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Bacteriological Spoilage of Zobo (A Nigerian Drink Prepared From The Calyces of *Hibiscus Sabdariffa* L. (Malvaceae)

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----------ABSTRACT----------
This study was undertaken to detect the bacteriological spoilage of zobo drink prepared from the calyces of Hibiscus sabdariffa using sugar, pineapple flavour and spices - Ginger (Zingiber officinalis), Black pepper (Piper guineense). The pH of the various products was analyzed over a six days period. The pH was found to reduce as the days went by due to bacterial activity, with product 2 having the lowest pH due to its high nutritive value. The effect of spices was noticed as they prevented bacterial activity. The bacterial count of the products varied from $8.1x10^4$ to $2.5x10^6$cfu/ml on the first day of storage. The change in colour and aroma of the products stored for 3 days was studied. In all the products, the colour was bright red on the 1st & 2nd day and on the 3rd day it became dark red. Product 1&2 had a berry like aroma on the 1st & 2nd day and on the 3rd day it became sour, Product 4&5 had spicy odour on the 1st & 2nd day and on the 3rd day it became off odour, Product 5 had a fruit odour on the 1st & 2nd day and on the 3rd day it became sour. The organisms isolated from the zobo products were:-Micrococcus, Bacillus, Proteus & Escherichia species. The results suggest that zobo drink if not properly processed is very susceptible to microbial deterioration due to its high nutrient content. It was found to have a shelf-life of 2days after which it became undesirable when stored at ambient temperature.

***KEYWORDS**: Bacteriological Spoilage, Zobo drink, Hibiscus sabdariffa*

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I. INTRODUCTION

Microbial spoilage is damage or waste caused by microorganisms like bacteria anf fungi (including yeast) in general due to their invasion. Various members of these groups may cause changes in the character of the food, which may be classified as "positive" or "negative."

The products of positive microbial transformation include cheese, yoghurt and wine, which can be seen as increasing the nutritional value or keeping quality of products with a short shelf life. Negative effects of microbial growth include food poisoning, mainly caused by different and less widespread bacteria.As they grow, microorganisms release their own enzymes ito the liquid surrounding them and absorb the products of external digestion. This is the main basis of microbial spoilage which lowers nutritional value of the product. As a result, products develop undesirable flavors, odours, appearances or textures via microbial action. Bacteria and moulds may also produce waste products, which act as poison or toxins, thus causing the renowned ill effects.

Fruits and vegetables are normally susceptible to infection by bacteria, fungi and viruses (Pelczer, *et al*., 1986, 1993). Deterioration of raw vegetables and fruits may result from –physical factors, action of their own enzymes, microbial action, or combination of all of these (Frazier and Westhoff, 1978).Frazier and Westhoff, (1978) reported the variety of microorganisms present in deteriorating fruits and vegetables. They are:- *Pseudomonas, Yeast, Rhizopus, Streptococcus, Bacillus, Erwinia, Aspergillus, Chromobacteria, Penicillium, Fusarium, Flavobacterium, Xantomonas, Enterobacter*, etc. The character of the spoilage will depend upon the product attacked and the attacking organism. *Hibiscus sabdariffa* L. Malvaceae is a tropical plant of considerable economic importance (Faye and Neela, 2004). The main edible part is the fleshy sepal, called a calyx surrounding the seed. The calyx is bright red, acid and closely resembles the cranberry (*Vacinium* spp) in flavour (Morton, 1987; Faye and Neela, 2004; McCaleb, 1996). In the West Indies, Sorrel is a prized drink during the Christmas holidays (McCaleb, 1996), and calyces can be chopped and added to fruit salad (Morton, 1987). In Africa, they are frequently cooked as a side dish eaten with pulverized peanuts for stewing as sauce, for making a fine-textured sauce or juice (zobo), syrup, jam, marmalade, relish, chutney or jelly. The seeds are somewhat bitter but have been grounded to a meal for human food in Africa and have also been roasted as a substitute for coffee.

Zobo drink has become a household name in almost every Nigerian home in recent times. The drink if not preserve is, however very susceptible to deterioration by food-borne microorganisms. Therefore, the aim of this study is to:

1. Isolate and identify food-borne micro-organisms associated with the spoilage of zobo drink.
2. Check the effect of sugar, spice and flavour on the bacteriological spoilage of zobo drink.

