

Effect of *Citrus aurantifolia* juice on the shelf-life of zobo drink produced locally in Afikpo, Ebonyi State, Nigeria

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To cite this article:

Stanley Chukwudozie Onuoha, Kayode Fatokun. Effect of *Citrus Aurantifolia* Juice on the Shelf-Life of Zobo Drink Produced Locally in Afikpo, Ebonyi State, Nigeria. *American Journal of BioScience*. Vol. 2, No. 2, 2014, pp. 45-48. doi: 10.11648/j.ajbio.20140202.14

Abstract: The bacterial quality of Zobo drinks produced locally and the effect of *Citrus aurantifolia* juice (lime juice) on the bacteria associated with the drinks were investigated. Zobo drinks were obtained from local market and analyzed bacteriologically according to standard methods. The total viable count was 2.77×10^4 cfuml⁻¹ and the total coliforms was 2.55×10^4 cfuml⁻¹. Bacteria isolated from zobo drink samples included *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp, *Klebsiella* spp. The isolates in decreasing order of occurrence were *Staphylococcus aureus* (22%), *Escherichia coli* (19%), *Enterobacter* spp. (18%), and *Pseudomonas aeruginosa*. (16%), *Klebsiella* spp. (13%) and *Bacillus* spp (11%). The total coliforms and total viable counts generally decreased in values following treatment of zobo drink samples with different concentration of lime juice. The total coliforms count for 0.5 concentration was 2.0×10^2 cfuml⁻¹ while, 2.0 concentrations had zero cfuml⁻¹. The total viable count decreased rapidly from 6.0×10^2 to 0.1×10^2 as the juice concentration increased from 0 to 2.0 respectively. The study revealed that lime juice can be used to prolong the shelf-life of Zobo drinks.

Keyword: Bacteria, Hibiscus Sabdariffa, Lime Juice, Shelf-Life, Zobo Drinks

1. Introduction

Zobo drinks are local beverage quite popular in Northern Nigeria and are enjoyed at various social gatherings. Its popularity has recently spread across the entire country because of its purported medicinal value, as well as the increasing cost of other available soft drinks (Adegunloye *et al.*, 1996).

Zobo drink is prepared from the calyx of the roselle hemp plant *Hibiscus sabdariffa* (Malvaceae) by boiling the dry calyces in water for about 10-15 minutes to extract the pigment or embedded flavor. The filtrate after extraction may be taken hot as tea or as refreshing drink when cooled or chilled and packaged in plastic sachet or container. The sharp sour taste of the raw extract is usually sweetened with sugar cane or granulated sugar, pineapple, orange or other fruits depending on choice. The sweetness of Zobo drink does not last long due to spoilage by microbial activities (Oboh and Elusiyani, 2004).

The calyces of *Hibiscus sabdariffa* have been found to be rich in vitamins and other antioxidants (Wong *et al.*, 2002). It is also rich in minerals (Babaola *et al.*, 2000). The

leaves are used as vegetables and the seed are source of oil, there is an increase demand for Zobo drinks due to its low prices, nutritional and medicinal properties. (Osueke and Ehirim, 2004).

The greatest limitations for large- scale production of Zobo drinks is the rapid deterioration of the drink. The shelf- life of Zobo is approximately twenty-four hours following production if not refrigerated. The spoilage may be attributed to microorganisms associated with the dried calyx, the processing for the production and other factors. Apart from the fact that most chemical preservatives are expensive and usually not affordable by the local people that produce this drink (Zobo) and may have adverse effect on human.

There is need for alternative source of preservation that is natural, cheap or affordable and readily available and safe. The aim of this study is:-

- i to isolate the possible bacteria that can cause deterioration of Zobo drink.
- ii to investigate the effect of lime juice on the bacterial quality of Zobo drinks with the view to improve the shelf- life of the drink.

2. Materials and Methods

2.1. Source of Samples

Twenty (20) Zobo drinks were purchased randomly from Eke Afikpo Market in Ebonyi State, and were taken to the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic Unwana for Microbiological analyses.

2.2. Microbiological Analyses

One milliliter (1 mL) of Zobo drink sample was transferred into 9 mL of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution (10^2), 0.1 mL of the sample was transferred from each test tube serially into sterile petri dish and inoculated with prepared Nutrient and MacConkey agar, spread evenly to solidify. The inoculated nutrient agar plates were incubated at 30°C for 48 hrs while the inoculated MacConkey agar plates were incubated at 35°C for 48 hrs. After the period of incubation, the colonies on Nutrient agar were counted and recorded as colony forming units per milliliter (cfumL^{-1}). Colonies of lactose fermenting organisms (red or pink colonies) on MacConkey agar plates were also counted and recorded as coliforms (Harrigan and McCane, 1996).

