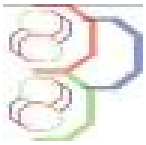

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Screening of natural spices for improving the microbiological, nutritional and organoleptic qualities of the *Zobo* drink

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ABSTRACT

Objective: The study aimed at standardizing the *Zobo* -making process and improving its nutritional, organoleptic and microbial qualities thereby increasing its shelf life using natural spices, 4 natural spices namely, ginger, garlic, cinnamon and nutmeg.

Methodology and Results: At 1%, w/v concentration per spice, a chemical preservative, sodium benzoate at different concentrations (1%, 0.1% and 0.01%) and control to which no preservative was added were stored at ambient temperature and control samples were stored in the refrigerator at 4˚C for 28 days. Samples were drawn periodically after preparation for microbiological analyses. Serially diluted samples were inoculated in duplicates onto Nutrient Agar (NA) and Potato dextrose agar (PDA) for bacteria and fungi respectively, the NA plates were incubated at 37^0C for 18-24 hours and the PDA plates at 30^0C for 3-6 days before reading the plates. The spoilage organisms were identified using standard cultural, staining and biochemical techniques. The spoilage bacterial organisms were identified as *Micrococcus luteus, Corynebacterium xerosis, Bacillus sphaericus, Veillonella spp, Aeromonas veronii, Pseudomonas spp, Corynebacterium kutscheri, Bacillus marinus, Bacillus megaterium* and *Bacillus pumilus* while the fungal spoilage organisms identified from the *Zobo* samples include *Aspergillus niger, A flavus, A. fumigatus* and *Geotrichum spp.*

Conclusion and application of study: The natural spices were more effective than refrigeration in delaying microbial deterioration of *Zobo.* Nutmeg was the most effective. Moreover, the results showed that *Zobo* will spoil after 12 days, and that treatment of *Zobo* with nutmeg is most effective (critical difference=0.058; $p \leq 0.05$) at extending the shelf life of *Zobo* while maintaining the nutritional and organoleptic properties of the drink.

Application of results: The study shows the possibility of large-scale production of *Zobo* using treatment with nutmeg as a safe, low-cost means of preservation.

Key words: *Zobo*, shelf life, natural spices

INTRODUCTION

Zobo drink, a non-alcoholic local beverage, is produced from the dried petals of *Hibiscus sabdariffa (Plate 1)*. *Zobo* drink has been shown to be a good source of natural carbohydrate, protein and vitamin C (Ogiehor *et al.*, 2007). It is locally called "*Zobo* rodo" (Hausa), "Isapa" (Yoruba) and Sorrel in English and is a delicacy in many parts of Nigeria (Adebayo-tayo and Samuel, 2008). The *H. sabdariffa* plant, commonly known as Roselle, while native to India and Malaysia is now found in many tropical and subtropical countries of Africa, Asia and the Americas (Bola and Aboaba, 2004). It is a dicotyledonous plant belonging to the sub-class Archichlamydea, Order Malvale and Family, Malvaceae (Shivali and Kamboj, 2009). *Zobo* drink is prepared first by boiling the dried leaves of the Roselle, followed by cooling and filtration. The filtrate, which is red in color, is sometimes sweetened to taste with pineapple, orange or sugar and spiced up with ginger. It is further allowed to cool and is best served chilled (Egbere *et al.*, 2007). Due to increased religious and health campaigns against alcoholic beverages in Nigeria and the consequent decrease in the consumption of alcoholic beverages in certain areas, *Zobo* drink has great potential as a local alternative to imported red wines in particular and alcoholic beverages in general (Egbere, *et al* ., 2007). Moreover, production of this and similar local beverages has become the main source of income in many homes in the rural communities and more recently in the urban areas where these have grown to cottage business proportions due to support from the government through the poverty alleviation schemes, thereby alleviating poverty among the people (Essien *et al.,* 2011). Phytochemical analyses of extracts of various parts of the Roselle plant reveal the high nutritive and medicinal value of this plant. The flower is reported to contain carbohydrates and sugars like sucrose and mannose. It also contains proteins, fat and vitamins with other acids. The seeds contain starch, cholesterol, cellulose, some acids (oleic acid, formic acid) and alcohols. The leaves contain alcohols, malic acid, fibre and ash. The fruits contain acids (formic acid, acetic acid), alcohols, pectin and minerals. The roots contain tartaric acid and saponin (Shivali and Kamboj, 2009). Medicinal value of aqueous extracts from the Roselle plant has been reported to include anti-hypertensive, antiseptic astringent diuretic and purgative activities remedy for cancer, abscesses, cough, dysuria, laxative, scurvy and fever (Osueke and Ehirim, 2004). In spite of its health and nutritional benefits, *Zobo* drink is often contaminated with enteropathogenic microorganisms with as much as $2.49x10^4$cfu, which could be harmful to persons who consume large quantities of the drink (Bukar *et al*., 2009). Major points of contamination of the *Zobo* drink include: the packaging material, as most retailers package the drink in already used plastic bottles and polyethene bags, which are not properly, disinfected prior to packaging (Nwafor and Ikenebomeh, 2009). The dried calyces are also a major point of contamination as they harbor spoilage organisms such as *Penicillium* and *Aspergillus* sp. (Amusa *et al*., 2005) and the retailers, who seldom prepare the drink under aseptic conditions and often do not do enough boiling to reduce the microbial load in the preparation of the beverage. Some of the microbes commonly found in the drink include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa and Aspergillus* sp. amongst a host of others (Nwachukwu *et al*., 2007). The shelf life of the *Zobo* drink depends on various factors such as the packaging material, contamination during preparation and refrigeration to mention a few, however, it has an average shelf life of 24 to 48 hours after which spoilage organisms may begin to reduce the quality of the *Zobo* (Nwafor and Ikenebomeh, 2009). On the other hand, plant extracts of spices such as ginger, garlic, cinnamon and nutmeg have been developed and proposed for use in foods as natural antimicrobials (Hsieh *et al* ., 2001). Moreover, the extracts of traditional natural spices have been shown to have a broad spectrum antibacterial activity, including effects on *Escherichia, Salmonella, Staphylococcus,*

