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Investigation of Bacteria Associated with the Spoilage of Zobo Drink Fortified with Scent Leaf and Ginger

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Authors' contributions

This work was carried out in collaboration between all authors. Author FOI designed the study and wrote the protocol. Author AMO reviewed the experimental design and all drafts of the manuscript. Author OKA performed the statistical analyses and wrote the first draft of the manuscript. Author OEG handled the laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at investigating the bacteria responsible for the spoilage of "zobo" a Nigerian non-alcoholic beverage fortified with scent leaf and ginger.
Methodology: The plant materials were air dried and grinded mechanically then incorporated into the zobo milieu produced using traditional procedures. Afterwards, the samples were stored at room temperature and in the refrigerator for 5 days and microbial analyses carried out on the samples at 24 hours intervals using pour plate technique.
Results: The microbial load of the various zobo samples during storage revealed that there was no significant difference ($p \leq 0.05$) between the counts encountered on all the samples as the count ranged from 3.10 x 10^3cfu/ml (refrigerated sample) to 4.02 x 10^3cfu/ml (control) on day 0. There was a significant ($p \leq 0.05$) increase in the bacterial load on the control sample while those fortified with ginger and scent leaf respectively remained fairly constant throughout the storage. Furthermore, six bacteria species were isolated from the freshly prepared zobo and during storage, they were *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris,*

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Streptococcus species and *Escherichia coli*. Out of these, *S. aureus, P. vulgaris* and *B. subtilis* persisted in the stored zobo samples during the storage period.
Conclusion: From the foregoing, there is can be safely concluded that *S. aureus, P. vulgaris* and *B. subtilis* are the main spoilage organisms of zobo drink and ginger as well as scent leaf may not be suitable to curtail the spoilage that may be caused by these organisms in zobo drink.

Keywords: Zobo drink; fortification; bacteria; spoilage; storage.

1. INTRODUCTION

Zobo drink is a Nigerian non-alcoholic local beverage made from dried calyces of the flower of *Hibiscus sabdariffa* by boiling and filtration [1,2]. The red succulent calyx is boiled with sugar to produce the local nutritious drink [3]. The drink have been found to be rich in vitamins, natural carbohydrate, protein, vitamin C and other antioxidants [4]. Various medicinal uses of the drink have been reported such as being a diuretic, cholerectic, febrifugal, hypertensive, anti-helminthic, and antimicrobial, decreasing viscosity of the blood and stimulating intestinal peristalsis [5].

Demand for Zobo drink is largely based on its nutritive value, flavor, aroma and colour [6]. More importantly, its consumption may takes an active role in bone and teeth formation as it is a rich source of vitamin C, calcium, magnesium and zinc [7]. Oboh and Obahiagbon [8] found the glycemic index of Zobo drink to be 33 ± 3 which consequently made it possibly suitable for maintenance of normal blood sugar, weight reduction in athletes due to its low glycemic index. There are changes in beverage consumption patterns over the past several decades which may be related to the high prevalence of obesity related diseases [9,10] and it is believed that Zobo may displace other carbonated beverages in the market, due to benefits derived from it which is lacking in other beverages taken for their thirst quenching properties and stimulating effect [11].

The economic and religious situation in Nigeria has also made the Zobo drink gain wide acceptance in different occasions. It is used as refreshment, entertainment in parties or as appetizer before the main dish is served and it is also sold in market to various consumers [12]. The Zobo drink is prepared by boiling the dry calyces of *H. sabdariffa* in water (80ºC) for about 10-25 min from which the pigment or flavor embedded juice is extracted. After extraction, the filtrate may be taken hot as tea or allowed to cool.

Zobo drink has become a household name in almost every Nigerian home in recent times. There is increase in the demand for Zobo drinks due to its low prices, nutritional and medicinal properties [13]. It is gaining wide acceptance, being consumed by several millions of people from different socio-economic classes and background.

