Journal of Global Biosciences
ISSN 2320-1355
Volume 7, Number 8, 2018, pp. 5573-5584
Website: www.mutagens.co.in

Research Paper

MICROBIAL, SENSORY AND NUTRITIONAL PROPERTIES OF LABORATORY PREPARED SORREL (ZOBO) DRINKS FORTIFIED WITH SPICES AND SUGAR

Ekanem, Jonathan Okokon

Department of Microbiology,
University of Uyo,
PMB 1017, Uyo, Akwa Ibom State,
Nigeria.

Abstract

The microbial load of laboratory prepared zobo drink produced from the extract of *Hibiscus sabdariffa* mixed was examined. The physiochemical, nutritional and sensory properties of the zobo drink was investigated. The moisture content (82.4%) was high while other parameters ash (0.93%), crude fat (1.31%), crude protein (6.8%), carbohydrate (8.54%), calorie (73.1kcal), TTA (0.22mg/l) was determined. The pH was low as the freshly prepared zobo drink harbored no significant microbial load. The spiced zobo drinks were free from any microbial contamination. The result from the sensory parameters evaluated showed preference of refrigerated zobo drink to that stored at room temperature with respect to taste, flavor, color, consistency and overall acceptance.
Key words: microbial, zobo drink, sensory parameters and nutritional.

INTRODUCTION

Zobo drink, a non-alcoholic local beverage is produced from the dried petals of *Hibiscus sabdariffa* (Linn Roselle) by boiling and filtration [1]. *Hibiscus sabdariffa* (Roselle) is a Vegetable plant of West Africa origin. It has however been noted that the crop is native to India but was introduced to other part of the world such as central Africa, West Indies, Australia, Africa and many tropical countries as it is best grown in tropical and subtropical regions [2]. It has the most widespread acceptance in the Roselle producing areas of the Nigerian savanna region where it is grown as a vegetable crop [2]. The Roselle is an annual herbaceous, upright plant growing up to two metres belonging to the family Malvaceae. Its habitat is variable and the leaves also vary in shape and size. The flowers are usually yellowish, sometimes occurring with dark red pigmentation at

the center [3].

Zobo drink has gained wide acceptance, being consumed by several millions of people from different socio-economic classes and background. Zobo drink has been shown to be good source of natural carbohydrate, protein and vitamin C which constitutes the major reason for consuming soft drink and fruit juice [4]. Many researchers have reported on the preparation and preservation of zobo drinks with different food items, such as lime. [5] revealed that total coliforms and total viable counts generally decreased in values following treatment of zobo drink samples with lime juice. Large-scale production of zobo drink is limited by its tendency for rapid deterioration. It has a shelf-life of approximately twenty-four hours following production, if not refrigerated.

The drink is prone to microbial contamination which can lead to food spoilage [2]. A lot of factors such as, packaging mode before retailing, and the poor hygienic practices as well as lack of portable water, proper storage and waste disposal facilities at preparation and service points have resulted in poor unsanitary conditions and thus served as potential contaminants and increased risk to public health in commercial Zobo drinks sold [6]. Several researchers have reported high microbial counts on commercial zobo drinks sold in various markets and locations in the country ([8], [9] and [7]). The present study is aimed at evaluating the microbiological and nutritional properties of home-made zobo drinks prepared using standard procedures and also the effect of spices on the sensory parameters of the zobo drink under different storage conditions.

MATERIALS AND METHODS

Sample Collection

Samples of the calyces of *Hibiscus sabdariffa* and sugar were obtained at Ator market, Ikot Ekpene, Akwa Ibom State. Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) were also obtained from the same market. The samples were kept in clean transparent polythene bags and stored at room temperature of 28^{o}C and were subsequently professionally verified. The organisms were maintained on Nutrient Agar slants at 4^{o}C in the refrigerator.

Preparation of Zobo drink

The calyces sample was prepared by sorting which involves the removal of unwanted particles such as dirt, stones, etc. The calyces of *Hibiscus sabdariffa* was extracted for its anthocyanin components using the hot water extraction method. Sixty grammes (60g) of the dried calyces were put into a conical flask containing 1000ml distilled water. It was allowed to stand for 40minutes at 100^{o}C in a water bath as outlined in the method of [10], the calyces were removed from the solution by filtration using a sieve cloth.

