Microbial Profile of Commercially Sold Zobo Drink (Hibiscus Sabdariffa) In Auchi, Edo, Nigeria.

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Abstract

The microbiological assessment of different zobo drinks sold in Auchi and Benin metropolis, was carried out using the pour plate technique. Five zobo samples were randomly bought from five retail sellers in the market. Therefore, one gram (1g) of each sample were weighed out and subjected to a 10-fold serial dilution. A measured volume was then inoculated into Nutrient agar (NA), MacConkey agar (MCA), De Man-Rogosa and Sharpe agar (MRS) and Potato - Dextrose agar (PDA) using the pour plate methods for total aerobic plate count, lactic acid bacteria (LAB) and fungal count (FC) respectively. The mean total ranged from $9.0 \times 10^7 - 2.6 \times 10^9$ cfu ml^{-1} while fungal count ranged from no growth to $0 - 7.74 \times 10^7$ cfu ml^{-1} in Auchi and Benin markets respectively. A total of eight organisms were identified in the market samples using their morphological characteristics on the nutrient agar plate. The isolates of bacteria from all the markets includes the following Streptococcuc Spp, Corynebacterium Spp, Bacillus Cereus, Bacillus Subtilis, Bacillus Megaterium, Micrococcus Luteus, Bacillus Polymyxa, Staphylococcus Aureus, while the isolated fungi include Penicillium Spp, Aspergillus Flavus, Aspergillus Niger, Yeast cells. To guarantee food safety and consumer protection, the producers and merchants in Estako West LGA of Edo State are strongly advised to use additional additives and maintain a hygienic processing environment. Sterilized packing materials are also advised.

Keywords: Pathogens, Microbial quality; local beverage; Proximate analysis; public health.

1. Introduction

Zobo drink is a common street drink across Nigeria and West Africa. Many research efforts have been done towards the proof of the use of Hibiscus Sabdariffa plant species in medicinal treatments in recent years. It has been studied as a plant with many health benefits (Sabzghabaee et al., 2013). Hibiscus Sabdariffa is relatively easy to grow and can be grown as a part of a multi-cropping system (Mohammed et al., 2021). In China the seeds are used for oil and the plant is used for its medicinal properties (Li et al., 2019, Tseng et al., 2000), while in West Africa the leaves and powdered seeds are used in meals, pharmaceutical and food industries (Adegunwa, M.O et al., 2018; Oboh, G. et al., 2007).

Hibiscus Sabdariffa has over three hundred species known to man (Faddegon Inc). It is an annual dicotyledonous herbaceous shrub belonging to the plant family Malvaceae (Duke, J.A 1983). Popularly known as ‘gongura’ in Hindi and ‘roselle’ or ‘sorrel’ in English (Ochani and D’Mello, 2009; Satyavati et al., 1987; Ross 1999). It is harvested from late November onwards. The chemical sensory properties of hibiscus beverages (zobo drink) are largely dependent on the methods of harvest, raw material quality and processing variables. Calyx drying methods particularly plays a very important role (Ramirez-Rodriguez et al., 2011; Plotto et al., 2004). In Africa, shelled calyxes are often spread over mats or plastic sheets placed on the ground (Ana et al., 2017). This allows contact with different materials and its quite exposed.

In Nigeria and other West African countries, zobo drink is a locally produced beverage. It is prepared through indigenous methods from plant (Roselle) flowers by untrained individuals without carrying sanitary procedures in food handling and production. Zobo drink is preferred by people compared to carbonated drinks because it is rich in natural carbohydrates, protein, anti-oxidant, vitamin c, calcium, magnesium and zinc. It is non -alcoholic, medicinal and has a low glycemic index (Wong P, 2002; Oboh et al., 2011). Zobo is

economically affordable and attractive to many people more than soda hence its acceptability in social gathering and commercial consumption (Olayemi et al., 2011).

The need to increase sustainability and value to traditional food that can potentially claim health benefits in both domestic and foreign markets has led to the implementation of several research projects in countries where production of hibiscus (zobo drink) is important, namely; Nigeria, Mexico and Senegal (Cisse et al., 2012; Diessana et al., 2015; Perez-Ramirez et al., 2015; Ramirez-Rodriguez et al., 2011). While these projects aim to study the physic-chemical and phytochemical composition of calyx extracts, the optimization of consumer acceptance of hibiscus beverage has scarcely been undertaken, particularly in recent years (Babajide et al., 2005; Bechoff et al., 2014; Mounigan et al., 2006; Ramirez et al., 2010; Wong et al., 2003).

The consumer health and acceptance of zobo drinks are largely determined by raw material quality and processing variables. Commercially sold zobo must have undergone cross contamination during the preparation and handling (CDC, 2021). The process of drying the calyx must have made it susceptible to diseases and microorganisms (Salami and Afolayan, 2020) like *Steptococcus Spp*, *Corynebacterium Spp, Bacillus Spp, Micrococcus Spp, Staphylococcus Aureus* and Fungal contaminants from water or materials used during production, like Penicillium Spp, Aspergillus Spp, Yeast cells. Regarding this matter, the studies objectives were to determine the microbial quality of control of Hibiscus Sabdariffa (zobo drink) locally produced in Auchi, Edo State, determine the characterisation of bacteria associated with Hibiscus Sabdariffa (zobo drink), proximate analysis and hygiene of zobo vendors and producers in Auchi, Edo State, Nigeria.