Ocimum gratissimum belongs to the family Leguminocoeae commonly known as “alfadaca” it is naturally used in the treatment of different diseases which include upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin disease, pneumonia tooth and gum disorder, fever and as mosquito repellants [14]. *O. gratissimum* is found in the tropical and warm temperature regions such as India and Nigeria. The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes*, *Shigella*, *Salmonella* and proteus for fungi *Candida albicans* [15].

Ginger (*Zingiber officinale*) is a flowering plant whose rhizome, is widely used as a spice or a folk medicine. It is a herbaceous perennial which grows annual stems about a meter tall bearing narrow green leaves and yellow flowers [16]. Ginger is in the *family Ziayiberaceae*, to which also belongs to turmeric (*Curcuma louga*), *Cardamom elettaria Cardamomum,* and *galamgal*. Ginger is among the heal theist (and most delicious) spices on the planet earth. It is loaded with nutrients and bioactive compounds that have powerful benefits for the body and brain. It contains gingerolia substance with powerful medicinal properties i.e it has a powerful anti-inflammatory and anti-oxidant effects. Ginger may reduce muscle pain and soreness as well as help to treat chronic indigestion [17].

This study is therefore designed to assess the bacteria responsible for the spoilage of zobo drink fortified with scent leaf and ginger and determine whether these plants can be used as

preservative agents in the prevention of bacterial spoilage of zobo drinks.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials for Zobo Drink Production

The zobo flower and other ingredients used for the zobo drink production were purchased from Oja Oba market in Akure, they were then transported to the Food Processing Laboratory of Department of Food Science Technology, Rufus Giwa Polytechnic, Owo for further analyses.

2.2 Collection and Preparation of Plant Samples

Fresh leaves of *Ocimum gratissimum* was harvested at botanical garden of the Department of Science Laboratory Technology (Environmental Biology unit), Rufus Giwa Polytechnic, Owo while the ginger rhizome was purchased from ojakoko market in owo, Ondo state. The plant materials were then authenticated at the Environmental Biology Unit of Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owoand voucher specimens (OG101L- *O. gratissimum* Leaf and ZO2912R- ginger rhizome) were deposited at the Department of Forestry Resources Technology, of the same institution. The leaf and rhizome were rinsed thoroughly in distilled water and air dried for three weeks in the laboratory. The dried samples were then ground into powder with the aid of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

2.3 Reagents and Chemicals

All reagents and chemicals were of analytical grade and were obtained from the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State Nigeria.

2.4 Experimental Design

2.4.1 Production of zobo drink samples

Five hundred grams (500 g) of zobo flower was rinsed lightly in water so as to avoid washing away the colour. Before soaking, the zobo was divided into four equal parts thus: ZGE contained 50 g of ginger rhizome, ZSE contained 50 g of Scent leaf, two parts did not receive any additives. Each division (125 g) was then soaked in one litre (1L) of distilled water in a plastic container for 3hrs. Thereafter, it was heated and brought to a boiling point (100ºC) where it was held for 30 minutes. The heat was turned off and was allowed to cool for about 15 minutes. Afterwards, with the aid of a bowl and muslin cloth it was sieved. The drained mixture was poured into a bigger bowl where additional water, sweeteners (sugar) and flavouring (eve cola flavour) was added to taste. It was tested to gauge the level of sweetness, packaged and refrigerated.

