

**Bacteriological Quality of locally-produced  
Sorrel Beverage (Zobo) Vended in Awka  
Campus of Nnamdi Azikiwe University Awka,  
Nigeria**

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## **ABSTRACT**

*The bacteriological quality of locally-produced sorrel beverage (Zobo) vended in Awka Campus of Nnamdi Azikiwe University, Nigeria was studied. Nutrient agar was used as the growth medium while the spread plate technique was employed in the isolation of the bacteria. A sample aseptically produced at the microbiology laboratory of the University was used as the control. The pH of the vended and control samples were also determined. The bacteria counts ranged between 10 and 30 cfu/ml while the control sample had no bacterial in it. Sample 4 had the highest count of 30 cfu/ml while samples 2 and 3 had the lowest counts of 10 cfu/ml. The pH of the vended samples ranged between 2.2 and 2.4 while that of the control sample was 2.1. The bacteria isolated from the samples were characterized and identified on the basis of their morphology and biochemical characteristics as Pseudomonas spp, Escherichia spp, Proteus spp and Klebsiella spp. Pseudomonas spp, Escherichia spp and Proteus spp were most frequently isolated in the samples examined. The bacteria may have entered the samples through the air, soil, water, packaging containers and handlers and have been reported to cause diseases of man and animals through the production of toxins. Good manufacturing practices must therefore be observed during the production, packaging and handling of Zobo beverage. The incorporation of effective food preservatives into the drink is also recommended.*

**Keywords:** *Bacteriological Quality, locally-produced, sorrel beverage, Awka Campus, Nnamdi Azikiwe University.*

## INTRODUCTION

Roselle (*Hibiscus Sabdariffa*) is an annual, erect, bushy, herbaceous plant species from the family Malvaceae. It has smooth or nearly smooth, cylindrical and tropically red stems, with a green or red coloured stalk and a red or pale yellow calyx that is edible (1, 2).

Roselle is widely grown in almost all the warm countries such as India, Malaysia, Saudi Arabia, Thailand, Egypt, Mexico and in the North Eastern and Middle Belt regions of Nigeria (3,4,5). The most exploited part of a roselle plant is its calyx. Calyxes are used for the preparation of herbal drinks, cold and warm beverage and jellies (1,6) due to their brilliant red colour and unique flavor. In Nigeria, the dried roselle calyxes are prepared into a nutritious refreshing drink called "Zobo". It is locally called "Zoborodo" by the Hausas, "Isapa" by the Yorubas and Sorrel in English. It serves as a source of income for many women.

The calyxes of *Hibiscus sabdariffa* have been found to be rich in vitamins, carbohydrates, proteins, minerals, flavonoids and other antioxidants (1,6,7,8,9) and are good in reducing hypertension (10,11), serum cholesterol levels (12), treatment of urinary bladder stones, heart diseases, cough, bronchitis, sores and wounds (13,14). They have also been reported to inhibit the in vitro growth of *Staphylococcus epidermidis* (15).

Zobo is an indigenous non-alcoholic drink made from a hot water extract of roselle calyxes. The extract is usually sweetened with sugar and may be flavoured with other materials such as pineapples. The drink is very popular in Nigeria because of its medicinal value (16,17) and is commonly hawked in market places, motor parks, schools, hospitals and other places in already used plastic containers. The dried calyxes are susceptible to deterioration by food-borne microbes which can lead to reduction in quality of the drink in terms of colour, taste and nutrition.

One of the greatest limitations for large scale production of Zobo is its rapid rate of deterioration. The drink deteriorates after twenty four hours of its production if unrefrigerated. Other challenges include the unhygienic conditions employed during

the production process, making the product to be of unpredictable bacteriological quality (18). Bacterial spoilage of Zobo drink results to changes in its composition and appearance as a result of growth and metabolism (19). The characteristics associated with the spoilt drink include changes in taste, colour, development of foul odour and increased turbidity.

