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CHEMICAL AND MICROBIOLOGICAL PROFILE OF 'LEGO'-A LOCALLY PRODUCED FLAVOURED DRINKS

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ABSTRACT

The study assessed the chemical and microbial safety of street vended local drink 'Lego' collected from three different vendors in Abeokuta metropolis. A control sample was prepared in the laboratory and nine samples of three different flavours (orange, pineapple and blackcurrant) were collected. Total Vibrio count, total faecal Coliform and total staphylococcus count were estimated in all the samples. Total Vibrio cholera is completely absent while Five samples were found to harbour total staphylococci counts of bacteria within the range of 0.7-1.5x10^{4}cfu/ml. All the samples exhibited the presence of Coliform counts within the range of 1.0-9.5x10^{4}cfu/ml and *Staphylococcus aurous*, *Micrococcus acidophilus* and *Klebsiella aerogenes* species were bacteria isolated from the samples collected. Heavy metals were also estimated in all the collected samples. Chromium and lead were absent but cadmium was present and is within 0.01-0.025mg/g. The lowest is from orange flavour at camp with 0.01 mg/g while the highest is from Lafenwa in pineapple flavour with 0.025mg/g. Total titratable acidity ranges from 0.175 – 0.219 and the pH ranges from 4.21±0.14 and 5.40±0.00. Hence, these implies that Lego flavoured drink is bacteriologically and chemically unsafe for consumption.

Keywords: 'Lego' drink, microbial load, heavy metals, flavour, safety.

INTRODUCTION

Fruit juices are nutritious drinks with great taste and health benefits (Suaad and Hamed, 2008). The consumption of fruit drinks could have both positive and negative effect on the part of consumers. There are several reports of illnesses due to the food borne diseases associated with the consumption of fruit drinks at several places around the globe (Mosupye and Holy, 2000; Muinde and Kuria, 2005; Chumber *et al*., 2007). Several factors can act as source of contamination such as use of unhygienic water for dilution, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust. Such drinks are prone to harbor bacterial pathogens notably *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* (Buchmann *et al*., 1999; Sandeep *et al*., 2004; Barro *et al*., 2006). Fruit juices are well recognized for their nutritive value, mineral, and vitamin contents. Fruit juices processed under hygienic condition could play important role in enhancing consumers' health through inhibition of

breast cancer, congestive heart failure (CHF), and urinary tract infection (Saenz and Sepulveda, 2001; Hyson, 2011).

'Lego' is a flavoured drink, sold by vendors and street hawkers in Abeokuta metropolis. This drink is produced by addition of water and flavour which may be associated with diarrhoea diseases which occur due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants before packaging and disregard of good manufacturing practices (GMPs) and good hygiene practices (GHPs). The producer of these flavoured drinks are often poorly educated, unlicensed, untrained in food hygiene, and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease. Most of the foods are not well protected from flies, which may carry food borne pathogens. Water used in the preparation of this drink can be a major source of microbial contaminants including *Coliforms, Faecal coliforms*, *Faecal streptococci*, *Vibrio cholera* (Tasnim *et al*., 2010). While the quality of fruit drinks is strictly being maintained in the developed countries under several laws and regulations, unfortunately, in many developing countries including Nigeria, the manufacturers are not much concerned about the microbiological safety and hygiene of fruit drinks because of lack of enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks becomes a serious problem (Tasnim *et al*., 2010).

In Abeokuta metropolis, there is a high demand for these flavoured drinks especially among the school children and unfortunately in the roadside shops, recreational areas (parks), and in the busy market places, the microbiological quality of the supplied juices remains questionable. Food borne diseases may be associated with the consumption of this flavored drinks sold by the vendors and the street hawkers due to poor processing and handling. This underscores the need for safety assessment of this emerging beverage mostly consumed by the school children.

In developing countries like Nigeria, drinks, meals, and snacks sold by street food vendors are widely consumed by millions of people. Hence, the present study aimed to assess the chemical and microbial safety of street vended Lego drink in Abeokuta metropolis.

MATERIALS AND METHODS

Sample Collection

Three different samples each of the Lego flavoured drink was collected from three different hawkers at three different locations (Camp, lafenwa, kuto) around Abeokuta metropolis. Three varieties of flavoured drink (strawberry, orange and blackcurrant) were chosen based on the consumer demand.