2. Methods and materials

2.1. *Sample collection*

Five samples of zobo were purchased from Uchi market, Eboreime market, Poly Road and Sabo in Etsako West LGA and New Benin market in Benin City. The samples were put into a clean container and taken to the laboratory for immediate analysis.

2.2. *Reagents*

The reagents that were used during this experiment were peptone water, Nutrient Agar, MacConkey agar, De Man-Rogosa and Sharpe agar and Potato - Dextrose agar.

2.3. *Preparation of stock culture*

1g of each sample was weighed using a weighing balance into a sterile beaker and steeped with 9 ml of distilled water. Each of the beakers were sealed with aluminium foil and were allowed to stand for 5-10 minutes. These all served as the samples' stock cultures.
1ml of the extract from each of the samples were transferred into different test tubes each containing 9 ml of distilled water.

2.4. *Preparation of media*

The media that were used during this experiment were Nutrient Agar, MacConkey agar, De Man-Rogosa and Sharpe agar and Potato - Dextrose agar.
The various media were prepared according to manufacturer's specifications as shown below:
28g of Nutrient Agar, 39g of Potato Dextrose Agar, 50g of Mac Conkey Agar, 67.15g of M.R.S Agar were each dissolved separately in one litre (1L) of distilled water and 20 ml each of the mixtures were dispensed into Bijou bottles and sterilized by autoclaving at 121°C for 15 minutes. The media were allowed to cool at 47°C, mixed well before pouring into the Petri dishes.

2.5. *Isolation and enumeration of microbes (bacteria and fungi)*

2 drops of the diluted sample culture were pipetted into the Petri-dish, the cool molten medium poured into the Petri-dishes was allowed to solidify. The plate containing nutrient agar was inoculated at a temperature of 37°C for 24 hours, the bacteria colonies developed within this period were counted as a colony forming unit per ml (cfu ml^{-1}) using the colony counter.
The process was repeated for fungi isolation using potato dextrose agar and was inoculated at room temperature for 48-72 hours, the fungi colonies developed within this period were counted as a colony forming unit per ml (cfu ml^{-1}) using the colony counter.

2.6. *Identification of microbes*

Culture plates were examined. The isolates were identified using their colonial morphological and biochemical reaction for bacteria. The process went through sub-culturing to get more isolates and finally the pure isolates were cultured in agar slant each.

2.6.1. *Morphological test*

- Gram Staining.
- M.R.S test is a selective agar that allows only growth of Lactobacillus Spp.
- MacConkey Agar Test is a selective agar that allow only the growth of lactose fermenter.

2.6.2. *Biochemical test*

- The catalace test degrades hydrogen peroxide and releases oxygen which is detected as effervescence.
- Oxidation fermentation test identifies the oxygen requirement of the organism.

3. Results

Table 1. Enumeration / counting of colonies

Samples	Bacteria	Fungi
A. Eboreime	6.3×10^8 cfu ml^{-1}	2.5×10^5 cfu ml^{-1}
B. Uchi	9.0×10^7 cfu ml^{-1}	2.13×10^8 cfu ml^{-1}
C. Sabo	5.3×10^8 cfu ml^{-1}	2.5×10^5 cfu ml^{-1}
D. Benin	2.6×10^9 cfu ml^{-1}	7.75×10^7 cfu ml^{-1}
E. Poly Road	1.0×10^8 cfu ml^{-1}	Nil

Table 2. Morphological and Biochemical Characteristics of Bacteria Isolated from The Preparation

Isolate No	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
Gram reaction	+	+	+	+	+	+	+	+	+	+
Catalyst test	_	_	+	+	_	+	+	+	+	_
MC Test	1FL						1FL			
M.R.S Test							8FL		9FL	

Interpretations: Positive (+), Negative (−), Lactose Fermenter (LF)

Table 3. Biochemical characteristics of bacteria / fungi isolated from the preparation

Bacteria Isolate	Identified Bacteria	Bacteria Oxidation Fermentation	Fungi Isolate	Identified Fungi
A1	Streptococcus Spp	Facultative Anaerobic	A3	
A2	Corynebacterium Spp	Anaerobic	A4	Penicillium Spp
B1	Bacillus Cereus	Aerobic	B3	
B2	Bacillus Subtilis	Facultative Anaerobic	B4	
C1	Streptococcus Spp	Aerobic	C3	
C2	Bacillus Megaerium	Aerobic	C4	Aspergillus Flavus
D1	Micrococcus Luteus	Facultative Anaerobic	D3	
D2	Bacillus Polymyxa	Aerobic	D4	Aspergillus Niger
E1	Staphylococcus Aureus	Facultative Anaerobic	E3	
E2	Streptococcus Spp	Facultative Anaerobic	E4	