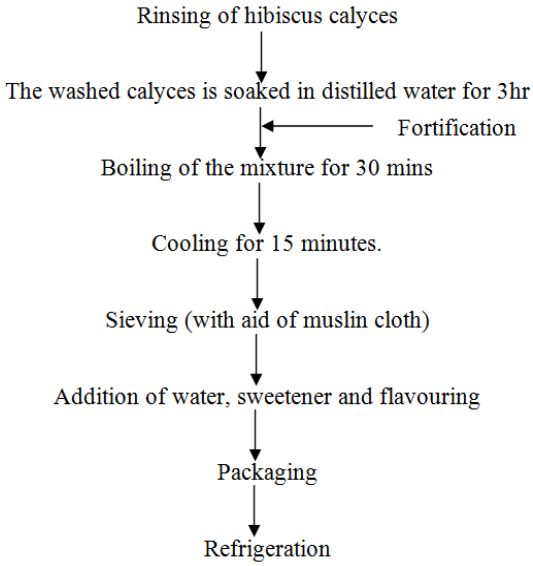

Fig. 1. Flowchart for the production of zobo drink

2.4.2 Microbiological analysis

All the samples including the controls (those without extracts) were subjected to microbiological analysis at regular intervals using serial dilution and pour plated in triplicates on the following media (1) Nutrient agar (Oxoid UK) for estimation of total heterophilic bacteria, (2) Eosin methylene blue agar (Oxoid, UK) for enumeration of coliforms, these were prepared using manufacturer's specifications and incubated invertedly at 37ºC for 24 hrs.

2.4.3 Identification of isolates

Discrete colonies were picked with a sterilized wire loop and transferred aseptically to fresh agar plates to get pure colonies. The

identification of microorganisms was based on such tests as:- Gram Reaction, Cultural characteristics, Cell Morphology, Biochemical tests and Sugar fermentation reactions. The identification sequence for bacterial culture in the manual for identification of medical bacteria was used in identifying the isolates [18].

2.5 Statistical Analysis

Unless otherwise indicated results are expressed as means ± SD of three replicates. Data were subjected to one –way analysis of Variance (ANOVA) using SPSS version 16.0. The Duncan's Multiple Range test was used to separate the means at the 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Bacterial Load on Stored Zobo Drink Fortified with Ginger and Scent Leaf

The microbial load of the various zobo samples during storage is presented in Table 1. There was no significant difference ($p \leq 0.05$) between the counts encountered on all the samples as the count ranged from 3.1 x 10^3cfu/ml (refrigerated sample) to 4.02 x 10^3cfu/ml (control) on day 0. These figures were lower than the ones reported by [19] and [20] who carried out assessment of bacterial quality of zobo drinks in Oyo and Osun states respectively in Nigeria, albeit their samples were street vended samples which may have been contaminated by the vendors. However, there was a significant increase in the bacterial load on the control sample while those fortified with ginger and scent leaf respectively remained fairly constant.

On the fifth day of storage, the refrigerated sample had the lowest bacterial load (2.90 x 10^2), this could be a result of controlled environment in the refrigerator while the control sample had the highest count of 1.33 x 10^6cfu/ml which might be due to unhindered proliferation of the organisms in the zobo drink. These results suggest that after two day storage, the fortified zobo drinks (ZGE and ZSE) may not be satisfactory for consumption since they had a count in the range of 104 cfu/ml. According to Qi et al. [21], only zobo drinks with bacterial load of <104 cfu/ml is satisfactory for human consumption.

3.2 Bacteria Isolated from Stored Zobo Drink Fortified with Ginger and Scent Leaf

The bacterial species isolated from the samples were *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris, Streptococcus species* and *Escherichia coli* (Table 2). All these were present in the samples immediately after production and some disappeared at various stages of the storage. The presence of pathogenic organisms like *E. coli* and *S. aureus* suggests a possible environmental and fecal contamination during handling process, it has been documented that polluted water can contribute significantly to high level of indicators in zobo juice [22]. This is a major concern for public health since biological contaminant of bacterial origin present in any juice is a major course of diseases giving rise to acute or chronic illnesses such as dysentery and typhoid [23].

The presence of *Bacillus subtilis*, *Micrococcus luteus* and *Proteus vulgaris* is an indication of soil contamination since they are part of normal flora of the soil. *M. luteus* is a benign environmental commensal while *P. vulgaris* is a known saprophyte implicated in the spoilage of many food substances. However, *Bacillus subtilis* have been incriminated in contributing towards life threating diseases.