Microbiological Analysis

Microbiological analysis included enumeration, characterization and identification of potential pathogens according to standard procedures (AOAC, 2012) for the number of bacteria including *Staphylococcus aurous, Micrococcus acidophilus* and *Klebsiella aerogenes* species **.**

Ashing of Sample

2g of blended sample was weighed in a tarred silica dish and was transferred to a temperature controlled muffle furnace, the muffle was kept at 300^{o}C. After the material was dried and charred, the temperature was allowed to rise to 450^{o}C and ashed at this temperature.

After complete ashing, the dish was removed from the muffle furnace, cooled and covered with glass (AOAC, 2012)

Sample Digestion for Atomic Absorption Determination

Two grams (2g) of each food sample was weighed into a 100ml digestion flask. 15ml of concentrated trioxonitrate (v) acid (HNO_3) was added. The digestion flask containing the sample was then placed on a bath (Gerhardt) in a fume cupboard, and sufficiently heated until a clear solution was obtained (AOAC, 2012).

Atomic Absorption analysis of digested samples

After digestion, each of digested samples was carefully poured into a100ml volumetric flask and made up to the mark. The metal elements (Lead, Cadmium and Chromium) were analyzed by using Atomic Absorption Spectrophotometer (Thermoscientific S series, S4AA system) at wavelength of 217.0nm, 228.8nm and 193.7nm respectively using acetylene gas, air and N_2gas.(AOAC, 2012).

Statistical Analysis

All analysis was reported as means of two replicates. Data analysis was carried out using one-way analysis of variance (ANOVA) based on Completely Randomized Design (CRD) using Statistical Package for Social Sciences (SPSS) version 16.0 Mean separation was by Duncan's New Multiple Range Test (DNMRT) and significance accepted at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the microbial loads of three different 'Lego' flavoured drink samples obtained from three different hawkers in Abeokuta metropolis (Camp, Kuto and Lafenwa) and the control was produced in the laboratory. The total *staphylococcus* counts, total *Coliform* counts and total *vibrio cholera* were estimated, which ranges from $0.7x10^4$-$1.5x10^4$ Cfu/ml, $1.05x10^4$-$9.5x10^4$ Cfu/ml and no visible growth for *vibrio cholera* respectively. Most of the fruit drink samples exhibited equal or much higher bacterial than permitted. For staphylococcus counts, five samples had no counts at all while the lowest is the control with $0.7x10^4$ Cfu/ml. The highest bacterial load for the staphylococcus counts for hawked fruit drink was found in pineapple (L2) collected from Lafenwa and pineapple flavour from camp (C1) which was $1.5x10^4$Cfu/ml. While the total Coliform counts were within $1.0x10^4$ – $9.5x10^4$Cfu/ml. Pineapple sample for Lafenwa market had the lowest count $1.0x10^4$ Cfu/ml while orange samples from Kuto had the highest Coliform counts with $9.5x10^4$Cfu/ml. Total *Vibrio cholera* was completely absent; there was no visible growth on all the samples.

It shows that the samples obtained from Kuto market had more microbial contaminants compared to samples from Lafenwa and camp. All the samples from Kuto had the highest count for total Coliform counts compared to other samples. According to Rashed et al, (2013), these values does not conformed to the recommended microbiological standards for any fruit drink; all numbers are as per ml of juice consumed (Gulf Standards, 2000).

The variation in the microbial loads obtained in the drinks might be due to poor handling, unhygienic environment during the cause of processing and the use of unclean water. Since it is a locally produced drink, the producers are untrained personnel and are ignorant of Good manufacturing practices, Good hygienic practices as well as food safety

which are paramount in the cause of food processing and improper handling. Pathogens may enter during processing, like using dirty container for mixing, using unwashed hands, unsterilized packaging materials and utensils for mixing the additives, using mouth in blowing the packaging materials before sealing manually or tied.

Table 1: Total *Staphylococcus* count, *Vibrio* count and *Coliform* Count

Sample No	Type of drink	Sampling area	Total Staphylococcus Count (Cfu/ml)	Total Vibrio Count (Cfu/ml)	Total Coliform Count (Cfu/ml)
A	Pineapple	Control	0.7x104	NG	2.5 x104
C1	Orange	Camp	NG	NG	1.05x104
K1	Orange	Kuto	NG	NG	9.5x104
L1	Orange	Lafenwa	1.2x104	NG	7.5x104
C2	Pineapple	Camp	1.5x104	NG	8.5x104
K2	Pineapple	Kuto	NG	NG	9.0x104
L2	Pineapple	Lafenwa	1.5x104	NG	1.0x104
C3	Blackcurrant	Camp	NG	NG	6.5x104
K3	Blackcurrant	Kuto	NG	NG	8.0x104
L3	Blackcurrant	Lafenwa	1.3x104	NG	8.5x104

NG-No Growth

Inadequate storage facilities may also harbour microbes because the drinks contain additives and when it is sealed it must be kept freezed and/or iced so as to maintain its quality, failure in doing this might leads to loss in its quality and make it unsafe for consumption.

