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Hazard Analysis and Critical Control Point of Zobo Drinks Sold in Samaru, Zaria, Kaduna State, Nigeria

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ABSTRACT

A hazard analysis of zobo drink preparation was carried out for retailers around Samara, Zaria, Nigeria. This analysis entailed observation of the raw materials and environment of production through a well prepared flow chart, watching all steps of the preparation and packing, recording temperatures during cooking and display and collecting samples of zobo dring for aerobic mesophillic count, fungal count and isolation of probable organisms. The aerobic mesophilic bacteria count increased with time from immediately after collection of the raw roselle leaves and reduced upon boiling, an increase in count was also observed at the stage of addition of flavours and starring which was due to cross contamination. Coliforms, *Eschericia coli* and *Staphylococcus aureus* were isolated, coupled with *Aspergillus* spp, the mean count of bacteria was found to be within the range of the standard given by the ICMSF which is for the bacterial count not to exceed 10^5 cfu/ml or g. Though the level of counts appears safe, the presence of coliforms S. *aureus* and E. *coli*, preparation in a highly contaminated environment and holding at ambient temperature for sale could be risky. Education of producers on the hazards, critical control points and the importance of hygienic environment cannot be overemphasized. The control measures and monitoring procedures for zobo drink preparation are suggested

Keywords: Aerobic mesophilic count; Fungal count; Coliforms; *Escherichia coli*; *Staphylococcus aureus*; *Aspergillus* spp; ICMSF

INTRODUCTION

Hazard analysis and critical control point system is designed specifically for minimizing food safety risk. The HACCP system works best in an environment where certain component of an overall system of food safety related controls are supported as manufacturing practice or good hygienic practice.

Hazard analysis as defined by the national advisory committee on microbial criteria for food is the process of collecting and evaluating information on hazards associated with food or drinks under consideration to decide which are significant. For a hazard to be listed among the significant hazard that should be addressed in the HACCP plan, the hazard should be such a nature that its prevention, elimination or reduction to an acceptable level is essential to the production of a safe product.

The name "zobo" is derived from the local Hausa name for the roselle plant that is "zoborodo". Zobo drink is a local non-alcoholic drink made from hot water extract of the roselle

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calyces. The extract is usually sweetened with ginger, pineapple or strawberry.

In Northern Nigeria, zobo drink is quite popular enjoying patronage at various social gatherings and occasions such as wedding, birthdays, thanksgivings and naming ceremonies. It is also hawked in motors parks, school premises and market places. Its popularity has spread across the entire country with an increase in sale and consumption due to high cost of other available soft drinks whose concentrates constitute a drain in the economy. The non-alcoholic nature of zobo drink makes it readily consumed by both Muslims and Christians.

The different parts of the roselle plant are the leaves, seeds and calyces and these have been used for different purposes such as; vegetables, source of oils, refreshing drinks and food preservatives. The calyces of the rosella plant has been found to contain moisture, protein, fats, ascorbic acid, calcium, niacin, riboflavin, iron, phosphorus, pigment (mainly anthocyanin). These make zobo drink very nutritive and provide a good medium for microbial growth.

Despite the increasing popularity and consumption of zobo drink, due to its nutritional and medicinal values, a great concern is in the way the locally made drink is handled during production in addition to other sources of contamination resulting from processing, packaging and storage conditions that could contribute to the spoilage and bad quality of the final product. Zobo drink is a locally non-alcoholic drink made from the hot water extract of the dried calyx of *Hibiscus sabdariffa* (roselle). It is usually sweetened with ginger, pine apple, and other flavor depending on choice. The drink is quite cheap compared to other bottled soft drinks and is widely consumed for economic and health reasons but its acceptability is still limited because of its short shelf life lund [1-6].

MATERIALS AND METHODS

Collection of samples: The procedure for the production of "zobo" drink was carefully observed and a flow chart was designed. Samples were then collected at different but strategic points in the production chain and these samples that were collected included:

- Dried zobo leaves which are the main raw materials.
- Washed zobo leaves.
- Boiled juice.
- End product after sieving and addition of ingredients and flavour.
- Chilled ready to drink "zobo" drink.

Samples were then transported to the laboratory for microbial analysis (**Figure 1**).

