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Original Research Article

Effect of Fortifying Zobo (*Hibiscus sabdarifa*) Drink with Pineapple and Watermelon on the Mineral Compositions and Microbial Quality

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Abstract

This study was aimed at assessing the effect of fortifying zobo drink with pineapple and watermelon on the mineral composition and microbial quality. Dried calyces of *H. sabdariffa* (zobo) leaves were obtained from Lafenwa market in Abeokuta, Nigeria. The leaves were boiled, cooled, filtered and stored in 3 different containers labelled A, B and C respectively. Pineapple and watermelon juice were extracted and added to the zobo drink labelled B and C respectively in ratio 1:4. The samples were immediately transferred to the laboratory where they were allowed to stand for 72 hours (3 days) before analysis. Staying for 3 days before analysis was to give room for the growth of microorganisms. The pH was measured daily for 3 days while the mineral composition and microbial counts were determined after 3 days using standard methods. The results of this study showed that zobo drinks fortified with pineapple and watermelon respectively had higher concentrations of essential minerals than the unfortified one. This could make them to have greater therapeutic agents than the unfortified one. However, the higher microbial loads observed in the fortified drinks will reduce its shelve life and place them as possible causes of infection if consumed after 48 hours of preparation and stored under room temperature. Thus, we encourage the consumption of zobo drinks fortified with pineapple and watermelon within 24 hours of production before the growth of microbes begins.

Keywords: Microbial Growth, Mineral Composition, Pineapple Juice, Watermelon Juice, Zobo Drink

INTRODUCTION

Boiling and filtration are used to make Zobo drink, a non-alcoholic native beverage from dried flowers of *Hibiscus sabdarifa* (Linn Roscelle) (Onabanjo and Airaodion, 2022). It is gaining widespread recognition in the West African sub-region, where it is drank by millions of people from various socio-economic classes and backgrounds, particularly among the youth, who consider zobo drink as a cheap and soothing non-alcoholic drink in social gatherings (Ogiehor and Nwafor, 2004). With recent government policies emphasizing the need to rely less on imported foods and beverages, zobo beverages appear to be economically and socially attractive. Figure 1

Minerals are inorganic compounds found in all tissues and fluids, and their presence is required for the proper functioning of certain physicochemical processes. Minerals are chemical components that the body uses in a variety of ways. Despite the fact that they produce no energy, they serve a crucial part in a variety of bodily functions (Eruvbetine, 2003). For their normal life activities, all forms of living matter require certain organic

Figure 1. Dry Zobo (*Hibiscus sabdariffa*) Leaves (Onabanjo and Airaodion, 2022)

elements or minerals (Ozcan, 2003). Minerals are divided into macro (major) and micro (trace) elements. The ultratrace elements are the third group. Calcium, phosphorus, sodium, and chlorine are macrominerals, while iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, and sulphur are microminerals (Eruvbetine, 2003).

The macro minerals must be in concentrations larger than 100 mg/dL, whereas the micro minerals must be in concentrations less than 100 mg/dL. Boron, silicon, arsenic, and nickel are ultra-trace elements that have been detected in animals and are thought to be important for them. The evidence concerning the requirements and importance of other elements such as cadmium, lead, tin, lithium, and vanadium is inconclusive (Murray *et al*., 2000).

Zobo beverage has been demonstrated to be a rich amount of natural carbohydrate (11.07%), protein (0.73%), (Onabanjo and Airaodion, 2022) and vitamin C (Okoro, 2003; Ogiehor and Nwafor, 2004), which are the main reasons why soft drinks and fruit juices are consumed. The actions of the accompanying microbes may intensify the increase in these components as the storage period lengthens. Several bacteria have been shown to be connected with zobo beverage during storage (*Bacillus, Steptococcus, Staphylococcus, Leuconostoc, Lactobacillus, Aspergillus, Penicillium, Geotrichum, Fusanum,* and *Alternana*) (Akinyosoye and Akinyele, 2000; Ogiehor and Nwafor, 2004). The related microorganism's multiplication accelerates deterioration and contributes to the relish beverage's short shelf life (24–48 hours).

It has been stated that indigenous spices are used to inhibit the activities of bacteria in foods (Akpomedaye and Ejechi, 1998; Nwafor and Ogiehor, 2003). Spices are thought to have medical values (particularly in African contexts) and to have desirable determinative effects on the overall organoleptic quality of food when employed, in addition to their antibacterial characteristics. Furthermore, the use of low-temperature storage to slow and stabilize microbial development in food has been well-documented (Ogiehor *et al*., 1998; Ogiehor *et al*., 2004). Thus, using spice extracts alone or in combination with low-temperature storage to limit microbial activity associated with zobo drink while maintaining nutritional and economic quality could be a viable option. As a result, the goal of this study was to see how fortifying zobo drink with pineapple and watermelon extracts affected the mineral composition and microbiological quality of the drink.