Due to the illiteracy of the producer, codes of practices provided for food safety, guidelines and packaging must have been neglected. The packaging materials might also have low barrier properties thereby embodying pathogen into the drink unlike large scale that goes for high density packaging materials. Proper use of additives in preparing drinks preserves food and prevents it from spoilage when used without measurement is harmful to the consumer's health.

The results revealed Lego flavoured drink might potentially increase the risk of food borne disease due to the presence of food pathogens found there-in. It is hard to affirm to the sanitary conditions during the cause of production and the packaging materials used. Most of the street drinks are not officially admitted and vendors operate unauthorized with adequate policy (Obadina et al., 2011).

Characterization of the microbial counts

Table 2 shows bacteria species that were isolated from the samples collected which are *Staphylococcus aurous, Micrococcus acidophilus* and *Klebsiella aerogenes* species. This is similar to (Rashed *et al*., 2013) who worked on fruit juices from different vendor and found *E. coli*, *Klebsiella* and *Staphylococcus* spp. in addition to that, *Micrococcus acidophilus* was found Pineapple flavours from the three locations harbour this three species (*Staphylococcus aurous, Micrococcus acidophilus* and *Klebsiella aerogenes*).

Table 2: Characterisations of the isolates from flavoured 'Lego' drinks

Sample	Flavour	Gram Stain	Shape	Motility	Catalase	Oxidase	Coagulase	Urease	Indole	Methyl Red	V P	Gelatin	Starch	Caesin	Probable Identification
C1	Orange	+	C	-	+	-	+	+	+	-	-	+	+	-	S A
C1	Orange	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
K1	Orange	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
KI	Orange	-	R	-	-	+	-	+	+	+	-	+	-	-	K A
L1	Orange	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
L1	Orange	+	C	-	+	-	+	+	+	-	-	+	+	-	S A
C2	Pineapple	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
C2	Pineapple	-	R	-	-	+	-	+	+	+	-	+	-	-	K A
K2	Pineapple	+	C	-	+	-	+	+	+	-	-	+	+	-	S A
K2	Pineapple	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
L2	Pineapple	+	C	-	+	-	+	+	+	-	-	+	+	-	S A
L2	Pineapple	-	R	-	-	+	-	+	+	+	-	+	-	-	K A
K3	Blackcurrant	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
K3	Blackcurrant	+	C	+	+	+	-	-	-	-	-	+	+	-	M A
L3	Blackcurrant	-	R	-	-	+	-	+	+	+	-	+	-	-	K A
L3	Blackcurrant	+	C	+	+	+	-	-	-	-	-	+	+	-	M A

KEYS: - Negative, + Positive, C- cocci, R- Rod, SA- *Staphylococcus aurous, MA-Micrococcus acidophilus, KA- Klebsiella aerogenes*

Colony morphology and biochemical traits of the isolates following incubation for 24 hours, typical pink, circular, convex colonies on MacConkey Agar, yellow colonies on Mannitol Salt Agar were initially considered as Coliforms, and Staphylococcus spp, consecutively. Based on the biochemical characteristics, isolates were confirmed as *Klebsiella aerogenes*, Staphylococcus spp and *Micrococcus acidophilus.*

Heavy metals in the sampled 'Lego' drinks

Table 3 shows the result of heavy metals found in the collected samples. Cadmium, lead and chromium were tested on the samples but chromium and lead was absent in all the samples while cadmium was present in every samples except the control.

The highest value of cadmium in the sample was found in pineapple drink which is 0.025mg/g and it was collected from Lafenwa. The lowest is 0.01mg/g from camp and it is found in orange drink collected at camp. The control and black currant conformed to recommended limits U.S.E.P.A (1994) which is 0.1ppm.

According to Food Safety Authority of Ireland, heavy metals are food contaminant and they are regarded as substance that gets into food unintentionally which might be chemicals, stones, insects and debris. Carelessness in the cause of production might be the possible causes of the presence of the heavy metal (cadmium) in the drink as the result of contamination by exposure to dust particles before packaging and according to (Hanaa et al., 2000) this is due to human activities. It might occur naturally and drinks containing high levels of essential metals, or toxic metals such as cadmium, chromium and lead may be hazardous to human health.