Streptococcus, Klebsiella, Proteus, Clostridium, Mycobacterium and *Helicobacter* species (Groppo *et al* ., 2002; Sagdic, 2003; Shan *et al* ., 2007).

These magnitudes of success observed in vitro have not been replicated in vivo particularly in food items that must be cooked before eating. For example, while Bello and Osho (2012) reported some measure of antimicrobial activity of spices on spoilage organisms isolated from moin-moin in vitro, Ayoade *et al*., (2012) however, observed no effects of these spices on the progression of spoilage in this food item in vivo. The absence of antimicrobial effect in vivo was ascribed to the sustained heat used in cooking the moin-moin which is thought to have a suppressing effect on the antimicrobial properties of these spices. This kind of wide differences observed in vivo and in vitro is not expected in food items like *Zobo* where no cooking is required. Transitioning *Zobo* from a locally marketed to commercial product status is hampered due to its poor shelf life of 24 to 48 hours, which would require very little inventory. The present work is aimed at standardizing the *Zobo* -making process with a view to improving the microbial quality and safety of this product, thereby increasing the shelf life while also maintaining its excellent nutritional and organoleptic properties using natural spices.

MATERIALS AND METHODS

Laboratory preparation of Zobo drink: Six hundred (600) g dried calyces of *Hibiscus sabdariffa* was boiled with 12 litres of clean water. Three (3) whole pineapples were thoroughly cleaned, cut with a clean knife and the peels were boiled along with calyces to give the drink pineapple flavour. The mixture was boiled for 30 minutes and sieved. Immediately, 1075g of granulated sugar was added to taste and stirred to dissolve while the drink was still hot. It was kept to cool in a clean bowl. A litre each of this stock was partitioned into 8 separate clean bowls and labelled with each treatment that was applied. For the spice treatments, the natural spice to be used (i.e., ginger, garlic, cinnamon and nutmeg) were grated using a clean grater and 100g of each spice was added to each bowl according to their labels to give 1% w/v concentration per spice. For the chemical preservative treatment, sodium benzoate at different concentrations (1%, 0.1% and 0.01%) was applied and a control to which no preservative was added, making eight (8) treatments. Each *Zobo* drink was stored at ambient temperature and control samples were stored in the freezer for the 4 weeks duration of the study. Samples were drawn at intervals on the same day of preparation (day zero), 3rd, 7th, 14th, 21st and 28th day after preparation for microbiological analyses. Proximate analyses were performed on fresh samples of the various treatments. The flow diagram for *Zobo* preparation is shown in Figure 1.

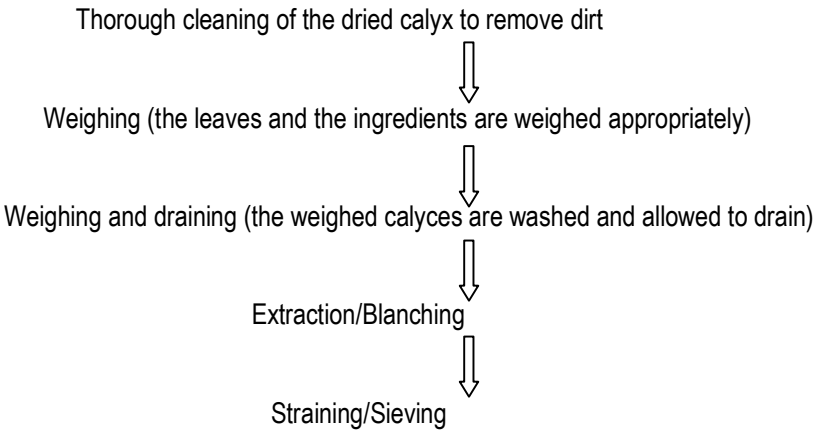

Figure 1: A flow diagram for the preparation of *Zobo* drink.

Plate 1: A picture of dried calyces of *Hibiscus sabdariffa* (left) and that of the finished product- *Zobo* drink (right).