MATERIALS AND METHODS

Preparation of Zobo Drinks

Dried calyces of *H. sabdariffa* (zobo) leaves were purchased from Lafenwa market in Abeokuta, Nigeria and were identified by a botanist. They were manually cleaned by handpicking stones and other unwanted debris. They were thoroughly washed using sterile de-ionized water. About 400g of the washed calyces zobo leaves were boiled in 2000 mL of water for 15 minutes as described by Onabanjo and Airaodion (2022), and was left to cool for 15 minutes before removing the calyces using a white muslin cloth and left to stand in a vessel. The cooled zobo drink was stored in 3 different containers and were labelled A, B and C respectively.

The pineapple and watermelon were also purchased from the same market. They were separately washed, peeled and chopped into small bits with a clean stainless knife. The seeds of the watermelon were removed. The chopped pineapple and watermelon were separately blended with a blender with stainless steel blades until juice and pulp was obtained. The juice was filtered using a white muslin cloth and the resulting extract was stored in a clean bottle (Onabanjo and Airaodion 2022). Pineapple juice was added to the zobo drink labelled B and watermelon was added to the zobo drink labelled C in ratio 1:4. Sample B therefore contained 80 mL of zobo drink and 20 mL of pineapple juice while sample C contained 80 mL of zobo drink and 20 mL of watermelon juice. The samples were immediately transferred to the laboratory where they were allowed to stand for 72 hours (3 days) before analysis. Staying for 3 days before analysis was to give room for the growth of microorganisms.

Determination of Mineral Composition of Zobo Drink Samples

Mineral elements were determined using wet-acid digestion method for multiple nutrients determination as described by Airaodion *et al*. (2021). About 0.5 mL of the sample was measured into a 150 mL Pyrex conical flask. 5 mL of the extracting mixture (H_2SO_4–Sodium salicylic acid) was added to the sample. The mixture was allowed to stand for 16 h. The mixture was then placed on a hot plate set at 30°C and allowed to heat for about 2 hours. 5 mL of concentrated perchloric acid was introduced to the sample and heated vigorously until the sample was digested to a clear solution. 20 mL of distilled H_2O was added and heated to mix thoroughly for about a minute. The digest was allowed to cool and was transferred into a 50 ml volumetric flask and made up to the mark with distilled water. The digest was used for the determinations of calcium (Ca) and magnesium (Mg) by the ethylenediaminetetraacetic acid Versanate Complexiometric titration method. AOAC (2006) method was used to determine sodium (Na) and potassium (K) by using a flame photometer (model PFP7 Digital, Jenway, UK). All other minerals were determined by atomic absorption spectrophotometer (model 3030, Perkin Elmer, Norwalk USA).

Determination of pH of Zobo Drink Samples

The pH values of the samples were determined every 24 hours for 3 days using Airaodion *et al.* (2019a) approach. Using a previously standardized pH meter, 10 mL of the sample was poured into a beaker and the pH was calculated (JenWay 3505). A phosphate buffer of pH 4.0 and 7.0 was used to calibrate the pH meter.

Microbiological Analysis

Microbial count was determined according to standard methods of Collins and Lyne cited in Das and Dash (2015). About 1 mL of each sample was poured on plate count agar and incubated for 24 hours at 37^{o}C. The plates were examined for the presence of bacteria colonies.

RESULTS AND DISCUSSION

Minerals are chemical components that organisms require as critical nutrition to accomplish vital tasks (Umar *et al*., 2020). Because they are vital and cannot be synthesized by the body, they must be consumed. Table 1 shows the results of the mineral profile of zobo drink samples. Sodium was the most concentrated mineral, followed by potassium, and copper was the least. These minerals are vital in several biological functions and are very crucial for health, according to Airaodion *et al*. (2021). The potassium concentration in unfortified zobo in this study was 5.11 mg/100g, which is similar to the 5.11 mg/100g found by Nwankwo *et al*. (2022), but substantially higher than the 2.00 mg/100g found by Adeniji (2017). This figure is much lower than Borokini *et al*. (2019)'s figure of 28.10 mg/100g potassium. In this study, the potassium concentration of unfortified zobo beverages was lower than that of zobo drinks fortified with pineapple and watermelon juice, which were 6.86 and 6.08 mg/100g, respectively. Nwankwo *et al*. (2022) earlier observed that adding ginger and clove to zobo drinks boosted potassium concentrations.

