.1 x 10^3, 3.8 x 10^3, and 3.6 x 10^3 CFU/g, respectively. These initial counts indicated differences in the baseline microbial load introduced by the various treatments. Throughout the 9-day study period, the impact of different treatments on the Staphylococcus count was apparent. On Day 3, at ambient temperature, Zobo without treatment (ATZC) displayed a Staphylococcus count of 4.2 x 10^3 CFU/ml, while refrigerated Zobo without treatment (RTZC) showed a count of 2.7 x 10^4 CFU/ml. This pattern persisted on subsequent days, with ambient-stored samples generally exhibiting lower Staphylococcus counts compared to refrigerated samples. Storage temperature played a critical role in influencing the Staphylococcus count of Zobo samples. On Day 7 to 9 there was a consistent trend of higher Staphylococcus counts in ambient-stored samples compared to refrigerated ones as the storage duration increased underscored the importance of temperature control in preserving microbial quality. Throughout the study, Zobo samples treated with Ginger and Garlic (ZGG) generally exhibited lower *Staphylococcus* counts compared to other treatments, suggesting potential antimicrobial effects of these additives according to Braide et al., (2012). The isolation of Staphylococcus species from zobo drinks, which may be enterotoxin producers responsible for staphylococcal foodborne illness, suggests that these beverages can be a source of biotoxins leading to adverse health effects through acute or chronic exposure. This isolation is indicative of inadequate personal hygiene practices, suboptimal storage facilities, employment of low-quality raw materials, and unsanitary environmental conditions. Furthermore, samples that were refrigerated consistently exhibited reduced Staphylococcus counts in comparison to those stored at ambient temperatures, underscoring the efficacy of refrigeration in inhibiting the proliferation of Staphylococcus bacteria.

A comprehensive analysis of the cultural and biochemical characteristics of microbial isolates from Zobo drinks subjected to various treatments and storage temperatures revealed valuable insights into the microbial diversity and potential spoilage organisms present. The dominant bacterial genera identified across the different conditions included *Bacillus*, *Staphylococcus*, *Micrococcus*, *Proteus*, *Lactobacillus*, and *Escherichia coli*. These findings are consistent with previous reports by Nwafor and Ikenebomeh (2009), Egbere et al. (2007), Ayandele (2015), and Nwachukwu et al. (2007).

The current study demonstrated notable microbial heterogeneity, with *Micrococcus* (25%), *Bacillus* (20.8%), and *Staphylococcus* (20.8%) emerging as the most prevalent isolates. Additionally, *Proteus*, *Lactobacillus*, and *Escherichia coli* constituted 12.5%, 8.3%, and 12.5% of the total isolates, respectively. This observed microbial diversity highlights the complexity of the microbial ecosystem in Zobo beverages.

Importantly, the type of treatment applied to the Zobo samples had a noticeable impact on the distribution of microbial species, suggesting that specific treatments may selectively inhibit or promote certain organisms. Further investigations are warranted to elucidate the mechanisms underlying these treatment-specific effects, with a view to developing more effective preservation strategies. Moreover, storage temperature was identified as a key determinant of microbial composition, emphasizing the temperature sensitivity of these microbial communities and the critical role of temperature control in maintaining microbiological quality.

Further investigations into the temperature-dependent dynamics of microbial growth and survival could provide nuanced insights into the design of optimal storage conditions for these beverages. Understanding the microbial composition is crucial for assessing the shelf life and overall quality of commercially made zobo drinks. The presence of potentially pathogenic organisms, such as *Escherichia coli*, emphasizes the importance of proper handling, storage, and treatment procedures to ensure consumer safety. Moreover, the role of beneficial microorganisms like Lactobacillus suggests potential avenues for enhancing the functional qualities of these beverages through targeted fermentation processes. Comparing the results across different treatments and storage temperatures allows for a nuanced understanding of microbial dynamics. For instance, the prevalence of *Staphylococcus* and *Bacillus* at varying percentages across treatments may indicate specific susceptibilities or resistances to certain preservation methods. The total coliform count in Zobo remained relatively consistent over the study period, with some variations between treatments. The findings suggest that the addition of ginger and garlic may have a positive impact on inhibiting bacterial growth in Zobo drink. Overall, these results provide valuable insights into the microbial stability and shelf life of these popular beverages, offering guidance for manufacturers and consumers alike.