Preparation of Spices

The ginger rhizomes and garlic bulbs were washed with potable water repeatedly. Their outer covering was peeled off with a sterile knife and then sliced into cutlets and dried using a hot air oven at 65^{o}C for 48 h. An electric blender, the dried ginger and garlic bulb were pulverized into powder.

Storage of Samples

The Zobo juice samples with the spices were stored at room temperature (28^{o}C ±2^{o}C) and refrigeration temperature for 5 days.

Experimental Set up for Sensory and Microbiological Analysis

The zobo drink was then aseptically divided into four parts containing 200 mls each. The four parts were labeled A, B, C and D respectively. Sample A (ordinary Zobo drink) serves as control, Sample B (Zobo drink and Sugar), Sample C (Zobo drink, Sugar and Garlic) and Sample D (Zobo drink, Sugar and Ginger).

Product Samples	**Experimental Design**
A	200ml of Zobo drink (control)
B	200ml of Zobo drink + 15g of Sugar
C	200ml of Zobo drink + 2g of Garlic
D	200ml of Zobo drink + 2g of Ginger

Key: A = Zobo drink, B = Zobo drink + Sugar, C = Zobo drink + Sugar + Garlic, D = Zobo drink+ Sugar + Ginger

Physicochemical analysis of fruit juice

Determination of pH: Ten milliliters of the juice was dispensed into a beaker and the pH was determined with a previously standardized pH meter (JenWay 3505). The pH meter was calibrated using phosphate buffer of pH 4.0 and 7.0 [11].

Moisture Determination:

Ten ml of sample was measured in a clean crucible using sensitive balance. The crucible with the sample was placed in an air-dry oven at 105°C and left to stay overnight. Then crucible was transferred to oven again and weighted after 2 hours, this was repeated until constant weight was obtained.

Calculation:

$$\text{Moisture Content \%} = \frac{(W_2-W_1)-(W_3-W_1)\times 100}{W_2-W_1}$$

where:

W_1 = weight of empty crucible

W_2 = weight of crucible + sample

W_3 = weight of crucible +dry sample

Determination Total Titratable Acidity (TTA): Standard method of ([26], [27]), was used to measure the titratable acidity. Five of the sample of fruit juice were homogenized in distilled water (20) and filtered through whatman No. 1 filter paper. Phenophthalein was added to 20 of the filtrate as indicator and titrated against 0.05 M NaOH. Titratable acidity was calculated using the equation:

$$TA = \frac{MNaOH \times NaOH \times 0.09 \times 100}{\text{Juice sample}}$$

Where:

TA = Titratable acidity

MNaOH = Molarity of NaOH used

NaOH = Amount (in) of NaOH used

0.09 = Equivalent weight of lactic acid

Determination of Total Ash: The ash content was determined from the loss in weight that occurred during incineration of the evaporated sample at a temperature high enough to allow all organic matter to be burnt off without allowing appreciable decomposition of the ash constituents. Ashing was carried out in a muffle furnace subjected to heat at 550°C for 6 h [11].

Determination of Fat: This was carried out using the method of [11]. Clean and dried thimble was weighed (W_1) and 5 g oven dried sample was added and re-weighed (W_2). Round bottom flask was filled with petroleum ether (40-60°C) up to ¾ of the flask. Soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boiled gently, the sample was put in the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 h. After the barrel of the extractor was emptied, the condenser was removed and the thimble removed, taken into the oven at 100°C for 1 h and later cooled in the desiccator and weighed again (W_3):

$$\text{Fat (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Estimation of Crude Fibre: About 2g of the sample was defatted with petroleum ether for 2 hours It was then boiled under reflux for some minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml solution. The solution was filtered through a cloth on fluted funnel and washed with boiling water until they were no longer acidic. The residue was transferred to a beaker and boiled for another 30 minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. The final residue was then filtered and washed with boiling water several times until it was no longer basic. The residue was finally washed twice with methanol and quantitatively transferred into a pre-weighed crucible and dried at 105oc (1a). The furnace was incinerated at 550oc and was allowed to stand at this temperature for 2 hours. It was then cooled in a dessicator and weighed as 1o [11].

Calculation

$$\frac{1a\text{-}1o \times 100}{\text{Weight of original sample taken}}$$

Where; 1a = weight of empty crucible

1o = weight of crucible and its content after incineration

Determination of Carbohydrate: This was determined as the difference obtained after subtracting total organic nitrogen (protein), Lipid, Ash, Moisture and Fibre from the total dry matter [11].