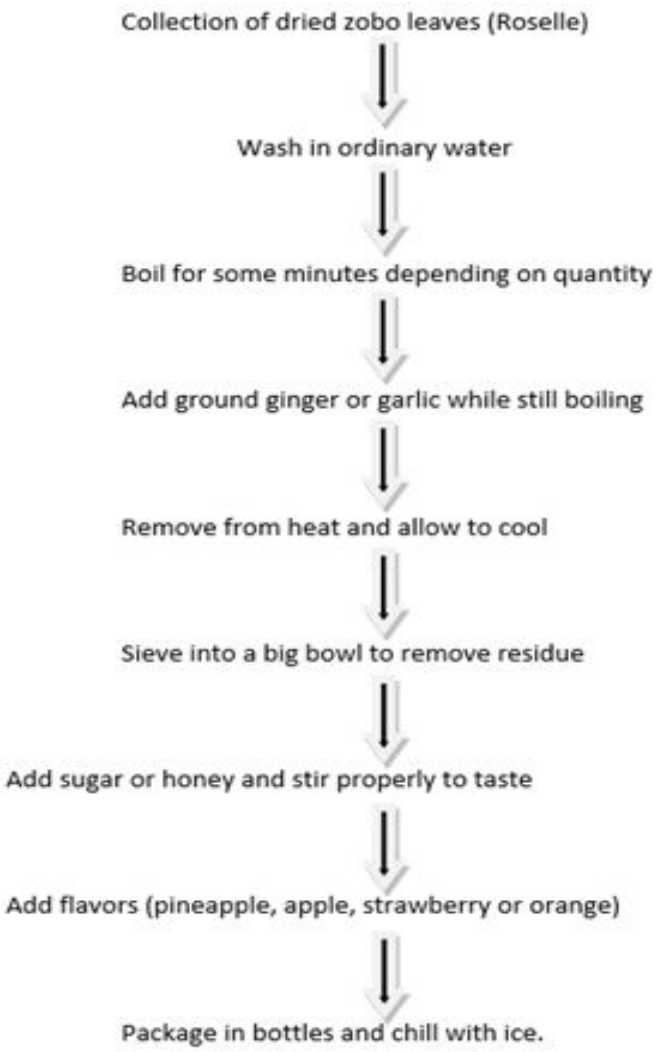

Figure 1: Production of zobo drink.

Total Aerobic Mesophilic and Fungal Counts

One (1) gram of dried zobo leaves was weighed and transferred into 90 ml peptone water, and 10 ml of the liquid samples gotten from the other four strategic stages of the production of the drink was also transferred into 90 ml peptone water to obtain the stock. Serial dilutions were now done on each sample collected from the different strategic

stages in universal bottles (four each for all samples. Up to 10^4 dilutions was made from the stock and 0.1 ml each of the 10^4 dilutions of each of the samples was then transferred using sterile pipettes into labelled sterile petri dishes. Using the pour plate technique as described by Plate Count Agar (PCA was used for bacterial enumeration and Sabouraud Dextrose Agar (SDA for fungal enumeration. The plates inoculated using NA was incubated for 24 hrs at 37°C for bacterial counts while the potato dextrose agar plates were incubated at room temperature for 5 to 6 days. After incubation, the colonies on the different were counted and recorded in colony forming units per gram (cfu/g for the first sample and colony forming unit per ml (cfu/ml for the other four liquid samples [7,8].

Isolation and Identification of Bacteria

Pre enrichment: 1 ml of the samples were obtained from the stock and transferred into pre enriched media to induce the growth of the microorganisms to be isolated. Tryptic soy broth was used to pre enrich *E. coli* and peptone broth was used for the pre enrichment of S. *aureus* and they were both incubated at 37°C for 24 hours.

Culture on selective media: After pre enrichment of the organisms, a loop full of the colonies was obtained from the pre enrichment medium and inoculated into selective media (Eosine methylene blue was used for E.*coli* and manittol salt agar for S.*aureus* and incubated at 37°C for 24 hours.

Gram staining: A thin film of each bacterial isolate was prepared by picking a colony of growth from the slant culture onto a clean grease free glass slide using a sterile wire loop and was allowed to air dry before heat fixing it on the bunsen flame. The slides were flooded with crystal violet dye and allowed to stand for about 60 seconds before there were washed off with clean water. Lugol's iodine (mordant was flooded on the slides and allowed to stand for about 30 seconds before it was washed off with water. The smears were decolourized with alcohol decolourizer or acetone and washed off with water. The smears were finally flooded with neutral red dye or safranin dye, allowed to stand for about 60 seconds before they were washed with water and allowed to air dry. The slides were microscopically observed for both Gram positive and Gram negative bacteria, using 100x objective.

Biochemical Characterization of Isolates

Citrate utilization test: The test organisms were picked with a sterile wire loop and stabbed into a slant of prepared Simmon's citrate agar in a bijou bottle and the bottle was incubated at 37°C for about 24-48 hours; space allowed for oxygen penetration through the cover. A colour change from green to bright blue indicated a positive test reaction and otherwise was a negative reaction.