The 1.22 mg/100g magnesium found in unfortified zobo drink in this study is similar to the 1.19 mg/100g found by Nwankwo *et al*. (2022), but much higher than Borokini *et al*. (2019)'s 0.69 mg/100g. The value is likewise comparable to the 1.17 and 1.00 mg/100g obtained from zobo beverages including watermelon and pineapple, respectively. The magnesium content in zobo beverages has been reported to be low (Bassey *et al*., 2020). In reality, Akujobi *et al*. (2018) found no potassium in zobo beverages, both fortified and unfortified. The magnesium level in this study was lower than the recommended daily intake (130-340 mg/dL) in all of the samples examined, despite the fact that magnesium is a cofactor for several enzymes that catalyze key biological events. Magnesium has been shown to work with calcium to help in muscle contraction and blood clotting. It functions as a cofactor for a number of enzymes (Wardlaw and Smith, 2012).

This study's mean calcium concentration of 0.96 mg/100g is similar to Borokini *et al*. (2019)'s 0.95 mg/100g in unfortified zobo beverages, but lower than Adeniji (2017) and Olayemi *et al*. (2011)'s 2.8 and 4.00 mg/100g, respectively. Calcium concentrations in watermelon and pineapple-fortified zobo beverages were 0.76 and 0.97 mg/100g, respectively. Borokini *et al*. (2019) found calcium content of 0.92 mg/100g in zobo drink supplemented with clove, while Nwankwo *et al*. (2022) found 0.92 mg/100g in zobo drink fortified with pineapple bark. This indicates that adding flavor to zobo beverages had no meaningful influence on calcium

Table 1. Mineral Composition(mg/100g) of Fortified Zobo Drinks

Mineral	Zobo Only	Zobo + Pineapple	Zobo + Watermelon
Potassium (K)	5.11±0.67	6.86±0.15	6.08±0.26
Magnesium (Mg)	1.22±0.06	1.00±0.02	1.17±0.01
Calcium (Ca)	0.96±0.00	0.97±0.00	0.76±0.00
Iron (Fe)	2.01±0.05	2.68±0.23	1.89±0.02
Copper (Cu)	ND	0.08±0.00	0.61±0.01
Manganese (Mn)	0.21±0.01	0.19±0.00	0.30±0.01
Sodium (Na)	24.98±3.27	25.25±1.78	29.01±2.04
Zinc (Zn)	0.19±0.00	0.35±0.03	0.29±0.01
Na/K	4.89±0.51	3.68±0.84	4.77±0.63

Results are presented as means ± standard deviation (SD) of triplicate analysis (n = 3)
ND = Not Detected