II. MATERIALS AND METHODS

Sample Collection

Dried zobo plant calyces, the red variety of *Hibiscus sabdariffa*, Ginger *Zingiber officinalis*), Black pepper (*Piper guineense)* were bought from Swali market in Yenagoa, Bayelsa State, Nigeria. They were subsequently professionally verified.

Preparation of Zobo drink

Thirty grammes (30g) of the dried calyces were put into a conical flask containing 500ml distilled water. It was allowed to stand for 40minutes at 100^{o}C in a water bath as outlined in the method of Faye and Neela, (2004). The calyces were removed from the solution by filtration using a sieve cloth. The zobo drink was then aseptically collected into 250ml sample bottles. To one of the bottles 15g of grounded sugar was added (Product 1) and to the other none (Product 2). The method described above was used in preparing spicy zobo drink, but before been placed in the water bath, the spices were added. 2g each of the spices was added. The spicy zobo drink was collected into 250ml bottles after filtration. To one 15g of grounded sugar was added (Product 3) and to the other none (Product 4). The method described above was also used in preparing the spicy, flavoured zobo drink. To the spicy zobo drink in 250ml sample bottle, 1ml of pineapple flavour and 15g of sugar was added to make a complete zobo drink.

Determination of pH

The pH of the zobo products 1-5 was measured using the pHs– 25, digital pH meter.

Viable Count of Bacteria

The method used for the serial dilution of samples was that described by Pelczar, et. al., (1993). The spread – plate method was used in determining the viable count of the various samples of zobo drink. One milliliter of the various dilutions were transferred to sterile nutrient agar in Petri dishes that had been oven dried at 37^{o}C for 30 minutes. A bent sterile glass rod was used in spreading the diluted samples properly on the surface of the nutrient agar plates. The plates were incubated at 37^{o}C for 24 – 48 hours. Different bacterial colonies present in the plates were counted and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count.

Isolation of Microorganisms

The streak plate method was used in isolating pure cultures. The organisms were isolated from the zobo drink samples on the first day of storage. Loopful of the samples (Product 1-5) were aseptically collected and streaked on agar plates. The plates were incubated at 37^{o}C foe 24 hours.

Identification of Isolated Microorganisms

Discrete colonies were picked with a sterilized wire loop and transferred aseptically to fresh agar plates. The essence being to obtain pure cultures of organisms that can be used for further analysis. The identification of microorganisms was based on such tests as:- Gram Reaction, Colonial Morphology, Cell Morphology, Biochemical tests and Sugar fermentation reactions. Biochemical tests carried out included:- motility citrate utilization, methyl red, vogues posteur, indole, coagulase and catalase tests. The identification sequence for single bacterial culture in the manual for identification of medical bacteria (Cowan and Steel, 1974) was used id identifying the isolates.

III. RESULTS AND DISCUSSION

The pH values of the various zobo products stored for six days are shown in Table1. Zobo product 1 had a pH value of 0.68. Product 2 had the lowest pH value of 0.53. The spicy zobo drink had a pH value ranging from 0.69 in Product 4 to 0.71 in Product 3 and 5. The microbial load of the various Products on the first day of storage is shown in Table 2. Product 1 had a bacterial load of 8.1 x 10^{4}cfu/ml. Product 2 had the greatest microbial load of 1.21x 10^{5}cfu/ml. The spicy zobo drink had a lower microbial load ranging from 2.5 x 10^{4}cfu/ml in Product 5 to 3.2 x 10^{4}cfu/ml in Product 4.

The change in colour and aroma of the various zobo products store for 3 days is shown in Table 3. The cultural, biochemical and sugar fermentation characteristics of bacterial isolates of the various zobo products is shown in Table 4 – 8. The organisms isolated from the five zobo product were *Micrococcus, Bacillus, Proteus and Escherichia* species. *Micrococcus* sp was isolated in Product 1, 2 & 4. *Bacillus* sp was isolated in Product 1 & 3. *Proteus* sp was isolated in Product 1 & 5. *Escherichia* sp was isolated in all the Products.

The pH value of Product 2 was the lowest when compared to the others. Oxidation of sugar in Product 2 may have led to the decrease in the pH value. pH decreases with the presence of sugar molecules. Product 3 had a higher pH than Product 2 because it did not contain sugar. Product 3 and 4 shows the effect of spices on zobo drink with or without sugar. The spices hindered the bacterial activity thereby making it's metabolic activity to be reduced. Product 4 had a lower pH due to the presence of sugar which provides additional nutrients which may enable organisms to grow despite the high level of acidity; the spices in it hindered the bacteria from completely oxidizing it. When product 3 was compared to product 1, it was seen that spices had a great role to play in reducing bacteria population. Product 5 was seen to have pH value same as product 3, the pineapple flavoring agent must have contained some substance that have acted with the spices to reduce the metabolic activity of bacteria. The bacterial count of the zobo drink samples ranged from 2.5 x 10^6cfu/ml to 8.1x10^4cfc/ml.