Indole test: A colony of the test organism was picked with a sterile wire loop and inoculated into a bijou bottle containing about 3 ml peptone water, mixed and incubated at 37°C for about 24-48 hours. After incubation, the 0.5 ml of kovac's reagent was added and mixed. A ring red colour layer reaction within 10 minutes interval indicated a positive test reaction and otherwise was a negative test reaction.

Methylred-Voges Proskauer test: The test organisms were isolated and cultured in 10 ml of Methyl Red-Voges Proskauer (MRVP broth and incubated at 37°C for 48 hours. After incubation, 5 ml of the broth was transferred into serological tubes. To one tube, 5 drops of methyl red indicator was added and mixed properly and the development of an intense red colour indicated a positive test reaction, while otherwise indicated a negative test reaction. To the other tube, 5 drops of 5% alpha naphthol in ethanol and 5 drops of 40% potassium hydroxide were added and the tube was shaken gently and placed in a sloping position. The development of a red colour starting from the liquid air interface within 1 hour indicated a positive test reaction and otherwise indicated a negative reaction.

Catalase test: A drop of 3% hydrogen peroxide was placed on a clean glass slide and the test organisms (bacterial isolates were emulsified in it using a sterile wire loop. The slide was observed for gas bubbles which indicated a positive test reaction and otherwise indicated a negative test reaction.

Coagulase test: A drop of physiological saline was dropped on a clean glass slide and the test organisms were emulsified in it. A loop full of human plasma was added to the smear and the slide was tilted for about one minute. The clumping of the isolate indicated a positive test reaction while otherwise indicated a negative test reaction.

Isolation and Identification of Fungi

Culture method: 1 ml each of the samples was inoculated on Sabouraund Dextrose Agar (SDA and kept at room temperature for 5 days. It was the observed there after to check for its appearance on the plates and the mycelial growth of the organism on the culture medium.

Wet mount: Wet mount of the fungi isolates was prepared by emulsifying a portion of the isolates in a lacto phenol cotton blue on a clean glass slide. A cover slip was then placed on it to prevent penetration of air.

Microscopic observation: After wet mount has been made on the isolates it was now taken for observation under the microscope. The isolates were observed under the X10 and X40 objectives. The organism present would now be confirmed with the aid of a fungal atlas.

RESULTS

Preparation of zobo drink by retailers in Samaru, Zaria (**Figure 2**.

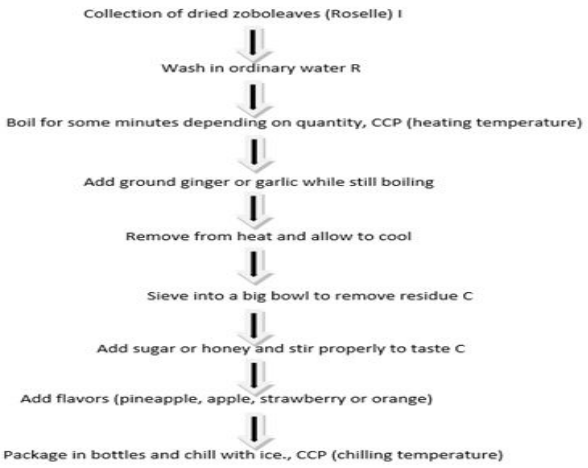

Figure 2: Stages involved in the production of zobo drink.

Where,

I: Initial spores present;
R: Reduction of microbial load after washing;
C: Cross contamination from kitche utensils used to sieve, stir and package;
CCP: Critical Control Point.

The result gotten shows no growth at the boiling state indicating a critical control point while there were high counts at the initial state before processing and a remarkable increase in count in the states of stirring and addition of flavor (**Table 1**).

Table 1: Total fungal and aerobic mesophilic counts of samples collected at different stages along zobo production chain.

Sample	Operation/Stage	AMC (CFU/ml)	FC (CFU/ml)
Dried zobo drink	Immediately after purchase	1.37×10^5	TNTC
Wet zobo leaves	After washing	2.1×10^2	2.0×10^4
Boiled extracted	After boiling Juice the leaves	No growth	No growth
Zobo drink	After adding water, flavours, stirring and sieving	1.0×10^2	1.0×10^2
Chilled zobo	After packaging and chilling for some time with ice	1.1×10^2	No growth

Key: AMC: Aerobic Mesophilic Count; FC: Fungal Count

This result revealed the organisms isolated from this study: Which are *Eschericia coli*, *Staphylococcus aureus* and *Aspergillus* SPP respectively (**Table 2**).

Table 2: Biochemical characterization of bacteria and morphological characterization of fungi isolated from zobo drink prepared at retail outlets in Samaru, Zaria.