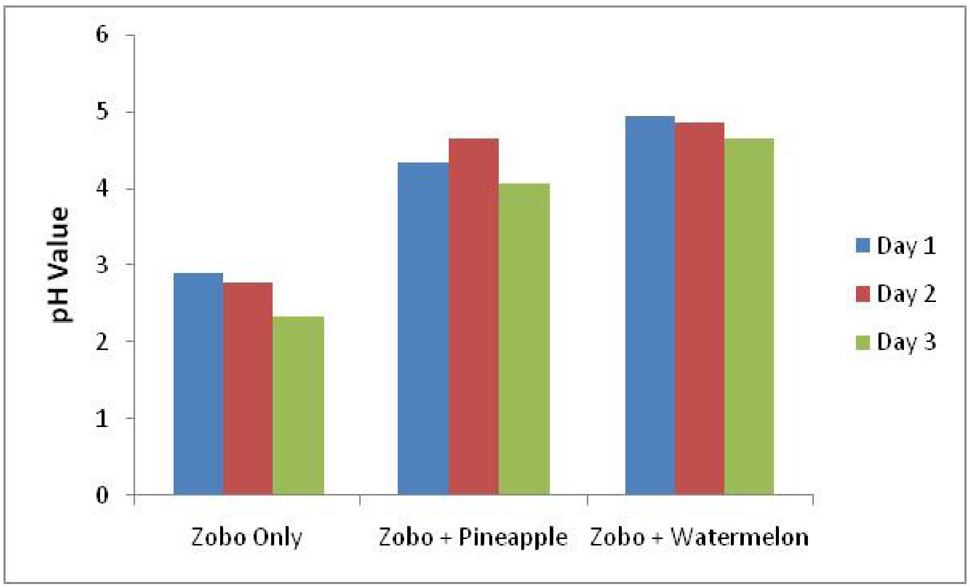

Figure 2. pH values of Zobo Drink Samples

Table 2. Microbial Quality (CFU/mL) of Fortified Zobo Drinks

Microbes	Zobo Only	Zobo + Pineapple	Zobo + Watermelon
Total Variable Count	6.94×10^4	9.25×10^4	12.77×10^4
Coliform Count	20.00	50.00	74.00
Total Aerobic	1.44×10^4	2.64×10^4	2.99×10^4
Total Anaerobic	1.71×10^4	2.41×10^4	2.60×10^4
Total Fungi	1.48×10^4	2.71×10^4	2.92×10^4
Salmonella typhi	14.00	26.00	28.00
Streptococcus spp	6.00	82.00	98.00
Mould and Yeast Count	22.00	38.00	36.00
Staphylococcus aureus	38.00	54.00	70.00

levels. Ossification, muscle contraction, and blood coagulation all require calcium (Rude, 2010). The skeleton contains the majority of calcium and magnesium.

Iron concentration in unfortified zobo drink was 2.01 mg/100g in this study, which is similar to the 2.00 mg/100g reported by Adeniji *et al.* (2017). This value is substantially greater than Olayemi *et al.* (2011)'s 0.67 mg/100g. The iron content of the seed makes it a valuable tool for preventing anemia. Iron is an essential mineral for hematopoiesis (Airaodion *et al.*, 2019b; 2019c; Airaodion and Ogbuagu, 2020). The iron level of the various samples was all below the recommended dietary limit for males (1.37 mg) and females (2.93 mg) (WHO, 2015).

All of the samples had copper levels that were lower than the recommended daily intake. Copper was not found in the unfortified zobo drink. This revealed that zobo drink is a poor source of copper, necessitating the use of alternate sources when consuming the beverage.

Furthermore, the mean salt content of unfortified zobo drink in this study (24.98 mg/100g) is similar to Borokini *et al*. (2019)'s 25.35 mg/100g, but lower than Olayemi *et al*. (2011)'s 35.78 and 50.67 mg/100g for red and wine colored zobo, respectively. This result did not differ substantially from the 25.25 mg/100g sodium record for zobo fortified with pineapple, but it did differ significantly from the 29.01 mg/100g sodium record for zobo fortified with watermelon. The sodium content of zobo fortified with pineapple juice in this study was similar to that of Borokini *et al*. (2019), who fortified zobo drink with pineapple bark and found 24.43 mg/100g sodium. Sodium and potassium regulate the water balance in bodily tissues and have a role in the transfer of various non-electrolytes (Airaodion *et al*. 2021).

In this investigation, the zinc concentration in unfortified zobo drink was 0.19 mg/100g, which is close to the 0.21 mg/100g reported by Borokini *et al*. (2019), but lower than the 1.93 and 1.20 mg/100g reported by Koopman (2017) and Adeniji, respectively (2017). Zinc concentrations were substantially greater in zobo fortified with pineapple (0.35 mg/100g) and zobo fortified with watermelon (0.29 mg/100g) than in the unfortified zobo drink. Akujobi *et al*. (2018), Bassey *et al*. (2020), and Koopman (2017) reported 0.92 mg/100g, 0.85 mg/100g, and 2.18 mg/100g for zobo drinks fortified with pineapple, ginger, and turmeric respectively. However, this study found the same zinc concentration (0.35 mg/100g) as Borokini *et al*. in a zobo drink fortified with pineapple (2019). Zinc is required for normal sexual development, particularly the growth of the testes and ovaries. In addition, it is required for reproduction (Airaodion *et al*., 2019d; Ekenjoku *et al*., 2019; Ogbuagu and Airaodion, 2020). Zinc promotes vitamin action, the creation of red and white corpuscles, good cardiac function, and normal growth (Lichten and Cousins, 2009).

As a result of this finding, it is clear that zobo drinks fortified with pineapple and watermelon have larger concentrations of critical minerals, which may be important in the body's daily operations. This is in line with the findings of Onabanjo and Airaodion (2022), who observed that adding pineapple and watermelon to zobo drink improves its nutritional quality and phytochemical composition.

