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Effect of Lime Juice on the Bacterial Quality of Zobo Drinks Locally Produced in Nigeria

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Abstract: The bacterial quality of zobo drinks locally produced and the effect of lime juice on the bacteria associated with the drinks were investigated. Zobo drinks were obtained from local market and analysed bacteriologically according to standard methods. The total viable counts was 2.79 $\log_{10}$ cfu mL^{-1} and total coliforms was 2.62 $\log_{10}$ cfu mL^{-1}. Bacteria isolated from zobo drink samples included *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus* sp. *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella* sp. The isolates in decreasing order of occurrence were *Staphylococcus aureus* (45%), *Escherichia coli* (40%), *Lactobacillus* sp. (37%), *Enterobacter aerogenes* (32%), *Pseudomonas aeruginosa* (30%), *Klebsiella* sp. (26%) and *Bacillus subtilis* (23%). The total coliforms and total viable counts generally decreased in values following treatment of zobo drink samples with lime juice. The study revealed that lime juice can be used to prolong the shelf-life of zobo drinks.

Key words: Zobo drinks, bacterial quality, lime juice, shelf-life

INTRODUCTION

Zobo drinks are aqueous extracts of calyx of roselle, *Hibiscus sabdariffa* which is annual herb that is widely cultivated in India and Africa. Zobo is a name derived from zoborodo which is the local hausa (Northern Nigeria) name for *Hibiscus sabdariffa* plant. The non-alcoholic drink or zobo is quite popular especially in Northern Nigeria and it is usually served chilled at various social gathering (Aliyu, 2000).

The zobo drink is prepared by boiling the dry calyces of *Hibiscus sabdariffa* in water for about 10-15 min from which the pigment or flavor embedded is extracted. After extraction the filtrate may be taken hot as tea or allowed to cool and packaged in plastic sachet containers then taken as a refreshing drink when chilled. 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 spoilage by microbial activities.

The calyces of *Hibiscus sabdarifta* have been found to be rich in vitamins and other antioxidants (Wong *et al*., 2002) and also minerals (Babalola *et al*., 2000). The leaves of roselle are used as vegetables and the seeds are source of oil.

There is increase in the demand for zobo drinks due to its low prices, nutritional and medicinal properties (Oboh and Elusiyan, 2004; Osueke and Ehirim, 2004). The greatest limitations for large-scale production of zobo drinks is the rapid deterioration of the drink. Its shell-life is approximately twenty-four hours following production if not refrigerated. Microorganisms associated with the dried calyx and the processing for the production of zobo drinks and other factors may contribute to its spoilage.

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Apart from the fact that most chemical preservatives may have adverse effect on humans, they are expensive and usually not affordable by the local people that produce this zobo drink. There is the need for alternative source of preservation that is natural, cheap or affordable and readily available and safe. Therefore the aim of this study is to investigate the effect of lime juice on the bacterial quality of zobo drinks with a view to improve the shelf life of the drink.

MATERIALS AND METHODS

Source of Samples

Twenty zobo drinks were purchased randomly from the local markets in Umuahia, Abia State, Nigeria and were taken to the laboratory for analyses. This study was conducted in the research laboratory, Michael Okpara University of Agriculture Umudike.

Microbiological Analyses

One milliliter of zobo drink sample was placed in 9 mL of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1 mL was inoculated separately on to nutrient agar and MacConkey agar plates and spread evenly using sterile bent glass rod. Each experiment was carried out in triplicates. The inoculated nutrient agar plates were incubated at 30°C for 48 h while the inoculated MacConkey agar plates were inoculated at 35°C for 48 h. After the period of incubation, the colonies on the nutrient agar plates were counted and recorded as colony forming units per millilitre (cfu mL^{-1}). Colonies of lactose-fermenting organisms (red or pink colonies) on MacConkey agar plates were also counted and recorded as coliforms (Harrigan and McCance, 1976). 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 and spore staining, catalase, coaqulase, oxidase, citrate utilization, indole production, methyl red, voges proskauer, starch hydrolysis and sugar fermentation (Harrigan and McCance, 1976; Baker and Breach, 1980). Isolates were determined by using standard techniques. Coliforms and *Escherichia coli* were determined on macconkey agar and the pink-red colonies with precipitation were subcultured by streaking. IMViC (indole, methyl red, voges proskauer, citrate) test was performed to identify and differentiate *Escherichia coli* and *Enterobacter aerogenes*. Golden yellow colonies isolated as *Staphylococcus* were inoculated on mannitol salt agar. Coagulase and catalase tests were performed to determine coagulase positive *Staphylococcus*. Then inoculated on DNase agar for the identification of *Staphylococcus auraus*. Oxidase positive colonies were considered for identification of *Pseudomonas aeruginosa* among other tests. Gram and spore stains, cell morphology motility starch hydrolysis, sugar fermentation reactions and nitrate reduction were among the tests carried out for determination of *Bacillus subtilis*, *Lactobaccillus* and *Klebsiella*.