Microbiological analysis: One (1) ml of each *Zobo* drink sample was aseptically transferred into a sterile test tube containing 9 ml of sterile water, thoroughly shaken using a vortex and serially diluted up to 10^{-9} dilution. 1 ml of dilutions 10^{-2}, 10^{-5}, 10^{-9} was inoculated in duplicates onto Nutrient Agar (NA) and Potato dextrose agar (PDA) for bacteria and fungi respectively, the NA plates were incubated at 37^0C for 18-24 hours and the PDA plates at 30^0C for 3-6 days before reading the plates. Pure cultures were obtained by re-streaking into fresh medium employing standard methods. The bacteria isolates were identified based on shape, colony, color, and Gram's staining reactions and biochemical tests such as methyl red, Vogues-Praskauer, Citrate, Urease, Indole, Motility, Catalase, Oxidase and Sugar fermentation tests. The fungal samples were examined macroscopically on the plates and recorded. Morphological characteristics observed include colony appearance, type of colonies, colony color (surface and backside colors), hyphal structures, type of spores and other cultural characteristics (Krieg *et. al.,* 1994). For microscopic examination, the fungal isolates were identified by placing a drop of cotton blue-in-lactophenol on a clean slide. Using an inoculating needle, a small piece of mycelium free of medium was picked and transferred to stain on slide carefully and evenly dispersed. This was gently covered with a cover slip to avoid air bubbles, and then viewed under the microscope for cultural characterization (Watanabe, 2010).

Proximate analyses: The proximate composition was carried out according to the method of Association of Official Analytical Chemists (2005). This includes determination of pH, moisture content, ash content, crude fat, fibre, fat, crude protein and carbohydrate was determined by difference. The nutritionally essential elements (Na, K, Ca and P) were determined using Atomic Absorption Spectrophotometer (AAS), *Shimadzu model AA-7000*.

Organoleptic evaluation: A 31 member panel consisting of students whom were regular *Zobo* drinkers was used to evaluate the drink. The panel tested the drink by sipping on the drink, then rinsing their mouth with water after testing each drink. It was ranked by the appearance, color, flavour, taste and over all acceptability on a modified 3-point Likert scale of 1-3. 3= Like very much, 2= Like, 1= Dislike.

Analysis of data: The frequency of occurrence for all bacterial and fungal isolates, the test for significance ($p \leq 0.05$) and the frequency in percentage, the overall acceptability of the *Zobo* samples after the sensory evaluation were analyzed using the IBM SPSS version 19.

RESULTS

The results obtained from the present study shows that in most cases there were no significant differences in the progression of microbial spoilage between *Zobo* samples treated with spices and maintained at ambient temperature and those kept refrigerated at 4°C. In some cases, the refrigerated samples without spices fared worse than the control and those treated with spices and kept at ambient temperature. This is observable in the garlic and cinnamon treatments kept at ambient temperatures, which by day 12 showed lower mean bacterial count than those of the same batch of treatments maintained at 4°C in the refrigerator and control samples (Table 1). The optimum concentration to obtain a consistent preservative effect of Sodium benzoate was 1%, at this concentration; the preservative effect was similar to those obtained from the spices (Table 1). Enumeration of the frequency of occurrence of the bacterial isolates in the various treatments revealed nutmeg emerged was the spice treatment with the least percent occurrence value for bacteria isolates, followed by cinnamon, garlic and ginger in ascending order. The low percent occurrence of bacteria isolates in the salt treatment with sodium benzoate at 1% concentration was similar to those observed for the nutmeg samples (Table 1). Bacterial count for *Zobo* drinks with different treatments kept at ambient temperature and refrigerated at 4^0C for 25 days. Data for refrigerated samples are shown in parenthesis. Data with similar alphabets are not significantly different at $p \leq 0.05$.

The spoilage bacterial organisms were identified as *Micrococcus luteus, Corynebacterium xerosis, Bacillus sphaericus, Veillonella spp, Aeromonas veronii, Pseudomonas spp, Corynebacterium kutscheri, Bacillus marinus, Bacillus megaterium* and *Bacillus pumilus* (Table 2). A general decline in the frequency of occurrence of bacterial spoilage organisms was observed in all treatments by the twelfth day for the samples maintained at 4°C including those kept at ambient temperature. Another increase in the frequency of occurrence was observed between the 18^{th} and 25^{th} day. The highest frequency of occurrence was recorded for *Aeromonas veronii* with frequencies of occurrence of 49% and 31% in the samples refrigerated and those kept at ambient temperature respectively. This is the only bacteria species that continued to increase throughout the 28 days when the study was terminated (Figs 2 and 3).

Table 1: Mean bacterial count for *Zobo* samples kept at ambient temperature and refrigerated at 4^0C for 25 days.

Number of days sample after preparation	Mean bacteria count (cfu/mL)/Treatment							
	Control	Ginger	Garlic	Cinnamon	Nutmeg	Sodium benzoate		
						1%	0.1%	0.01%
After day Zero (0)	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$2.5x10^{9a}$	$8.0x10^{9b}$	0.0^{a}	$0.5x10^{9a}$
After day 1	$(TNTC)^{b}$	$(6.00x10^{9})^{a}$	$(2.50x10^{9})^{a}$	$(2.00x10^{9})^{a}$	$(2.50x10^{9})^{a}$	$(2.00x10^{9})^{a}$	$(7.00x10^{9})^{a}$	$(3.50x10^{9})^{a}$
After day 3	$0.5x10^{10a}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$0.3x10^{10a}$	$0.3x10^{10a}$
	$(0.11x10^{10})^{b}$	$(0.00)^{a}$	$(0.15x10^{10})^{a}$	$(0.40x10^{10})^{a}$	$(0.00)^{a}$	$(0.05x10^{10})^{a}$	$(0.00)^{a}$	$(0.00)^{a}$
After day12	$0.2x10^{10ab}$	$0.4x10^{10b}$	0.0^{a}	$0.1x10^{10ab}$	$0.3x10^{10b}$	$0.2x10^{10ab}$	$0.2x10^{10c}$	$0.4x10^{10b}$
	$(0.00)^{a}$	$(3.00x10^{9})^{c}$	$(0.50x10^{9})^{a}$	$(1.50x10^{9})^{b}$	$(2.50x10^{9})^{bc}$	$(0.00)^{a}$	$(2.00x10^{9})^{bc}$	$(0.00)^{a}$
After day 18	$2.0x10^{9a}$	0.0^{a}	$1.0x10^{9a}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$4.0x10^{9b}$
	$(0.00)^{a}$	$(2.00x10^{9})^{a}$	$(2.00x10^{9})^{a}$	$(0.00)^{a}$	$(1.00x10^{9})^{a}$	$(1.00x10^{9})^{a}$	$(1.00x10^{9})^{a}$	$(1.00x10^{9})^{a}$
After day 25	$0.6x10^{10a}$	$0.5x10^{10a}$	$0.4x10^{10a}$	$0.4x10^{10b}$	$0.7x10^{10a}$	$0.2x10^{10a}$	$0.3x10^{10a}$	$TNTC^{c}$
	$(0.30x10^{10})^{a}$	$(0.30x10^{10})^{a}$	$(0.40x10^{10})^{a}$	$(0.35x10^{10})^{b}$	$(0.40x10^{10})^{a}$	$(0.10x10^{10})^{a}$	$(0.30x10^{10})^{a}$	$(0.20x10^{10})^{a}$