The colour of the zobo drink sample varied from bright red to dark red in the various zobo drink products (Table 3). The control sample was bright red. There was no difference in colour among the various samples from the 1 – 3 days of storage.

The aroma of the control was berry-like on the first and second day of the storage after which it became undesirable throughout the storage period. Zobo drink product 2 had a berry-like aroma on the first and second day of storage after which it became undesirable throughout the storage period. Zobo drink product 3 and 4 had a spicy odour on the first and second day of storage after which it became off odour throughout the storage period. The spicy odour in product 3 and 4 was due to the inclusion of spices on the zobo drink. The product 5 had a fruit odour due to the inclusion of pineapple flavors to the zobo drink on the first and second day of storage after which it became sour throughout the storage period. The gradual change in the aroma of the zobo drink products during storage was due to an increase in bacterial activity in the samples.

The bacteria isolated from the five zobo drink product were *Micrococcus, Bacillus, Proteus, and Escherichia* species. (Table 4 – 8).

Micrococcus species which were detected or isolated in product 1, 2 and 4 of zobo drink are harmless saprophytic bacteria occurring on the skin of humans and animals. *Bacillus* species which was isolated from zobo drink product 1 and 3 are habitants of soil and are able to withstand high temperature due to their ability to form spore (Pelczar *et. al.*, 1993). The presence of *Proteus* species in product 1 and 5 sample might have come from either the water used or from the ingredients used because it is found in water and soil. *Escherichia* species, which occurred in all zobo drink products are inhabitants of the mammalian intestine and are opportunistic bacteria. Some strains are known to cause gastro-enteritis while others cause urinary tract infections in humans. *Escherichia* is a concern to public health because it is an indicator organism in water and its presence in water is undesirable.The spoilage phase of various samples was found after 48 hours of storage at ambient temperature. The processing and storage condition influenced the presence of spoilage organisms in the zobo drink. From the result one can tell that zobo spoils after 2 days of storage at ambient temperature and that spoilage may be cause by the presence of the above-mentioned bacteria. Hence, zobo drink should be consumed within 48 hours of purchase at room temperature.

IV. CONCLUSION AND RECOMMENDATION

Most fruits and vegetables are very susceptible to deterioration by fungi and viruses, more especially by bacteria (Pelczar *et. al.*, 186, 1993). Zobo drink has become a household name in almost every Nigerian home in recent times. The drink if not well preserve is very susceptible to deterioration by food-borne microorganisms. It had been showed from the results that there were less microbial growth in products 3, 4 and 5; this is attributed to the fact that species such as ginger and black pepper had antimicrobial properties which hindered the growth of most bacteria. The bacteria organisms isolated from the various zobo drink samples were *Micrococcus, Bacillus, Proteus and Escherichia* species, which indicates the presence of contaminants from soil, water, humans and animals.

The pH reduces with increases in microbial population due to the metabolic activities of the microorganisms. From the result one can tell that zobo drink spoil after 2 days (48hours) of storage at ambient temperature, hence, zobo should be consumed within 48 hours of purchase at room temperature.

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Table 1: Effects of Sugar, Spices and Pineapple Flavour In the pH of Zobo Drink Samples

	pH						
SAMPLES	**DAY 0**	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**	**DAY 5**	**DAY 6**
Z - S 1	2.43	2.35	2.27	2.17	1.12	0.93	0.68
Z + S 2	2.43	2.36	2.24	2.14	1.11	0.87	0.53
Z- S + S 3	2.56	2.46	2.40	2.18	1.18	0.98	0.71
Z + S + S 4	2.50	2.45	2.38	2.16	1.27	0.92	0.69
Z+S + S + F 5	2.53	2.47	2.39	2.18	1.32	0.97	0.71

Key:

Z-S = Zobo drink without sugar
Z+S = Zobo drink with sugar
Z-S + S = Zobo drink without sugary with spices
Z+S+S = Zobo drink with sugar with spices
Z+S+S+F = Zobo drink with sugar with spices and with pineapple Flavour

Table 2: the Effect of Sugar, Spices and Flavour on the Bacterial Counts of Zobo Drink Product Samples on the first day of Storage