Effect of natural spices on the physicochemical quality of Zobo drink stored at different storage temperatures

A comprehensive assessment of key physicochemical characteristics on days 0 and 9 contributes significantly to understanding the shelf life and stability of commercially manufactured zobo beverages. Parameters examined encompass pH, Total Titratable Acidity ,Ascorbic Acid content, and Total Soluble Solids. Zobo juice treated displayed pH stability during the storage period. For example, sample Zobo Control maintained a consistent pH of 4.9 at both ambient and refrigeration temperatures on day 0, with minimal changes observed on day 9. This suggests the robustness of the beverage regarding acidity. However, Adesokan et al. (2013) in his study reported a pH ranged of 3.94 - 7.67 while Raimi. (2013). reported a pH range of 2.9 - 4.3 but with a mean pH value of 3.04 which agreed with our study. The data

suggests that the pH of zobo juice blended with ginger and garlic exhibits elevated values in comparison to zobo juice without spices, indicating a relatively acidic nature. This finding has implications for the consumption of zobo juice, as it may be advisable to pair it with a snack or consume it only after a meal. Furthermore, a study reported a pH range of 2.19-3.62 in commercially available fruit-flavored zobo beverages, highlighting the potential for variability in the pH levels of zobo-based products. Fasoyiro et al., (2005). The result showed a slight difference in pH of zobo drink stored at ambient and refrigeration temperatures, this corroborates with study of Seiyaboh, et al,(2013)

Total Titratable acid values for zobo drink treated remained relatively constant, indicating the preservation of acidity levels. Sample ZC exhibited a slight increase in TTA from 0.90 to 1.06 at ambient temperature on day 9. Similar trends were observed in other treatments (ZGA, ZGI, and ZGG). The high TTA in zobo juice may be due to the presence of various organic acids present in the ingredients used. Other ingredients like ginger and garlic may also contribute to its acidity. The combination of these ingredients and the natural acids they contain result in the TTA being high in zobo drink this is different from the report by Bolade et al., (2009), The present results of % TTA is low and it was attributed to low pH value and the status of zobo not being a product of fermentation.

The ascorbic acid content in zobo drink treatments showcased stability. For instance, sample ZC maintained its ascorbic acid content at 33.50 on day 0 and 33.51 on day 9 at ambient temperature. The ascorbic acid content is comparatively higher than that of Adesokan *et al.,* (2013) with 22.5, 25, 23.5 and 35.8 in Zobo without treatment (control), zobo with ginger, zobo with garlic and zobo mixed with both ginger and garlic respectively. This consistency is crucial for retaining the antioxidant potential of the beverage. The stability of vitamin C is predominantly influenced by pH levels, with higher pH values favoring oxidation processes of the vitamin (Leahu et al., 2013). While Hibiscus sabdariffa is recognized for its antioxidant properties (Oboh and Okhai, 2012), the addition of ginger further enhances the antioxidant content. Consequently, preserved zobo drinks are capable of resisting oxidation and safeguarding the integrity of vitamin C during storage. At ambient temperatures (approximately 20-30°C or 68-86°F), the degradation of ascorbic acid in zobo drink occurs at an accelerated pace due to elevated temperatures and potential microbial activity. This can result in a gradual decline in the ascorbic acid content over time.

On the other hand, storing zobo drink at refrigeration temperature (around 2-4°C or 36-39.2°F) significantly slows down the degradation process, preserving more ascorbic acid in the drink. However, it is essential to note that even refrigerated zobo drink will lose some of its ascorbic acid content over time, but at a slower rate compared to ambient temperature storage. The result also show that was slight difference among the zobo drinks stored at ambient and refrigeration temperatures. Zobo juice treatments demonstrated stability in TSS. Sample ZC maintained its TSS values at 9.20 on day 0 and 9.4 on day 9 at ambient temperature, with minimal changes observed in refrigerated storage. When the TSS in zobo drink is high, it may imply strong flavor, increased sweetness, higher nutrient content: thicker texture. Conversely, if the TSS in zobo drink is low, it may imply lighter flavor, reduced sugar content: thinner consistency, potential dilution. When stored at ambient temperatures, the TSS content in zobo drink may degrade faster due to the increased rate of chemical reactions occurring at higher temperatures. This degradation can lead to a reduction in the overall TSS content, causing the drink to lose some of its natural sweetness and flavor.

On the other hand, when stored at refrigeration temperatures (0-4°C), the TSS content in zobo drink is more likely to be preserved, as the lower temperatures slow down the chemical reactions responsible for the degradation of soluble solids. This means that the zobo drink will maintain its natural sweetness and flavor for a more extended period when stored at refrigeration temperatures.

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