Table 2: Identification of isolated bacteria; results of cultural, morphological and biochemical tests.

Isolates	GR	S	Ca	Ox	Gu	Mn	VP	Ci	SH	M	I	U	H_2S	MR	SF	Probable organism
1	+	C	+	-	-	-	+	+	-	+	-	-	+	-	-	*Micrococcus luteus*
2	+	R	+	+	-	-	+	+	-	-	-	-	-	-	-	*Corynebacterium xerosis*
3	+	R	+	+	-	-	-	+	-	+	-	+	-	-	+	*Bacillus sphaericus*
4	-	C	+	+	-	-	-	+	-	+	-	+	+	-	-	*Veillonella spp.*
5	-	R	+	+	+	+	+	+	-	+	-	+	-	-	-	*Aeromonas veronii*
6	-	R	-	+	-	-	-	+	-	+	-	+	-	-	-	*Pseudomonas spp.*
7	+	R	+	+	+	+	+	+	+	+	-	+	-	-	-	*Corynebacterium kutsceri*
8	+	R	+	+	+	+	-	+	-	-	-	+	-	-	+	*Bacillus marinus*
9	+	R	+	+	+	-	-	+	+	+	-	+	-	-	+	*Bacillus megaterium*
10	+	R	+	+	+	+	+	+	-	+	-	+	-	-	+	*Bacillus pumilus*

GR= Gram reaction, S= shape, Ca= catalase, Ox= oxidase, Gu= Glucose, Mn= Mannitol, VP= Voges- Proskauer, Ci= citrate, SH= starch hydrolysis, M= motility, I= indole, U= urease, H_2S= hydrogen sulphide, MR= methyl red, SF= spore formation, R= rods, C= cocci, += positive, - = negative