ZOBO DRINK SAMPLES	DILUTION	COLONIES ON PLATES	BACTERIAL COUNTS (CFU/ML)
Z - S 1	10^{-3}	81	8.1×10^{4}
Z + S 2	10^{-3}	121	1.21×10^{5}
Z- S + S 3	10^{-4}	26	2.6×10^{5}
Z + S + S 4	10^{-4}	32	3.2×10^{5}
Z+S + S + F 5	10^{-5}	25	2.5×10^{6}

Key: Cfu ---------- Colony forming units Others -------- Same as Table 1
Determinations were done in duplicates

Table 3: Changes in Colour and Aroma of Zobo Drink Samples Stored for 3 Days.

SAMPLES	COLOUR			AROMA		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Z – S 1	Bright red	Bright red	Dark red	Berry-like	Berry-like	Sour
Z + S 2	Bright red	Bright red	Dark red	Berry-like	Berry-like	Sour
Z – S + S 3	Bright red	Bright red	Dark red	Spicy-odour	Spicy-odour	Off odour
Z + S + S 4	Bright red	Bright red	Dark red	Spicy-odour	Spicy-odour	Off odour
Z + S + S + F 5	Bright red	Bright red	Dark red	Fruit odour	Fruit odour	Sour

Key: Same as Table 1

Table 4: Characteristics of Bacteria Isolated From Zobo Drink Product 1 on the first day of Storage

ISOLATE NUMBER	COLONIAL CHARACTERISTICS	GRAM REACT	CELLULAR MORPHO	MOTILITY	CITRATE	MR	VP	INDO	COAG	CAT	GLU	MAN	MALT	SUCRO	PROBABLE GENERA
1	Circular, cream clour	+	Cocci	+	+	+	-	-	-	+	AG	AG	AG	AG	Micrococcous species
2	Circular, yellowish colony	+	Rods	+	+	+	+	+	+	+	AG	AG	AG	AG	Baccillus species
3	Circular, cream colour	-	Rods	+	+	+	+	+	-	+	AG	-	-	-	Proteus species
4	Creamy colour colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: G – Gas Production, A – Acid Production, + Positive, -- Negative

Table 5: Characteristics of Bacteria Isolated From Zobo Drink Product 2 on the first day of Storage

ISOLATE NUMBER	COLONIAL MORPHOLOGY	GRAM REACT	CELL MORPHOLOGY	MOTILITY	CITRATE	MR	VP	INDO	COAG	CAT	GLU	MAN	MALT	SUCRO	PROBABLE IDENTIFICATION
1	Cream, colour colony	+	Cocci	+	+	+	-	-	-	+	AG	AG	AG	AG	Micrococcous species
2	Cream colour colony	-	Rods	+	+	+	+	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 4

Table 6: Characteristics of Bacteria Isolated From Zobo Drink Product 3 on the first day of Storage

ISOLATE NUMBER	COLONIAL MORPHOLOGY	GRAM REACT	CELL MORPHOLOGY	MOTILITY	CITRATE	MR	VP	INDO	COAG	CAT	GLU	MAN	MALT	SUCRO	PROBABLE IDENTIFICATIN
1	Circular, yellowish colony	+	Rods	+	+	+	+	+	+	+	AG	AG	AG	AG	Bacillus species
2	Circular creamy colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 4

Table 7: Characteristics of Bacteria Isolated From Zobo Drink Product 4 on the first day of Storage

ISOLATE NUMBER	COLONIAL MORPHOLOGY	GRAM REACT	CELL MORPHOLOGY	MOTILITY	CITRATE	MR	VP	INDO	COAG	CAT	GLU	MAN	MALT	SUCRO	PROBABLE IDENTIFICATION
1	Cream, colour colony	+	Cocci	+	+	+	-	-	-	+	AG	AG	AG	AG	Micrococcous species
2	Circular colour colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 4

Table 8: Characteristics of Bacteria Isolated From Zobo Drink Product 5 on the first day of Storage

ISOLATE NUMBER	COLONIAL MORPHOLOGY	GRAM REACT	CELL MORPHOLOGY	MOTILITY	CITRATE	MR	VP	INDO	COAG	CAT	GLU	MAN	MALT	SUCRO	PROBABLE IDENTIFICATION
1	Circular colour colony	-	Rods	+	+	+	+	+	-	+	AG	AG	AG	AG	Proteus species
2	Circular creamy, colour colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 4