Samples	IND	CIT	MR	VP	CA	CO	Fungal morphology	Inference
Dried zobo leaves	+	-	+	-	+	+	Black colour/ fluffywith reverse creamy colour	*E. coli, S. aureus Aspergillus* spp
Wet zobo leaves	+	-	+	-	+	+	Black colour/ fluffywith reverse creamy colour	*E. coli, S. aureus Aspergillus* spp

Boiled extracted juice	No growth on both NA and SDA							
Zobo drink	+	-	+	-	+	+	Black color/ fluffy with reverse creamy color	E. *coli*, S.aureus *Aspergillus* spp
Chilled zobo	+	-	+	-	+		No growth on SDA	E. *coli*, S. *aureus*

Key: EMB: Eosin Methylene Blue; MSA: Manittol Salt Agar; IND: Indole Test ; CIT: Citrate Utilization Test; MR: Methyl Red; +: Positive; -: Negative; SDA: Sabouraud Dextrose; PCA: Plate Count Agar; VP: Voges-Proskauer

DISCUSSION

The presence of microorganisms in food is a direct reflection of the activities carried out during the course of its production, storage, transportation and final packaging of the products. According to the result gotten from this study and in accordance to the flow chart that described the stages involved in the production zobo drink, it was observed that every activity involved in the stages of production affects the microbial load present either by increase or reduction. The result also showed that there were two critical control points in the production chain which were during the boiling of the leaves to extract the juice and chilling of the drink for packaging. A critical control point is a point, step, or procedure in a food manufacturing process in which control can be applied and as a result a food safety hazard can be prevented, eliminated or reduced to an acceptable level. At the boiling stage of the drink it was observed that there was no bacterial and fungal growth and at the point of refrigeration there was no fungal growth, but there were few bacterial counts which may have resulted from the packaging materials used. The temperature at which the drink was held during boiling was adequate to eliminate the initial microbial load present at collection and washing of the dried leaves. But there were further introduction of microorganisms at the stage of addition of water to dilute, addition of flavor, sieving and stirring of juice. This is as a result of the unsterilized utensils used at this stage and the various ingredients/flavors introduced to the drink that could have brought about cross contamination. The presence of Escherichia coli in the drink indicates the presence of faucal contamination as a result of the water used in the production of the drink; this has a great effect because most producers of zobo drink have no access to potable water supply and as a result they have no other choice than to use the available water which may be unsafe. The presence of *Staphylococcus aureus* also poses a great threat and this could result from the poor hygiene of the food handlers and as a result these organisms are introduced. The presence of *aureus* is very is of great complications because of its ability to produce potent toxins which results in food intoxication, food poisoning and food borne illness to the consumers of this product. It is also important to note that there was also presence of *Aspergillus* spp in the drink, but it was not isolated from the final product. The microorganisms isolated from this study was in agreement with the work of Peter and Elgba who isolated *Escherichia coli*, *Staphylococcus aureus*, *Bacillus sub ilis*, *proteus* spp and *Aspergillus niger* from zobo drinks. However, Osotogun and Abaoba also reported that the microorganisms found in association with zobo drink samples they worked on were *Aspegillus* spp and *Trichoderma* spp and that no bacteria was found in zobo drink samples which is an indication that the work was of little contrast with this study because there were presence of bacteria in association with fungi according to the results of this study on the other hand isolated *Aspergillus* spp, *Saccharomyces cerevisiae*, *Bacillus sub ilis*, *Esherichia coli* and *Staphylococcus aureus* from fermented zobo drinks which is also in agreement with this study because some of the microorganisms they isolated were also isolated from this study. Some of the bacteria isolated from this study may be part of the normal flora associated with the raw materials used to produce the drink and the unsterilized utensils used. These further highlights the need to safeguard the health of consumers by proper washing and contamination of raw materials and utensils used in the production of the drink despite the microbial counts and isolates obtained from the samples used in this study, it is important to note that the samples showed no visible signs of spoilage. Thus outward appearance alone may not be a reliable criterion for ascertaining the microbial quality of a product. The main concept of hazard analysis and critical control point is that if knowledge can be gotten about how a product can become unsafe if consumed, the control measures can be developed to prevent and detect such failures and keep food that presents an unacceptable risk from reaching consumers. As such the result gotten from this study has given information about the hazards associated with the production of zobo drink and the control measures to prevent these hazardous products from reaching consumers are hereby encouraged [9-12].

CONCLUSION

According to the result of this study microorganisms present in retailed “zobo drink” sold in samaru, Zaria were isolated and identified to be *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus* spp. The mean count of bacteria was found to be within the range of the standard given by the ICMSF which

is for the bacterial count not to exceed 105 cfu/ml or g. The presence of both bacteria and fungi indicates poor personal and public hygiene measures, ingredients and equipment's used in the course of production of the drink as well as poor handling processes.

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