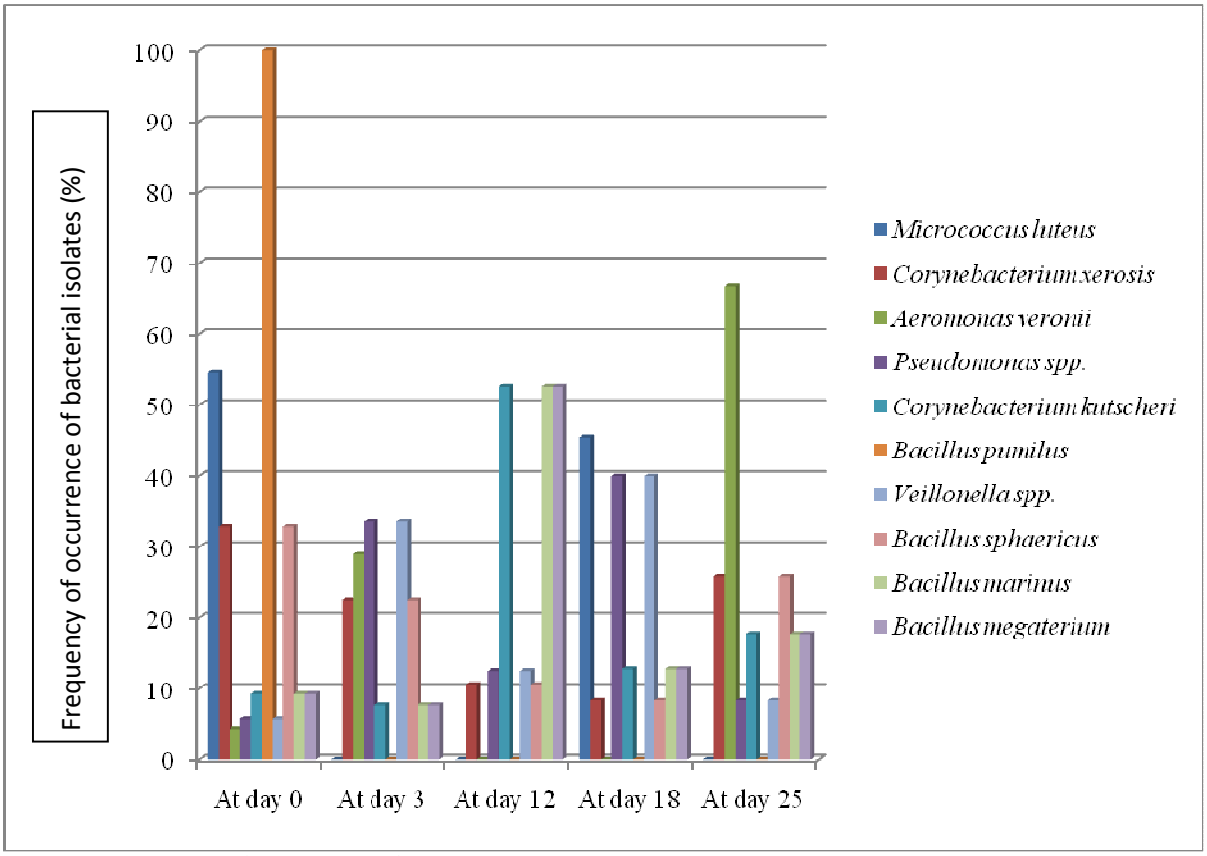

Figure 2: Frequency of occurrence of bacterial isolates in *Zobo* maintained at ambient temperature when sampled on days 0, 3, 12, 18 and 25.

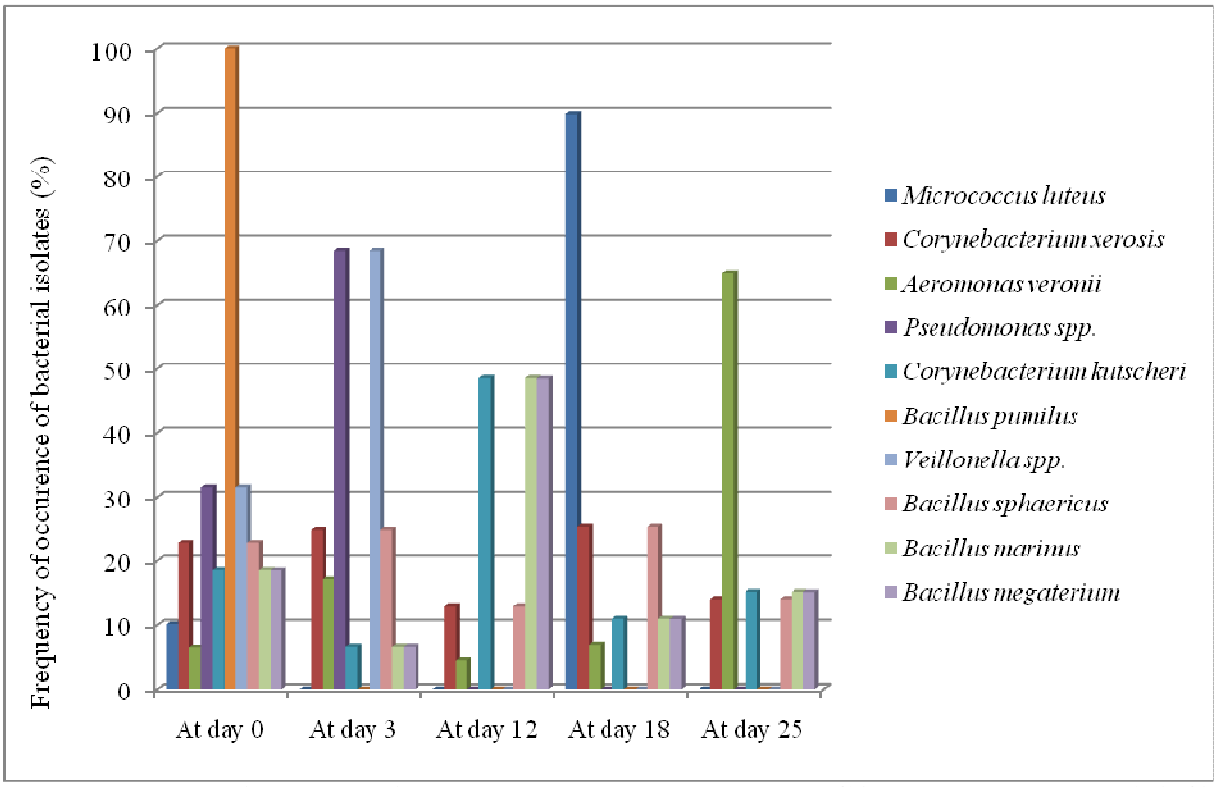

Figure 3: Frequency of occurrence of bacterial isolates in *Zobo* maintained at 4˚C when sampled on days 0, 3, 12, 18 and 25.

The fungal spoilage organisms identified from the *Zobo* samples include *Aspergillus niger, A flavus, A. fumigatus* and *Geotrichum species* (Table 3), *Geotrichum spp* and *Aspergillus fumigates* being the most frequently occurring species (Figs. 4 and 5). In a pattern similar to that observed for the bacterial spoilage organisms, the results show a general decline in the frequency of occurrence of fungal spoilage organisms by the twelfth day for the samples maintained at 4°C including those kept at ambient temperature, a surge in the frequency of occurrence was observed between the 18th and 25th day.

Table 3: Identification of fungal isolates.