The bacterial species commonly isolated from Zobo beverage include pseudomonas, Escherichia, Streptococcus, Staphylococcus and Bacillus (20). The presence of these organisms in the drink indicates unhygienic practices during preparation and calls for public concern in view of the health risks they may pose to consumers.

In view of the fact that this drink is consumed by many staff and students of the University, it is pertinent that the bacteriological quality be known, hence in this work, the bacteria in Zobo drinks sold in the Awka Campus of Nnamdi Azikiwe University, Nigeria were isolated, counted, characterized and identified.

## **MATERIALS AND METHODS**

### **Samples Collection:**

Samples were purchased from four different Vendors in the Awka campus of Nnamdi Azikiwe University, Nigeria. A control sample was produced in the laboratory of the Department of Applied Microbiology and Brewing of the same University.

### **Preparation of the control sample**

Fresh calyxes of the roselle (*Hibiscus sabdariffa*) were purchased from Eke Awka market in Awka, Anambra State, Nigeria and transported immediately to the laboratory, where they were washed, boiled in hot water for fifteen minutes and sieved under aseptic condition into sterile flasks and sweetened with pineapple flavour.

### **Isolation of bacteria from the samples**

One millilitre of each of the samples was transferred into sterile petri dishes containing molten nutrient agar and ketoconazole at concentration of 0.05mg/ml to inhibit fungal growth. The samples were spread on the agar surface using sterile glass spreaders after which they were incubated at room temperature for 24 hours.

The colonies that developed were counted and purified by repeated subculturing and later stored on nutrient agar slants for characterization and identification.

### **Characterization and Identification of the isolates**

The colonial morphology of the isolates was observed. The tests carried out were Gram staining, catalase, oxidase, motility, citrate utilization, indole, methyl red, voges proskauer and sugar fermentation tests. They were identified following the scheme of Holt et al (21).

### **pH Determination**

This was done using a pH meter that was standardized with a pH buffer. The meter was inserted into each of the samples and the pH values were taken and recorded.

## **RESULTS AND DISCUSSION**

The bacterial counts and pH of the Zobo drinks are presented in Table 1. The bacterial counts of the vended samples ranged between 10 and 30 cfu/ml while the pH ranged between 2.2 and 2.4. Sample 4 had the highest count of 30 cfu/ml as well as the highest pH of value of 2.4 while samples 2 and 3 each had the least counts of 10 cfu/ml as well as the pH values of 2.2. The control sample had no organisms in it and its pH was 2.1.

The morphological and biochemical characteristics of the isolates are shown in Table 2. The isolates were identified as *Pseudomonas* spp, *Escherichia* spp, *Proteus* spp and *Klebsiella* spp. Only the gram negative organisms were isolated from the samples. The presence of these gram negative organisms may be due to unhygienic practices during the preparation and handling of the drink as well as the use of unsterile containers for packaging after production. This result is in agreement with the work of Nwachukwu et al (16) who reported the presence of *Escherichia* spp, *Pseudomonas* spp, *Proteus* spp and *Klebsiella* spp in their Zobo drink samples.

The occurrence of the bacterial isolates in the Zobo drinks is presented in Table 3. *Escherichia* spp, *Pseudomonas* spp and *Proteus* spp occurred most in the samples studied while *Klebsiella* spp was the organism least encountered.

The producers of the drink admitted to the use of unsterile water to dilute the Zobo drink after boiling. This may be a possible source of contamination of the drink. Other possible sources of contamination include the handlers, the air, as well as the packaging containers which may not have been properly washed or sterilized before use.

The presence of the coliform bacteria *Escherichia* spp is an indication of recent contamination of the drinks with faeces. The organism may have been transmitted to the product through the water used in the dilution of the drinks and this can lead to the outbreak of diarrhoea especially in children less than five years of age. Most of these organisms isolated from the Sorrel drink are known to be the causative agents of food-borne gastroenteritis, therefore adequate hygienic practices must be observed during the preparation and handling of the Zobo drink.

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