Calculation

Carbohydrate % = 100 – value of moisture, protein, lipid, ash and fibre
i.e CHO = (M+P+L+A+F)

Determination of Caloric Value (Energy Level)
The caloric value was obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4:9:4 kcal respectively and taking the sum of the product.

Determination of Crude Protein: One gram of the sample was introduced into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixtures were digested on an electro thermal heater until a clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50 and 5 of this were transferred into the distillation apparatus, 5 of 2% boric acid was added into a 100 capacity conical flask (the receiver flask) and four drops of methyl red indicator were added. A 50% of NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Distillation was carried out in the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask will be about 50 after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1 M HCl and the protein content calculated ([11], [28]).

Determination of Total Solids: Total solids content was determined by evaporating a known weight of juice in an oven (Fisher Isotemp 175) at 105°C for 2-3 h. The solid left after evaporation was then weighed and used to calculate the total solids [11].

Determination of Total Bacterial and Fungal Counts

Samples were serially diluted aseptically using 1ml of kunu samples with 9ml of sterile distilled water to reduce the microbial load. After dilution, about 0.1ml of appropriate dilution was used to inoculate Nutrient agar (NA) and MacConkey agar (MAC) plates in triplicates for isolation of bacteria; and Potato dextrose agar (PDA) plates in triplicates for isolation of fungi. The culture plates for isolation of bacteria were incubated at 37°C for 24 hours while the PDA plates were incubated at 30°C for 96 hours for enumeration of colonies. The mean triplicate results on NA, MAC and PDA plates were then enumerated for total mesophilic bacteria, coliform bacteria and fungi respectively and recorded as colony forming unit per millimeter (cfu/ml) of kunu samples [12].

Sensory Evaluation

Sensory evaluation is unique source of product information concerned with measuring the response of people to products in terms of appearance, aroma, taste, texture and after and after taste without benefit of label, pricing or other imagery [25].

The sensory evaluation was carried out by 24 panelists composed of 12 males and 12 females recruited from the staff and students of the department of Microbiology, University of Uyo. There was a comparison test between the zobo juice stored in the refrigerator and that stored at room temperature. Zobo juice preserved with ginger/garlic was compared with zobo juice preserved with sugar. The evaluation was based on quality parameters such as taste, flavor, colour and overall acceptance (using questionnaire) by the panel of 24 testers. The test panelists were asked to rate the different juices presented to them on a 9 point hedonic scale ([13], [14]).

RESULTS

Table 1: Proximate and Physicochemical Composition of Home-made Zobo Drink

Parameter	Values
Moisture	82.42%
Ash content	0.93%
Crude fat	1.31%
Crude fibre	ND
Crude protein	6.80%
Carbohydrates	8.54%
Calorie	73.1kcal
Total Titratable acidity	0.22mg/l
pH	3.6

ND = Non detected

Table 2: Total Bacterial and Fungal Counts of Zobo Drinks Fortified With Sugar, Ginger and Garlic

Samples	Nutrient Agar	MacConkey Agar	Potato Dextrose Agar
A	0.5×10^2	0.5×10^2	No growth
B	No growth	No growth	No growth
C	No growth	No growth	No growth
D	No growth	No growth	No growth