Isolates	Cultural characteristics	Probable organism
Isolate 1	Typical black colony with white edges, yellow on the reverse plate	*Aspergillus niger*
Isolate 2	Typical yellow-green colony with white edges, white on the reverse plate	*Aspergillus flavus*
Isolate 3	Typical blue-green colony with white edges, white on the reverse plate	*Aspergillus fumigatus*
Isolate 4	Typical white colony, white on the reverse plate	*Geotrichum* sp.

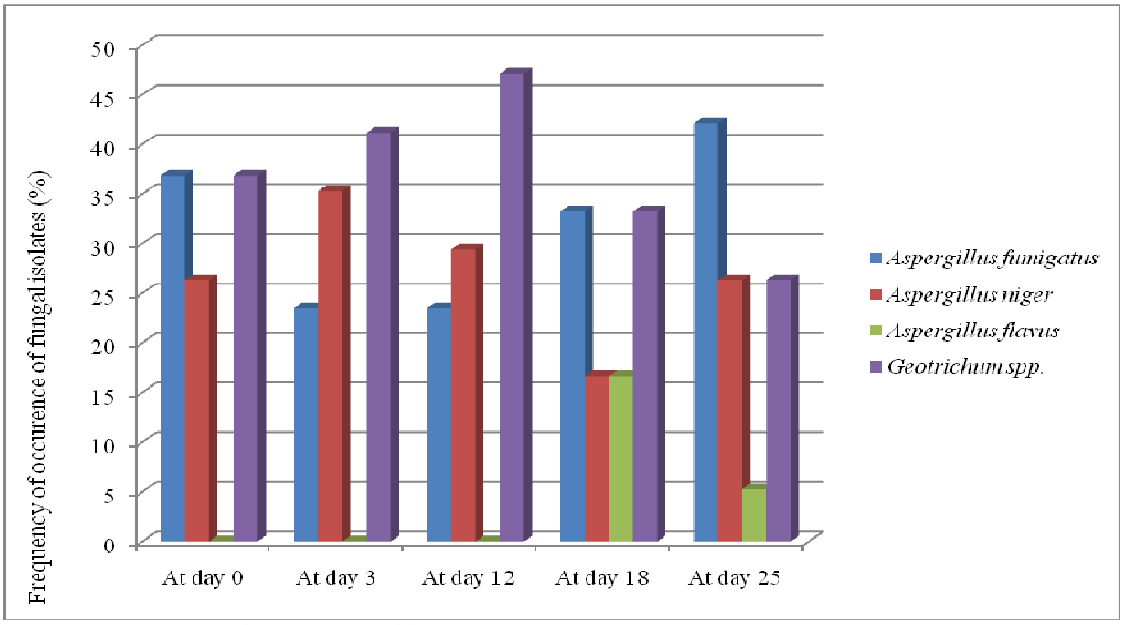

Figure 4: Frequency of occurrence of fungal isolates in *Zobo* maintained at ambient temperature when sampled on days 0, 3, 12, 18 and 25.

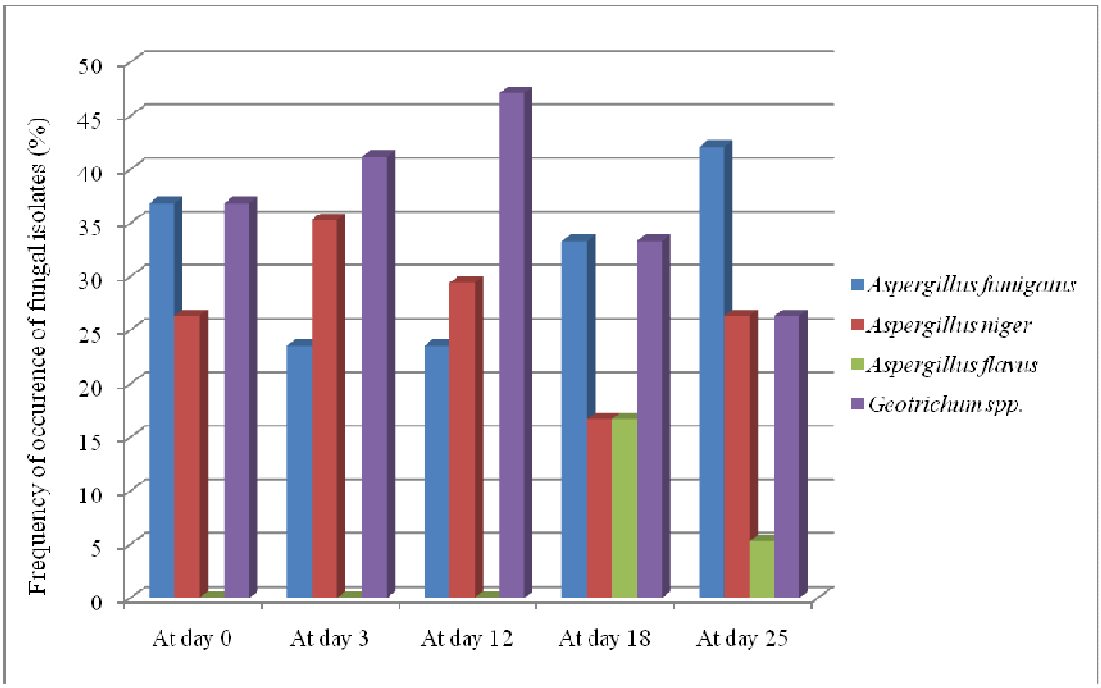

Figure 5: Frequency of occurrence of fungal isolates in *Zobo* maintained at 4˚C when sampled on days 0, 3, 12, 18 and 25.

