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Isolation And Identification Of Food-Borne Micro Flora From Zobo (A Nigerian Drink) Prepared From The Calyces Of *Hibiscus Sabdariffa* L. (Malvaceae)

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----------ABSTRACT----------
This study was undertaken to determine the different types of food-borne micro flora associated with Zobo, a Nigerian drink prepared from the calyces of Hibiscus sabdariffa. Five (5) different categories of Zobo products were prepared and the pour plate method was used in isolating pure cultures. The identification sequence specified for single bacterial culture in the manual for identification of medical bacteria was used in identifying the isolates. Four different organisms isolated were identified as: - Micrococcus sp, Bacillus sp, Proteus sp and Escherichia sp.

***KEYWORDS**: Food-borne Micro flora, Zobo drink, Hibiscus sabdariffa*

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I. INTRODUCTION

Moulds, yeast and bacteria exist almost everywhere on earth (Frazier and Westhoff, 1978). They flourish in the soil of the farm that grows our grains, fruits and vegetables, on the hides and feathers of our meat (animals) and on the fins and organs of the seafood we eat. Many of these microscopic floras create serious problems in close encounters with our food supply (Frazier and Westhoff, 1978).Microbial spoilage is damage or waste caused by microorganisms like bacteria and 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 into the liquid surrounding them and absorb the products of external digestion (Pelczar, et. al., 1986:1993). This is the main basis of microbial spoilage which lowers nutritional value of the product. As a result, products develop undesirable flavours, 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.

Hibiscus sabdariffa L. Malvaceae is a tropical plant of considerable economic importance (Faye and Neela, 2004; Stephen, 1994). 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, 1974). 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.This study was therefore carried out to characterize the various organisms associated with Zobo drink stored at ambient temperature. This information is vital in creating a regime for the preservation of Zobo drink.

II. MATERIALS AND METHODS

Sample Collection

Dried zobo plant calyces, the red variety of *Hibiscus sabdariffa*, Ginger (*Zingiber officinalis*), and 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.

Isolation of Microorganisms

The spread-plate method was used in isolating pure cultures. The organisms were isolated from the zobo drink samples on the first day of storage. Samples (Product 1-5) were aseptically collected and spread on agar plates using a bent sterile glass rod. The plates were incubated at 37^{o}C for 24 hours.

Identification of Isolated Microorganisms

Discrete colonies were picked with a sterilized wire loop and transferred aseptically to fresh agar plates. The essence was 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 in identifying the isolates.

III. RESULTS AND DISCUSSION

The results of the biochemical and sugar fermentation reactions of the various isolates from Product 1-5 showing a probable microbial identification at the end are presented in Tables 1-5 respectively.

In Product 1, Two (2) of the isolates were Gram positive while Two (2) were Gram negative, consisting of one (1) gram positive cocci; one(1) gram positive rod; and two (2) gram negative rods. Product 2 consists of one (1) gram positive cocci and one (1) gram negative rod. Product 3 consists of one (1) gram positive rod and one (1) gram negative rod. Product 4 consists of one (1) gram positive cocci and one (1) gram negative rod. Product 5 consists of two (2) gram negative rods. 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 Micrococci are found in water, soil and on mammalian skin. They are spoilage bacteria that are not thought to be pathogenic. They can grow in the presence of salt and are capable of spoiling foods, particularly cured meat, fish and poultry products. They are aerobic bacteria but can function as facultative anaerobes (Air Products, 2007).The members of the Bacillaceae produce spores as well as vegetative cells. The spores can survive adverse conditions such as drying and pasteurization. These organisms are common in the environment and in many foods. Some strains produce enterotoxins if allowed to grow, for example *Bacillus cereus* in rice dishes and high moisture flour products and members of the *B. subtilis* - licheniformis group in meat and pastry products and meat or seafood rice dishes.

The toxin of *B. cereus* is particularly heat resistant (126°C for 90 minutes) whereas the diarrhoeagenic toxin is inactivated by exposure to 56°C for 30 minutes. High levels (>105 per gram) are necessary to produce enough *Proteus* is found in water and in the soil. They are capable of spoiling foods, particularly fresh / raw meat and eggs (Air Products, 2007).Coliforms, faecal coliforms and *Escherichia coli* coliforms were one of the first groups of bacterial indicators used in the water and dairy industries (Air Products, 2007). They are those members of the Enterobacteriaceae that ferment lactose, and include *Enterobacter*, most *Eshcherichia*, *Klebsiella* and *Citrobacter* species. These organisms are not exclusively of faecal origin. Thermotolerant coliforms are those coliforms that can multiply at 44°C. This group includes *E. coli* types I and II and occasional strains of *Klebsiella* and *Enterobacter*. Most faecal coliforms are able to multiply at 44°C. Whilst the presence

of *E. coli* in food is generally undesirable because it indicates poor hygienic conditions, certain serotypes are pathogenic and may cause gastroenteritis (Air Products, 2007).

Verocytotoxin-producing strains of *E.coli* (VTEC) e.g. *E.coli 0157* may be particularly virulent and can cause symptoms ranging from mild diarrhoea to severe bloody diarrhoea (haemorrhagic colitis), sometimes progressing to haemolytic uraemic syndrome (HUS) and kidney failure.

IV. CONCLUSION

The organisms isolated from the various zobo products - *Micrococcus, Bacillus, Proteus and Escherichia* species indicates the presence of contamination from soil, water, humans and animals.These organisms have been implicated in food spoilage from the literature (Air Products, 2007), therefore their presence in the various zobo products can result in spoilage when the product is stored at ambient temperature.

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Table 1: Characteristics of Bacteria Isolated From Zobo Drink Product 1 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, 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 2: 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
4	Cream colour colony	-	Rods	+	+	+	+	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 1

Table3: 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 IDENTICATION
2	Circular, yellowish colony	+	Rods	+	+	+	+	+	+	+	AG	AG	AG	AG	Bacillus species
4	Circular creamy colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 1

Table 4: 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
4	Circular colour colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 1

Table 5: 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
3	Circular colour colony	-	Rods	+	+	+	+	+	-	+	AG	AG	AG	AG	Proteus species
4	Circular creamy, colour colony	-	Rods	+	+	+	-	-	-	+	AG	AG	AG	AG	Escherichia species

Key: Same as Table 1