According to our findings, the pH of unfortified zobo is 2.91, whereas the pH of zobo fortified with pineapple is 4.35 and zobo fortified with watermelon is 4.95. The pH of zobo samples decreased when they were permitted to stay from day one to day three, except those fortified with pineapple whose pH increased on day two but reduced in day three (figure 2). The reason behind the slight increment in the pH of zobo drink fortified with pineapple on day two is unclear. However, the low pH of the beverage observed in this study shows that zobobeverages, especially those that aren't fortified, are acidic (Onabanjo and Airaodion, 2022). Zobo plant is naturally acidic with a high concentration of organic acids (Olayemi *et al.,* 2011). This means that zobo drink should not be consumed without a snack or on an empty stomach. Those with peptic and stomach ulcers should avoid drinking unfortified zobo drink on a regular basis due to the low pH (Gbadegesin and Gbadamosi, 2017). Instead, they should have a zobo beverage flavored with pineapple and/or watermelon. The drop in the pH value of zobo beverages over time seen in the study agrees with Seiyaboh *et al*. (2013)'s findings.

Table 2 shows the results of the microbiological characteristics of zobo beverages. In general, the unfortified zobo drink had a lower microbe concentration than the fortified drinks. As a result, the unfortified drink's shelf life will be longer than the fortified drink's.

In this study, the number of coliform cells found in unfortified zobo drink (20 CFU/mL) was much lower than those found in zobo drink fortified with pineapple (50 CFU/mL) and watermelon (74 CFU/mL). This is consistent with the findings of Bassey *et al*. (2020), who found that flavoring zobo drinks with moringa dramatically enhanced the number of coliform cells when compared to unfortified drinks. This investigation only found a small amount of coliform cells. This could indicate that the drink is safe for human consumption, as coliform counts of 100 colony forming units per gram or above are regarded unsatisfactory for ready-to-eat food, according to HPA (2004). Furthermore, the counts reported in all of the zobo samples utilized in this investigation are lower than the 100 cfu/g coliform count limit specified by the International Commission on Microbiological Specification of Food (ICMSF, 2002). This indicates that the zobo beverages were made in a sanitary environment and are considered safe for human use. Mohammed *et al*. (2017) earlier stated that no coliform bacteria cells were found in their zobo drink since their experimental tubes remained constant during their trial period, with no gas formation in the Durham tubes. The HPA (2004) determined that a high bacterial cell count of 100 or more is an indicator of the overall degree of food microbial contamination. According to Mohammed and Ismail (2014), zobo drink contains bacteria such as Bacillus species.

The total aerobic and anaerobic activity measured in this study for both fortified and unfortified zobo drinks fell short of the 10^6 standard set by the International Commission on Microbiological Specification of Food. The mold and yeast count of 22 CFU/mL found in this study for unfortified zobo drink is similar to the 25 CFU/g found by Mohammed *et al.* (2017). This finding, on the other hand, was much lower than the 38 and 36 CFU/mL mold and yeast counts found in zobo drinks supplemented with pineapple and watermelon, respectively. This means that adding pineapple and watermelon to the beverage increases its spoilage.

Escherichia species, which were found in all zobo drink products, are opportunistic bacteria that live in the mammalian intestine. In humans, some strains cause gastroenteritis, while others cause urinary tract infections (Seiyaboh *et al*., 2013). Escherichia coli is a public health concern because it is a water indicator organism whose presence is undesirable. Microbial loads were higher in fortified zobo beverages than in unfortified ones in this investigation. Spices reduced the microbial burdens of zobo samples during the seven-day storage period, according to Adesokan *et al*. (2013). The flavors employed in this investigation are juice derived from raw pineapple and watermelon, rather than spices. This could have resulted in a higher microbial load than the zobo drink that wasn't fortified.

The presence of spoilage organisms in the zobo drink was influenced by the processing and storage conditions (Seiyaboh *et al*., 2013). The results show that zobo spoils within a few days of being stored at room temperature, and that this rotting may be caused by the presence of the bacteria stated above. As a result, zobo drink should be consumed at room temperature within 48 hours of purchase. The presence of microorganisms in the beverage revealed that the raw materials had bacteria, either directly from the source or during handling, and that these microbes could potentially be present in the containers utilized.

CONCLUSION

The results of this study showed that zobo drinks fortified with pineapple and watermelon respectively had higher concentrations of essential minerals than the unfortified one. This could make them to have greater therapeutic agents than the unfortified one. However, the higher microbial loads observed in the fortified drinks will reduce its shelve life and place them as possible causes of infection if consumed after 48 hours of preparation and stored under room temperature. Thus, we encourage the consumption of zobo drinks fortified with pineapple and watermelon within 24 hours of production before the growth of microbes begins.

Ethical Approval: Not Applicable

Consent for Publication: Not applicable

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTERESTS

Authors declare that they have no conflict of interests in this research and publication.

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AUTHORS' CONTRIBUTIONS

The research was carried out by both authors. Both authors read and approved the final manuscript.

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