Table 4. Cultural characteristics of bacteria isolates

Isolate	Microorganism	Cultural Characteristics of Bacteria Isolates
A1	Streptococcus Spp	For small creamy round
A2	Corynebacterium Spp	The small round creamy
B1	Bacillus Cereus	Big transparent
B2	Bacillus Subtilis	Big transparent
C1	Streptococcus Spp	transparent
C2	Bacillus Megaerium	Big creamy round
D1	Micrococcus Luteus	Big round, creamy colour
D2	Bacillus Polymyxa	Big round, creamy colour
E1	Staphylococcus Aureus	One big dull round
E2	Streptococcus Spp	Small dull round

Table 5. Percentage distribution of micro-organism isolated for bacteria

Micro-organism	A	B	C	D	E	Total	Percentage 100%
Streptococcus Spp	4		10	1	1	16	36.4
Corynebacterium Spp	2					2	04.5
Bacillus Cereus		2				2	04.5
Bacillus Subtilis		3				3	06.8
Bacillus Megaterium			9			9	20.5
Bacillus Polymyxa				4		4	09.1
Micrococcus Luteus			5			5	11.4
Staphylococcus Aureus				2	1	3	06.8

Table 6. Percentage distribution of micro-organism isolated for fungi

Micro-organism	A	B	C	D	E	Total	Percentage 100%
Penicillium Spp	3		20			20	56.10
Yeast Cells		4				4	09.75
Aspergillus Flavus		9				9	21.95
Aspergillus Niger	1			3	1	5	12.20

The result obtained from the study, Table 1. shows the colony count derived from the study, while Table 2, Table 3 and Table 4. shows the cultural, morphological and biochemical characteristics of the bacteria and fungi isolated from the preparation.

Further results obtained from the study revealed that all 5 (100%) of the samples analyzed showed growth presumed to be pathogenic bacteria. The occurrence of *Streptococcus Spp* was significantly higher (36.4%), followed closely by *Bacillus Megaterium* (20.5%) and *Micrococcus Luteus* (11.4%), followed by *Bacillus Polymyxa* (09.1%). *Staphylococcus Aureus and Bacillus Subtilis* both had (06.8%) respectively. Similarly, *Corynebacterium Spp* and *Bacillus Cereus* both had the lowest percentage (04.5%) (Table 5)
However, fungi growth was also seen to be present. *Penicillium Spp* (56.10%), *Aspergillus Flavus* (21.95%), *Aspergillus Niger* (12.20%) and Yeast cells (09.75%) (Table 6). The percentage of bacteria and fungi isolated were calculated separately.

4. Discussion

Zobo is a non-alcoholic drink prepared and consumed in large quantities in Auchi metropolis and Nigeria as a whole. It is widely accepted by different groups in Nigeria regardless of religion and ethnicity, hence it's been produced to supplement soft drinks and soda. The production system is sometimes done under unhygienic conditions (Oranusi et al 2003) with no agency to monitor the microbial quality and safety.

Zobo is produced under high temperature as the flowers are boiled above 100°C. This was enough to eliminate the isolated organisms found in the samples. The contamination was thus post processing during handling and packaging rather than during processing. Factors such as poor hygiene, improperly washed / cleaned food containers, contact surfaces and equipment, dirty environment, presence of animals in the cooking / packaging environment and source of water may have contributed to the contamination of the zobo drink (Oranusi et al., 2007).

The study revealed the presence of pathogenic microorganisms which are unsafe to public health. The occurrence of *Bacillus Spp* is of concern to public health and may be as a result of other ingredients added post production. Human life is at stake due to the isolation of these microorganisms. The isolation of yeast cells may be linked to contamination through air and dust, contaminated packaging material or poor hygiene and sanitation of the processing environment (El-Kholy et al., 2014). More importantly, is the fact that yeast can produce mycotoxins which may cause mycotoxicosis in humans.

5. Conclusion

Therefore, it is advised that in order to prevent microbial contamination, zobo drinks be prepared carefully. To prevent microbiological contamination, treated municipal water or pure water should be used for the processing and dilution of the processed drinks.

Spices like ginger and garlic should also be added to the processed zobo. Hygienic conditions should be maintained in the processing area, and additions like sugar, ginger, and garlic should all be adequately sanitized in addition to the packaging materials.

This beverage should be refrigerated for preservation and the upkeep of its organoleptic qualities due to its short shelf life and rapid deterioration and spoilage. To prevent microbiological contamination during the processing and dilution of the processed drinks, treated municipal water or pure water should be used.

6. Recommendations

- The water used in preparing zobo drinks must be potable and treated with chemicals to destroy every disease-causing bacterium.
- Adequate sanitary measures must be adopted/practiced during the processing and handling of the zobo drink to limit or prevent the introduction of bacteria.
- Regulatory agencies such as the National Agency for Food, Drug Administration and Control (NAFDAC) and Health Agencies should be mandated to regulate the sales and distribution of zobo drinks in helping stop food-borne illness outbreaks.
- It is highly recommended that zobo drinks be properly processed to avoid microbial contamination
- Quality control measures (albeit difficult) should be taken to ensure that zobo drinks are safe and free of pathogens before human consumption

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