Proximate and phytochemical analyses results revealed no significant differences in percent carbohydrate, moisture content, crude protein, ash, total solid, vitamin C and fat when the various samples of *Zobo* treated with natural spices and chemical preservatives (Tables 4 and 5).

Table 4: Proximate Nutrient composition determination and vitamin C contents of *Zobo* drink (*Hibiscus sabdariffa*) with natural spices and chemical preservatives

Treatment	Carbohydrate (%)	Moisture content (%)	Crude protein (%)	Ash (%)	Total solid (%)	Vitamin C (%)	Fat (%)
Garlic	10.98	87.50	8.00	0.44	13.5	42.02	0.40
Nutmeg	11.00	89.00	8.13	1.45	11.0	39.13	0.41
Ginger	11.12	88.00	8.13	1.33	12.0	36.23	0.38
Cinnamon	10.97	92.00	8.01	0.59	8.00	38.40	0.39
Sodium benzoate (0.01%)	10.99	92.00	7.81	1.32	8.00	36.96	0.39
Sodium benzoate (0.1%)	11.12	88.00	8.12	0.50	12.00	41.30	0.41
Sodium benzoate (1%)	11.87	87.50	8.20	1.75	13.50	41.96	0.42

Table 5: Phytochemical composition of *Zobo* drink (*Hibiscus sabdariffa*) treated with natural spices and chemical preservatives

Treatment	Alkaloids mg/ 100g	Flavonoids mg/ 100g	Saponins mg/ 100g	Tannins mg/ 100g	Sodium mg/100g	Potassium mg/100g	pH
Garlic	2.09 ±0.01	0.6±0.01	3.99±0.03	0.10± 0.01	10.98±0.20	9.87±0.20	2.69
Nutmeg	4.13±0.02	0.2±0.01	4.11±0.03	0.02±0.01	11.0±0.20	9.94±0.10	2.49
Ginger	3.01±0.02	0.12±0.02	3.98±0.02	0.021±0.0	11.12±0.21	10.08±0.20	2.51
Cinnamon	3.11±0.03	0.19±0.02	3.81±0.02	0.03±0.01	10.97±0.20	9.83±0.10	2.65
Sodium benzoate (0.01%)	2.91±0.02	0.99±0.02	4.01±0.02	0.01±0.01	10.99±0.21	9.97±0.20	2.58
Sodium benzoate (0.1%)	3.11±0.03	0.1±0.01	4.01±0.02	0.02±0.01	11.12±0.21	10.03±0.20	2.55
Sodium benzoate (1%)	3.91±0.03	0.12±0.02	4.21±0.02	0.04±0.01	11.87±0.21	9.95±0.20	2.52
Control	4.01±0.02	0.1±0.01	3.19±0.03	0.14±0.02	11.32±0.21	9.97±0.20	2.44

Sensory evaluation studies showed that the *Zobo* samples were still palatable after the spices were added. In terms of overall acceptability of the various *Zobo* treatments, results of the organoleptic tests reveal that the control sample (i.e. with neither spices nor salt) was the most acceptable with 96.8% of the tasters indicating that they 'like very much' followed by the Sodium benzoate treatments, ginger, nutmeg, cinnamon and garlic in descending order (Fig. 6). Among the spiced samples, ginger was the most palatable with 93.5% of the tasters indicating that they 'like very much'. The statistical analysis show that there was no significant difference between the control and the samples treated with ginger when compared based on acceptability ($p \leq 0.05$).