Each of the bacterial colonies on both Nutrient and MacConkey agar plates was subcultured and pure culture obtained. Isolates were identified by carrying out tests which included gram staining, spore staining, catalase, coagulase, oxidase, citrate utilization, indole production, methyl red, Voges proskauer, motility test and nitrate reduction test.

2.3. Treatment of Zobo Drinks with Lime Juice

Fifteen lime fruits were sterilized with 70% ethanol and peeled using a pre-sterilized knife. The fruits were then halved using a pre-sterilized knife and the juice was squeezed aseptically into sterile 100mL conical flask. In order to determine that the lime juice is not contaminated with bacteria, a loopful of the juice was inoculated on nutrient agar plate for 24 hrs. Ten fold dilution of the Zobo drink sample was obtained and 0.5, 1.0, 1.5 and 2.0 mL of the lime juice were added, respectively to each test tube of the fold dilution. The mixture was allowed to stand for 6 hrs at 30°C. Thereafter, from each treatment 0.1mL was inoculated into plates of Nutrient agar and MacConkey agar and incubated at 30°C and 35°C respectively for 48 hrs. The colonies were counted and recorded as colony forming units per milliliter (cfu mL^{-1}).

3. Results

Table 1 shows the total viable count and total coliforms obtained after the microbiological analyses. The total viable count was $2.77 \times 10^4 \log_{10} \text{cfu mL}^{-1}$ while the total coliforms were $2.55 \times 10^4 \log_{10} \text{cfu mL}^{-1}$ respectively.

Table 1. Total Coliforms and Total Viable Count (Cfu mL^{-1})

| Organisms | No. of samples | Microbial count(10^4cfumL^{-1}) |
|--------------------|----------------|---|
| Total coliforms | 20 | 2.55 |
| Total viable count | 20 | 2.77 |

Figure 1 shows the percentage occurrence of the isolated bacteria, their percentage occurrence were 11%, 13%, 19%, 18%, 22% and 16% respectively. From the result *Staphylococcus aureus* had the highest percentage occurrence while *Bacillus* spp had the least occurrence among the bacteria with 11% occurrence.

Table 2 showed the total coliforms and total viable count obtained after treatment with different concentration of lime juice. The result revealed a steady decrease in the number of bacteria in Zobo drink sample following treatment with lime juice. For instance at the high concentration of 2.0 ml^{-1} concentration there was complete inhibition of the coliforms, while there was a marked reduction on the growth of the bacteria as shown by the value of the total viable count (Table 2).

Table 2. Total coliforms and total viable counts after treatment of zobo drink with lime juice.

| Lime juice conc. (ml^{-1}) | Total coliforms count (cfumL^{-1}) | Total viable count (cfumL^{-1}) |
|---------------------------------------|---|--|
| 0 | 4.5×10^2 | 6.0×10^2 |
| 0.5 | 2.0×10^2 | 3.5×10^2 |
| 1.0 | 0.8×10^2 | 2.2×10^2 |
| 1.5 | 0.2×10^2 | 1.4×10^2 |
| 2.0 | 0 | 0.1×10^2 |

4. Discussion

Zobo drink is a refreshing beverage obtained from the Plant *Hibiscus sabdariffa*. The drink is becoming very popular with acceptability cutting across many ethnic group and socio-economic classes in Nigeria and other African countries. There is increase in the demand for Zobo drinks due to its low price, nutritional and medicinal properties (Oboh and Elusiyan, 2004; Osueke and Ehirim, 2004). The shelf- life of Zobo is approximately twenty-four hours following production if not refrigerated.

The spoilage may be attributed to microorganisms associated with the dried calyx, the processing for the production and other factors. Apart from the fact that most chemical preservatives are expensive and usually not affordable by the local people that produce this drink (Zobo) and may have adverse effect on human. There is need for alternative source of preservation that is natural, cheap or affordable and readily available and safe.

The effect of lime juice concentrations on the bacterial quality of Zobo drinks was investigated with a view to prolong the shelf-life of Zobo drinks. The main findings of

this study was that lime juice has the ability to inhibit the growth of spoilage bacteria found in the drink thereby extending the shelf-life of Zobo drink.

Bacteria isolated from Zobo drink sample in this study with their percentage occurrence included *Staphylococcus aureus*(22%), *Escherichia coli*(19%), *Bacillus spp*(11%), *Pseudomonas aeruginosa*(16%), *Klebsiella spp*(13%), and *Enterobacter spp*(18%) (Fig. 2). In a similar study, Nwachukwu et al (2007) isolated bacteria including *Lactobacillus spp* in his study with higher percentage occurrence in Zobo samples studied. Also, in another study, Amusa et al (2005) and Nwachukwu and Otokumefor (2002) reported that hawked Zobo drinks harbored similar bacteria including *Streptococcus*, *Proteus* species and *Lactobacillus* species. The presence of these bacteria in Zobo drink is therefore no longer in doubt and may be explained by the microbial quality of the ingredients used and personal hygiene, but studies should be pursued towards reducing the bacterial load.