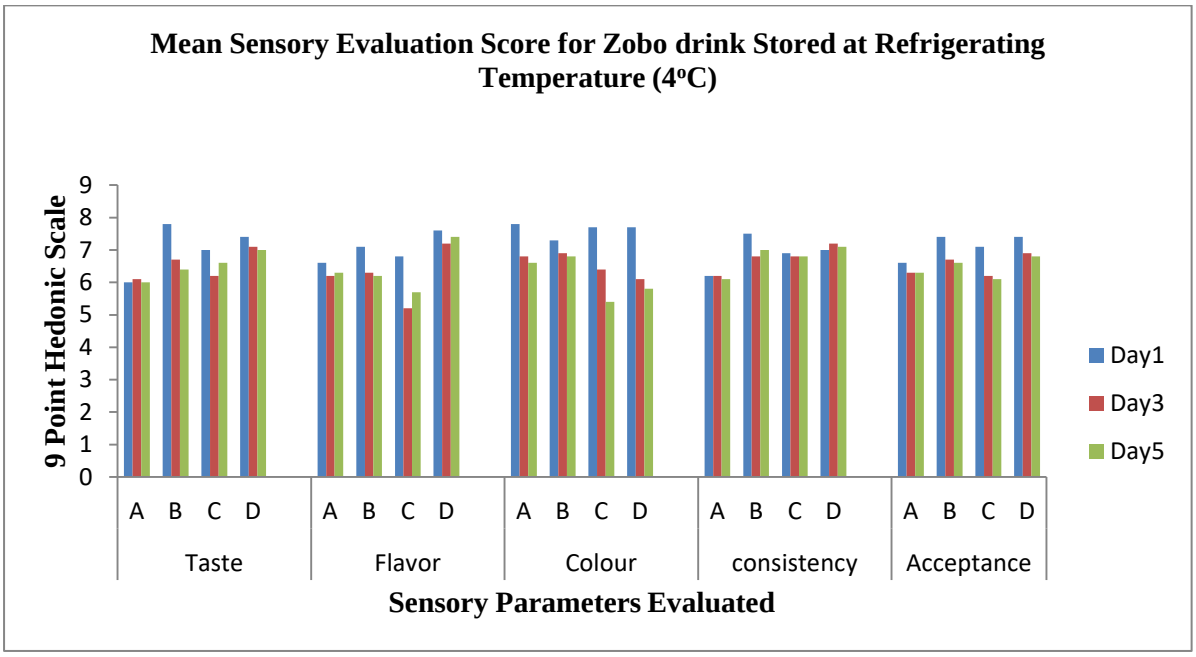

Figure 1: Mean Sensory Evaluation Score for Zobo drink Stored at 4°C

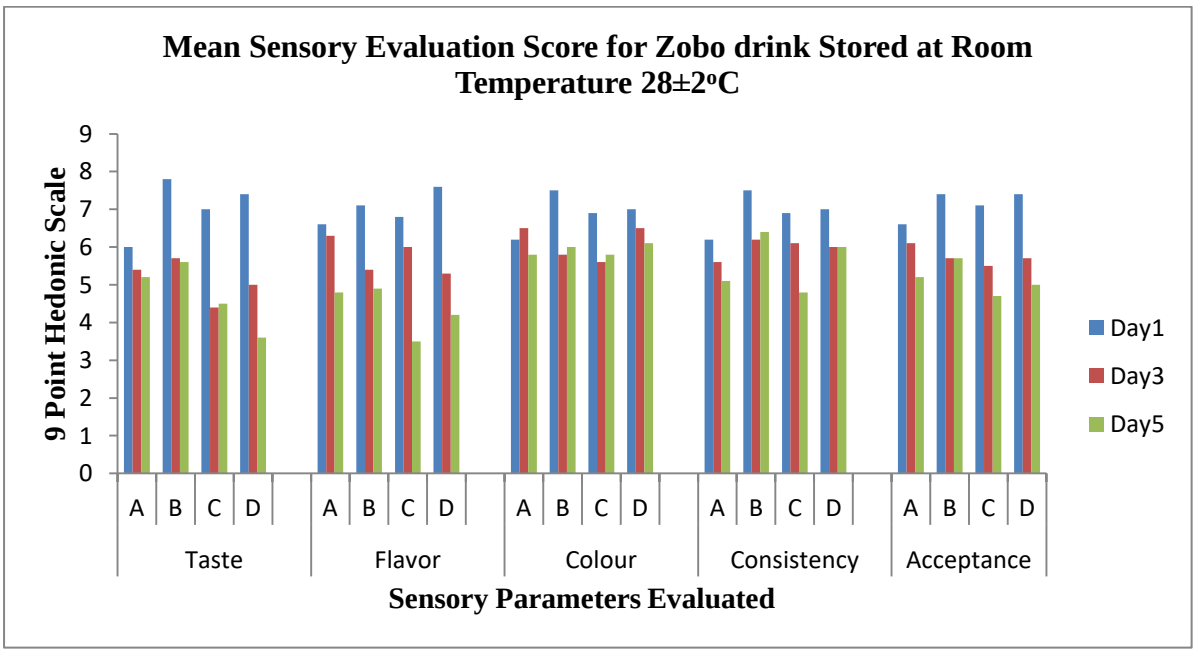

Figure 2: Mean Sensory Evaluation Score for Zobo drink Stored at 28±2°C

The pH of the zobo drink sample was 3.6, the moisture content was 82.42%, the ash content was 0.93%, the crude fat and crude protein were 1.31% and 6.80% respectively. Crude fibre was not detected while carbohydrate was 8.54%, calorie was 73.1kcal, titratable acidity was 0.22mg/l. The microbial counts from Zobo fortified with preservatives are presented in Table 2. For A it was 0.5×10^2 for total bacterial count, 0.5×10^2 for total coliform count and no growth for total fungal counts. Samples B, C and D had no growth on all the media used.