Treatment of Zobo Samples with Lime Juice

Thirteen lime fruits were surface-sterilized (70% ethanol) and peeled using a presterilized knife. The fruits were then halved (using a presterilized knife) and the juice squeezed aseptically (sterile gloves worn during operation) into sterile 100 mL conical flasks (Efiuvwevwere and Oyelade, 1991). 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 h. Ten fold dilution of the zobo drink sample was obtained and 0.1, 0.5, 1.0, 1.5 and 2.0 mL of lime juice were added, respectively to each test tube of the ten fold dilution. The mixture was allowed to stand for 6 h at 30°C. Thereafter, from each treatment 0.1 mL was inoculated onto triplicate plates of nutrient agar and macconkey agar and incubated at 30 and 35°C, respectively for 48 h. The colonies were counted and recorded as colony forming units per milliliter (cfu mL^{-1}). Data obtained were subjected to the analysis of variance using statistical analysis software.

RESULTS AND DISCUSSION

Table 1 showed that the total viable counts obtained from zobo drink sample was 2.79 $\log_{10}$ cfu mL^{-1} while the total coliforms was 2.62 $\log_{10}$ cfu mL^{-1}. Bacterial isolates were *Staphylococcus aureus*, *Lactobacillus* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* *Enterobacter aerogenes*, *Klebsiella* sp. and their percentage occurrence is shown in Table 2. Table 3 showed the total coliforms and total viable counts obtained after the treatment with different volumes of lime juice. The result revealed a steady decrease in the number of bacteria in zobo drink samples following treatment with lime juice.

The effect of lime juice concentrations on the bacterial quality of zobo drinks was investigated with a view to prolong the shell-life of zobo drinks. Since the zobo drinks deteriorate rapidly, may be due to microbial activities, especially when not refrigerated, the major problem is therefore how to preserve the drink. Bacteria isolated from zobo drink samples in this study (Table 1 and 2) included *Staphylococcus aurens*, *Escherichia coli*, *Bacillus subtilis*, *Psendomonas aeruginosa*, *Lactobacillas* sp. In another study, Amusa *et al*. (2005) reported that hawked zobo drinks harbored similar bacteria including *Streptococcus* and *Proteus* species. The presence of these bacteria in zobo drinks is therefore no longer in doubt but studies must be pursued towards reducing the bacterial load. This may be explained by the microbial quality of ingredients used and personal hygiene.

The occurrence of the different types of bacteria in zobo drinks is of public health importance. The result obtained indicated that isolation of *Staphylococcus aureus* from zobo drinks occurred frequently followed by *Escherichia coli* and other bacteria. The presence of these bacteria indicated possible contamination of the drink. *Staphylococcus aureus* in zobo drink could possibly be 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) Generally *Escherichia coli* is an indicator of water pollution (Hurst *et al*., 2002) and therefore, the presence of the organism in zobo drink is probably related to the sources or quality of water used for processing. In addition *Escherichia coli* isolated from water may have some health implications (Nwachukwu and Otokunefor, 2002).

Table 1: Total Coliforms (TC) and Total Viable Counts (TVC) Zobo drink samples

Organisms	No. of samples	Microbial counts ($\log_{10}$ cfu mL^{-1})
Total coliforms	20	2.62
Total viable counts	20	2.80

Table 2: Percentage occurrence of bacteria in Zobo drinks samples

Organisms	Occurrence of bacteria (%)
Bacillus subtilis	23
Enterobacter aerogenes	32
Escherichia coli	40
Klebsiella sp.	26
Lactobacillus sp.	37
Pseudomonas aeruginosa	30
Staphylococcus aureus	45

Table 3: Total coliforms and total viable counts after treatment of zobo drink samples with lime juice

Lime juice conc. (mL)	Total coliforms (cfu mL^{-1})	Total viable counts (cfu mL^{-1})
0	4.2×10^2	6.2×10^2
0.1	1.8×10^2	3.1×10^2
0.5	0.5×10^2	1.6×10^2
1.0	0.1×10^2	1.6×10^2
1.5	0	0.6×10^2
2.0	0	0.1×10^2

Moreover additives which were incorporated into the zobo drinks after extraction may be source of contamination. Furthermore, packaging materials which probably were not properly sterilized as well as containers and soil particles or the environment can serve as a source of additional microbial contamination of the zobo drinks (Frazier and Westhoff, 1995).

The result of the treatment of the zobo drink samples with different concentrations of lime juice (Table 3) reviewed that the bacterial load of the drink was reduced considerably. This finding therefore, suggests that the addition of lime juice, at appropriate concentrations, 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 tend to inhibit bacterial growth.

Therefore the addition of lime juice should be encouraged since this study has reviewed that lime juice can inhibit bacterial growth in zobo drinks. Moreover lime fruits are not hazardous and hence safe for human consumption. Very few studies have been carried out on the shelf-life of zobo drinks. Fasoyiro *et al.* (2005) determined the effect of three storage conditions and reported that the microbial load of samples at ambient and refrigeration increased with time. In there study no preservatives was used. The use of lime juice for this study was simply due to the reason that lime juice is acidic in nature and most micro organisms do not strive in acidic medium. How ever since few organisms such as fungi can survive acidic medium further studies should be carried out to test the effect of lime juice on fungi. There is also the need to investigate other natural preservatives especially of plant origin on the microbial quality of drinks. The result of the effect of lime on quality of zobo drinks is a finding that will be useful. Since zobo drinks is easy to produce at home, packaged in polyethylene containers and sold as source of income for most families, lime juice as preservative is recommended so as to prolong the shelf life. 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. Potable water should be used during processing to avoid bacterial contamination of the drink. Producers of zobo drinks 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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