Table 1. Total heterophilic bacteria count on stored zobo drinks

Sample	Day 0	Day 1	Day 3	Day 5
ZGE	$3.40\pm0.01^a \times 10^3$	$2.90\pm0.01^b \times 10^3$	$1.13\pm0.00^b \times 10^4$	$2.18\pm0.02^b \times 10^4$
ZSE	$4.01\pm0.01^a \times 10^3$	$6.20\pm0.03^c \times 10^3$	$2.90\pm0.05^c \times 10^4$	$5.15\pm0.00^c \times 10^4$
FRG	$3.10\pm0.02^a \times 10^3$	$2.07\pm0.01^a \times 10^2$	$2.05\pm0.02^a \times 10^2$	$2.90\pm0.01^a \times 10^2$
CNT	$4.02\pm0.01^a \times 10^3$	$2.30\pm0.00^d \times 10^4$	$1.19\pm0.15^d \times 10^5$	$1.33\pm0.01^d \times 10^6$

ZGE= zobo fortified with ginger extract, ZSE= zobo fortified with scent leaf, FRG= sample stored in fridge, CNT= control sample. Values with different superscripts on each column are significantly different

Table 2. Characteristics of bacteria isolated from fortified Zobo drink

Isolate code	Cultural characteristics	Cell morphology	Gram reaction	Ind	Coag	Cat	Cit	mot	Glu	Lac	Mal	Suc	Organism
ZB1	Circular, smooth, whitish	Coccus	+	-	-	+		-	AG	A	A	A	*Micrococcus luteus*
ZB2	Circular, yellowish, flat	Rod	+	+	-	+		-	AG	AG	AG	AG	*Bacillus subtilis*
ZB3	Circular, entire, yellowish, convex	Rod	-	+	-	+	+	+	+	-	-	-	*Proteus vulgaris*
ZB4	Circular, entire, whitish, flat	Coccus	+	-	+	+	+	-	AG	A	AG	A	*Staphylococcus aureus*
ZB5	Creamy, irregular, crenated, flat	Rod	-	-	-	+		+	AG	AG	A	-	*Escherichia coli*
ZB6	Cream, circular, entire, convex	Coocus	+		-	+		-	A	-	-	+	*Streptococcus species*

Key: += positive, - = negative, A= acid production, AG= acid and gas production

Table 3. Distribution and succession of bacteria during storage of fortifies zobo drink

	ZGE	ZSE	FRG	CNT
Day 0	ML,SA, SS, PV	ML,SA,EC,PV	ML,SA,EC,PV	ML,SA,PV,SS,EC
Day 1	SA,BS,PV	SA,BS,PV	SA,BS	SA,BS,PV,EC
Day 3	SA,BS	BS,PV	BS	SA,BS,PV,EC
Day 5	SA,BS	BS, PV	BS	SA,BS,PV

ML= Micrococcus luteus, SA= Staphylococcus aureus, BS= Bacillus subtilis, PV= Proteus vulgaris, EC= Escherichia coli, SS= Streptococcus species

During storage (Table 3), *M. luteus* and *Streptococcus* species disappeared after day 0 suggesting that they may just be environmental contaminant and may not be able to survive in the zobo drink. *S. aureus, P. vulgaris* and *B. subtilis* persisted in the stored zobo samples during the storage period suggesting that they are the main spoilage organisms of this drink. However, only *B. subtilis* survived throughout the storage period in all the samples. This signifies that it may be a major spoilage organism of zobo drink as it survives in the drink over a long period. This could be because the organism possesses the ability to produce resistant spores [24]. The function of ginger and scent leaf is to serve as preservatives in zobo drink since there have been reports of their antimicrobial activities but from this study it is evident that they may not deter microbial spoilage of zobo. This could be due to various chemical reactions between the phytochemicals present in zobo calyces and these plant materials. There is need to work further to unravel the reasons behind this observation.

4. CONCLUSION

The results obtained from this study suggests that *S. aureus, P. vulgaris* and *B. subtilis* are the main spoilage organisms of zobo drink. Also, scent leaf and ginger did not prevent the growth of these organisms in the zobo drink indicating that they may not be suitable as preservative agents for the elongation of shelf-life of this drink.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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