The mean value for the sensory evaluation is presented in figures 1 and 2 for both samples stored at refrigerating and room temperature respectively. The result of the sensory evaluation showed that the zobo drinks stored at the refrigerating temperature was preferred to that stored at room temperature regardless of the parameter examined as shown by the higher scores obtained.

DISCUSSION

The pH of the zobo drinks were all on the low side indicating and confirming the high acidity usually noticed in zobo drinks, it is found to be a naturally acidic fruit rich in organic acids ([14], [15]). The zobo drink also has some levels of protein and carbohydrates which confirms its nutritional value. The microbial counts recorded are far below that reported from most researchers on commercial zobo drinks sold ([8], [17], [18]). [19] reported average total count of 1.6×10^6 cfu/ml and 2.6×10^5 cfu/ml for aerobic bacterial and coliform counts respectively. This report on microbial counts is in agreement with that of [20] where counts of 2.0×10^2 were recorded for both laboratory prepared zobo and fortified zobo drinks during steeping and sieving while no growth was recorded during boiling and packaging. The low microbial load recorded in this research is due to the standard hygienic condition in which the processing and production of the zobo drink was done as the materials used for its production were prepared aseptically. The no growth observed in the fortified zobo drinks with spices and sugar is due to the antimicrobial properties from both the spices and sugar. According to [21] addition of some spices reduces microbial load significantly. Other researchers have also reported the inhibition of growth via fortification ([22], [23], [24]). The mean value of Twenty four (24) panelists showed that zobo drinks stored at refrigerating temperature were preferred to that stored at room temperature as

indicated by the higher scores obtained. It was also evident that majority of the panelist preferred the fortified zobo drink for all the sensory parameters analyzed.

CONCLUSION

It is therefore suggested that zobo drinks should be properly processed to avoid microbial contamination. Treated municipal water or clean water should be used during processing and dilution of the processed drinks to avoid microbial contamination. Addition of spices such as ginger and garlic to the processed zobo is also advocated. The processing environment should be hygienic, while the packaging materials should be sterilized and additives such as sugar, ginger, garlic and others should be properly sterilized. Owing to the quick deterioration and low shelf-life of this drink, it should be refrigerated for preservation and the maintenance of its organoleptic properties.

REFERENCES

[1] Ameh, A. O., Isa, M. T., Ahmed, A. S. and Adamu, S. B. (2009). Studies on the use of Troma in improving the taste of the Extract from *Hibiscus sabadariffa* Calyx; *Nigerian Journal of Pharmaceutical;* Vol 8No1, ISSN: 0189-823x

[2] Omemu, A. M; Edema, M. O; Atayese, A. O and Obadina, A. O. (2006). A survey of the microflora of *Hibiscus sabdariffa* (Roselle) and the resulting zobo juice. *African Journal of Biotechnology*. 254-259.

[3] Rice, R. P. (1990). Fruits and vegetables production is warm climates. 1st edition.Macmillam press Limited, London Uk. Pp. 40-65.

[4] Braide, W; Oranusi, U. S and Peter-Ikechukwu, A.L (2012). Perspectives in the hurdle techniques in the preservation of a non-alcoholic beverage, zobo. *African Journal of Food Science and Technology* 3(2): 46-52.

[5] Nwachukwu, E; Onovo, O. M and Ezeama, C.F (2007). Effect of lime juice on the bacterial quality of zobo drinks locally produced in nigeria. *Research Journal of Microbiology*, 2: 787-791.

[6] Omemu, A. M and Aderoju, S. T. (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, *Nigeria. Food Control*, 19:396–402.

[7] Raimi, O. R. (2013). Bacteriology quality of zobo drinks consumed in some parts of Osun State, Nigeria. *J. Appl. Sci. Environ. Manage*. 17:113-117.

[8] Ayandele A. A. (2015). Microbiological Analyses of Hawked Kunun and Zobo Drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 9 (10): 52-56.*

[9] Danladi, M. M., Ify, O. A., Joseph, O. A. and Eleojo, A. A. (2014). Antibiotic susceptibility pattern of bacteria isolated from Zobo drinks sold in Keffi, Nigeria. *Malaysian journal of microbiology,* 10(3): 169-173.