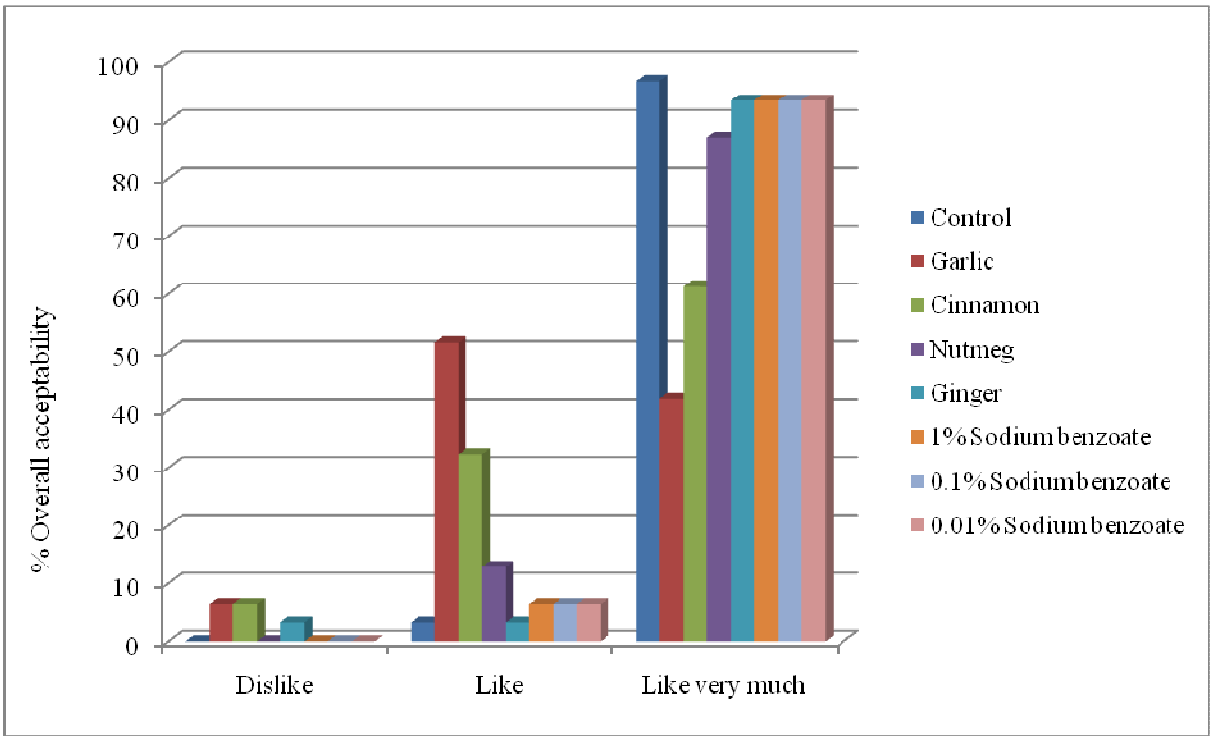

Figure 6: Percent overall acceptability of the *Zobo* samples after organoleptic test.

DISCUSSION

The natural spices namely ginger, nutmeg, cinnamon and garlic used in this study *in vivo* were able to reduce microbial spoilage of *Zobo* drink better than refrigeration. The magnitude of reductions observed with the spice treatments was similar to those of the control samples to which no spices were added, possibly due to the sanitary conditions under which the samples were prepared. Moreover, the fact that many of the spoilage organisms identified from these samples are soil-inhabiting organisms in spite of the strict sanitary conditions under which the present samples were prepared exemplifies the importance of cleanliness in preparation as a first important step in delaying spoilage of *Zobo.* The microbial count recorded for all the treatments including the control were significantly higher than the FAO recommended critical microbial count of 10 cfu/ml for ready-to-eat foods when samples were evaluated after the 12th day of preparation (FAO, 1979). The present results indicate that shelf life of *Zobo* will be preserved by spices up to the 12th day after which *Zobo* will spoil. In spite of the fact that all the spices which were used in the present study have been proven to have excellent antimicrobial activities *in vitro* (Gutierrez *et al.*, 2008), similar levels of antimicrobial activities was not observed *in vivo*. The disparity between the *in vitro* and *in vivo* antimicrobial activities of natural spices in delaying or suppressing microbial food spoilage was previously reported in the steamed beans pudding, moin-moin, where the excellent *in vitro* microbial activities of spices was not matched *in vivo.* The reduced *in vivo* antimicrobial effect of the spices was

attributed to the fact that these spices were added in their crude mixtures; as a result, other compounds apart from the antimicrobial compounds such as proteins might encourage the growth of microbes, thereby overshadowing the antimicrobial effects of the spices. Moreover, that the sustained heat used in cooking the moin-moin that is thought to have a suppressing effect on the microbial properties of these spices (Ayoade *et al.*, 2012). The observation that natural spices delayed the rate of deterioration of *Zobo* better than refrigeration (Tables 1, 2 and 3) is not surprising since fungi and psychotropic and spore-forming bacteria are known to be commonly associated with spoilage of food at refrigerated temperatures (APHA, 2001). Altunatmaz *et al.,* (2012) for example, reported mean value of up to 82.3 CFU/m3 and 54.6 CFU/m3 of fungi and psychrotrophic bacteria respectively that were found to be associated with spoilage of food at refrigerator temperatures. Phylogenetically, many of the well-known psychotropic organisms belong to the phylum Firmicutes; class Bacilli, which are commonly found to be associated with microbial and organoleptic spoilage of food (Martinez-Murcia *et al.*, 1993). Four species of the genus Bacillus were isolated from the *Zobo* samples examined in the present study namely, *Bacillus sphaericus, Bacillus marinus, Bacillus megaterium* and *Bacillus pumilus*. Moreover, the isolation of *Aspergillus* and *Geotrichum* species as the fungal spoilage organisms in the present study is consistent with earlier report that listed mould genera that have psychrotrophic species as including *Alternaria, Aspergillus, Botrytis, Cladosporium, Fusarium, Geotrichum, Monascus, Mucor, Penicillium, Rhizopus* and *Trichothecium* (Jay, 1996). The observation that *Aeromonas veronii* exhibited the highest frequency of occurrence and persistence throughout the duration of the study is consistent with reports that proclaim the dominating nature of this bacteria species when in competition with other bacterial species. This characteristic of *A veronii* has been exploited in medical microbiology where the medicinal leech, *Hirudo medicinalis* is used as an anticoagulant after plastic and reconstructive surgery. *A. veronii* co-habiting the human digestive tract with other bacterial members of the flora has been shown to be able to contain growth of other bacteria and remain the dominating flora. While without antibiotic treatment prior to leech therapy, patients are highly susceptible to infections caused by the bacteria, the specificity of the symbiosis of *Aeromonas veronii* biovar *sobria* (a non-pathogenic strain of this bacterium) and *Hirudo medicinalis*, the medicinal leech is the focus of research on exploiting the aggressive nature of this organism to human advantage (Indergand and Graf, 2000).

CONCLUSION

Results of the present study showed that treatment of *Zobo* with Nutmeg does not result in reduction of nutritional organoleptic characteristics of the drink. Moreover, nutmeg emerged as the most effective spice in delaying the bacterial and fungal deterioration of *Zobo.*

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