High exposure to these heavy metals has great consequences on human health. They affect minerals in the body. The metals of particular concern in relation to harmful effects on health are lead, cadmium, and chromium. The toxicity of these metals is in part due to the fact that they accumulate in biological tissues, a process known as bioaccumulation. This process of bioaccumulation of metals occurs in all living organisms as a result of exposure to metals in food and the environment.

pH and Total Titratable Acidity present in the 'Lego' drinks

Table 4 shows the total titratable acidity which is the amount of acid present in the drink and pH which is the measures of the strength of acid in the Lego drink.

There is a significant difference in the pH of all samples and this is attributed to temperature and duration of storage at such location. Lafenwa location, the group consuming commercially available pineapple showed the highest pH which ranges between 4.53±0.21 - 5.4±0.00. There was not a significant difference in the pH range as regard camp location. The pH ranges between 4.42±0.07 - 4.93±0.07.

The difference in pH of the fruit juice as consumed commercially could be attributed to the relatively lower intrinsic pH of commercially available fruit juices. Similar results have been shown by Lata and Amitha, 2003, Birgul *et al* (2003), Sanchez and Fermandez, (2003) and Birgul e*t al* (2008).

All the samples were acidic which might be due to the additives added. It contained sugar and can tend to fermentation after packaging and leads to its acidity.

The measure of the hydrogen ion (acid) concentration in water is called pH. A pH of 7 is neutral. Values above 7 are alkaline or basic; those below 7 are acidic. Therefore, it is noteworthy that location has inevitable effect on the fruit juice to resist pH change as observed in the study.

Table 3: Heavy Metals determination in flavoured 'Lego' drinks

Sample No	Type of drink	Sampling Area	Chromium (Cr) (mg/g)	Cadmium (Cd) (mg/g)	Lead (Pb) (mg/g)
A	A	Control	0	0.0±0.0	0
C1	Orange	Camp	0	0.01±0.00ab	0
K1	Orange	Kuto	0	0.012±0.00ab	0
L1	Orange	Lafenwa	0	0.015±0.007bc	0
C2	Pineapple	Camp	0	0.01±0.00ab	0
K2	Pineapple	Kuto	0	0.015±0.007bc	0
L2	Pineapple	Lafenwa	0	0.025±0.007c	0
C3	Blackcurrant	Camp	0	0.015±0.007bc	0
K3	Blackcurrant	Kuto	0	0.015±0.007bc	0
L3	Blackcurrant	Lafenwa	0	0.020±0.00bc	0

Values are means of duplicate analysis. Mean values having different superscripts show significant differences (P< 0.05) across the columns.

Table 4: pH and Total Titratable Acidity of flavoured 'Lego' drinks

Sample No	Type of drink	Sampling Area	pH	TTA
A	A	Control	4.42±0.57	0.0±0.0
C1	Orange	Camp	4.93±0.07	0.01±0.00ab
K1	Orange	Kuto	4.81±0.28	0.012±0.00ab
L1	Orange	Lafenwa	5.40±0.00	0.015±0.007bc
C2	Pineapple	Camp	4.42±0.07	0.01±0.00ab
K2	Pineapple	Kuto	4.53±0.28	0.015±0.007bc
L2	Pineapple	Lafenwa	4.53±0.21	0.025±0.007c
C3	Blackcurrant	Camp	4.47±0.14	0.015±0.007bc
K3	Blackcurrant	Kuto	4.21±0.14	0.015±0.007bc
L3	Blackcurrant	Lafenwa	5.11±0.14	0.020±0.00bc

Values are means of duplicate analysis. Mean values having different superscripts show significant differences (P< 0.05) across the columns.

CONCLUSION

From the result obtained it was concluded that Lego flavoured drink is bacteriologically and chemically not safe for consumption. The results shows high microbial load which shows *Staphylococcus aureus, Micrococcus acidophilus and Klebsiella aerogenes* as probable bacteria after identification but Vibrio cholera was absent. Heavy metals were within standards and there were no variation in recommended limits for the control and black currant because they conformed to recommended limits except cadmium while the pH and titratable acid are acidic. However, its safety can be improved upon.

RECOMMENDATIONS

Good manufacturing practices should be ensured in the production of Lego drink. Awareness of food safety and hygiene must be emanated among consumer and the producers. The water should be treated and the packaging material must be sterilized. Food handler should wash their hands before production and the drinks should be produced in a clean environment.

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