[10] Faye, D.H.C and Neela, B., (2004). Consumer acceptance and Physicochemical quality of processed red Sorrel / Roselle (Hibiscus sabdariffa) sauces from enzymatic extracted calyces. *Food Science Technology*, 4(4):141-148.

[11] AOAC. (2005). Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 978-0935584752.

[12] Harrigan, WF; McCance, ME (1976). Laboratory methods in food and dairy microbiology. Academic Press Ind. Ltd, London, pp: 25-29, 157, 285-303.

[13] Hashimi, M. S., Alam, S., Riaz, A and Shah, A. S (2007). Studies on microbial and sensory quality of mango pulp storage with chemical preservatives. *Pakistan Journal of Nutrition,* 6: 85-88.

[14] Larmond E (1977) Laboratory methods for sensory evaluation of foods (1st edn.). Food Research Institute Central Experimental farm, Canada. Department of Agriculture. Ottawa.

[15] Olayemi Foline, Adedayo Rachael, Muhummad Ruqayyah and Bamishaiye Eunice, (2011). The Nutritional Quality of Three Varieties of Zobo (*Hibiscus sabdariffa*) Subjected to the Same Preparation Condition. *American Journal of Food Technology, 6: 705-708.*

[16] Wong, P. S, Yusof, H. M, Cheman, Y. E (2002).Physico-chemical characteristics of roselle (Hibiscus sabdariffa L.).Nutr.Fd. Sci. 32: 68-73.

[17] Amusa, N.A., O.A. Ashaye, A.A. Aiyegbayo M.O. Oladapo, M.O. Oni and O.O. Afolabi, (2005). Microbiological and Nutritional Quality of Hawked Sorrel Drinks (Soborodo) (the Nigerian locally brewed soft drinks) widely consumed and notable drinks in Nigeria. *Journal of Food, Agriculture & Environment*, 3: 3-4.

[18] Umaru, G. A., Tukur, I. S., Akensire, U. A., Adamu, Z., Bello, O. A., Shawulu A. H. B., Audu, M., Sunkani, J. B., Adamu, S. G. and Adamu, N. B. (2014). Microflora of Kunun-zaki and Zobo Drinks in Relation to Public Health in Jalingo Metropolis, North-Eastern Nigeria. *International journal of food research,* 1: 16-21.

[19] Ezeigbo, O. R; Uhiara, S; Nwodu, J. A and Ekaiko, M. U. (2015). Bacteriological Assessment of Hawked Sorrel Drink (Zobo Drink) in Aba, South-East Nigeria, *British Microbiology Research Journal*, *5(2): 146-151.*

[20] Adeniji, P. O. (2017) Nutritional, Sensory and Microbiological Quality Assessment of fortified Zobo Drink: A Home-Prepared Traditional Nigerian Beverage. *J Nutr Food Sci* 7: 627.

[21] Adebesin, A.A., Saromi, O.T., Amusa, N.A. and Fagade, S.O. (2001). Microbiological quality of some groundnut products hawked in Bauchi, a Nigerian City. The Journal of Food Tech. in Africa 6:35–38.

[22] Oboh, G. and Elusiyan, C.A. (2004). Nutrient composition and antimicrobial activity of sorrel drinks (soborodo). J Med. Food 7(3):340-342.

[23] Zaika, L.L., Kissinger, J.C. and Wesserma, A.E. 1983. Inhibitory of lactic acid bacteria by herbs. J. Food Sci. 48:1455–1459.

[24] Adegoke, G.O. and Skura, B.J. (1994). Nutritional profile and antimicrobial spectrum of the spices Aframomum danielli, K. shum. Plant Foods for Human Nutrition 45:175–182.

[25] Iwe, M. O. (2002). Handbook of Sensory Methods and Analysis. Projoint Communications services Ltd, Enugu. pp. 70-72.

[26] Chandra,T.S. and Antony, U. (1997). Microbial population and biochemical changes in fermenting finger millet (*Eleusine coracana*). *World J. Microbiol. Biotechnol*., 13: 533-537.

[27] Ferrati, A. R., Tavolaro, P. Destro, M. T. Landgraf, M. and. Franco, B. D. G. M. (2005). A comparison of ready-to-use systems for evaluating the microbiological quality of acidic fruit juices using non-pasteurized orange juice as an experimental model. *Int. Microbiol*., 8: 49-53.

[28] Pearson, D (1970). The Chemical Analysis of Foods. 6th Edn., Churchill Livingstone Publ., London, UK., ISBN-13: 978-0700014576, p. 604.