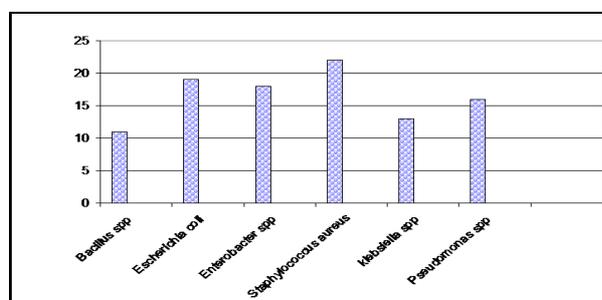


Figure 1. Percentage Occurrence of bacteria in Zobo drink samples

The occurrence of the different types of bacteria in Zobo drinks obtained indicated that isolation of *Staphylococcus aureus* occurred frequently followed by *Escherichia coli*, *Pseudomonas Sp.* and other bacteria. The presence of these bacteria indicated possible contamination of the drink. *Staphylococcus aureus* in zobo could be possibly through the processing methods which usually involved the use of hands since the organism is a common flora of the skin. The organism is responsible for staphylococcal food poisoning (Hobbs and Robert, 1993).

The occurrence of *Pseudomonas spp.* also could be as a result of contamination from food handlers or water since the organism is found in water and on skin surface as flora to the skin (Balcht and Smith, 1994).

The occurrence of *Bacillus spp* could be as a result of prevalence of their spores in environment (Jay, 1996). *Bacillus spp* formed spores which could survive high temperature of processing and have been isolated from non alcoholic beverages (Amusa et al., 2005).

Generally, *Escherichia coli* are an indicator or water pollution (Hurst et al., 2002) and therefore, the presence of the organism in zobo drink is probably related to the source or quality of water used for processing. In addition *Escherichia coli* isolated from water may have some health implication (Nwachukwu and Otokumefor, 2002).

Moreover, additives which were incorporated into the

zobo drinks after extraction may also be source of contamination. Packaging material and containers which probably were not properly sterilized as well as soil particles or the environment can serve as a source of additional microbial contamination of the zobo drink (Frazier and Westhoff, 1998).

The result of the treatment of zobo drink sample with different concentrations of lime juice (Table 2) reviewed that the bacterial load of the drink was reduced considerably. Different concentration of the lime juice inhibited the growth of the organisms to varying degrees. For instance, at a high concentration of 2.0 ml-1 lime juice exhibited complete inhibition of the total coliforms, while at the same concentration; it showed a near total reduction in the total viable bacteria count. This finding therefore suggest that the addition of lime juice at appropriate concentration in Zobo drinks may help in prolonging the shelf life of the drink. The possible explanation for the reduction of the bacterial load in Zobo drinks following addition of lime juice is the acidic nature of the lime juice. According to Jay (1996) the excellent keeping quality of fruits and soft drinks is due to low pH. This is because low pH tends to inhibit bacterial growth. Also, the possible explanation to the decrease in the microbial count and subsequent cessation of growth as observed suggest that the lime juice like preservatives which are used to store food substances create unfavourable microenvironment for the bacteria. They may have created hurdles which the organisms were not able to survive. The stepwise and gradual decrease in microbial counts observed in Zobo samples treated at different concentrations of lime juice (Table 2) is an indication of the difficulty encountered by the vegetative cells of the surviving organisms. This could be as a result of the unfavourable microenvironment created by the preservative (lime juice) at that concentration. It implies that the organism may have survived to some extent, the effect of the preservatives but further attempt to overcome the hurdles may cause them to become metabolically exhausted, hence the continuous decrease in population observed. Our finding corroborates earlier report by (Leister, 1994; Nwafor and Ikenebomeh, 2009; Nwafor, 2012). The differences in degree of inhibition at higher concentrations as compared with lower concentration suggest that the effect is concentration dependent. This finding is in line with the report of Nwafor, (2012).

5. Conclusion

Addition of lime juice to Zobo drink should be encouraged since this study has revealed that lime juice can inhibit bacterial growth in the drinks. Moreover, lime fruits are not hazardous and hence safe for human consumption. The use of lime juice for this study was simply due to the reason that lime juice is acidic in nature and most microorganisms do not thrive in acidic medium. The result of the effect of lime juice on quality of zobo drinks is a finding that will be useful since Zobo drinks is easily produced at home, packaged in containers and sold as

source of income for most families.

Lime juice as a preservative is recommended as to prolong the shelf-life and for more nutritional and medicinal values as it is also a source of vitamin C. Furthermore, in order to enhance the keeping quality of the Zobo drinks, the processing environment should be hygienic while the packaging materials and additives should be adequately sterilized. Portable water should be used during processing to avoid bacterial contamination of the drink. Producers of Zobo drinks also should be educated to know the importance of adherence to quality control measures during processing to avoid the hazardous effects of microbial contamination.

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