British Microbiology Research Journal
6(3): 147-153, 2015, Article no.BMRJ.2015.067
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antimicrobial Effect of Lime Juice Treatment on the Shelf-Life of Zobo Drink

O. R. Ezeigbo[1*], M. U. Ekaiko[1], N. G. Agomo[1], K. Ojukwu[1] and A. I. Nnadozie[2]

[1] Department of Biology/Microbiology, Abia State Polytechnic, Aba, Nigeria.
[2]Department of Biological Sciences, Federal University of Technology, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ORE designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MUE managed the analyses of the study, while authors NGA, KO and AIN managed literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/15534
Editor(s):
(1) Raúl Rodríguez-Herrera, Autonomous University of Coahuila, México.
Reviewers:
(1) Anonymous, Ghana.
(2) Adelekan Aminat Olabisi, Food Science And Technology, Bells University of Technology, Ota, Ogun State, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=829&id=8&aid=7585

Original Research Article

Received 3rd December 2014
Accepted 15th December 2014
Published 31st December 2014

ABSTRACT

Zobo drink is an indigenous non-alcoholic drink made from hot water roselle calyces of *Hibiscus sabdariffa*. The drink has gained wide acceptance, as it is consumed by several millions of people from different socio-economic classes and background in West African sub-region. The greatest limitation for large-scale production of zobo drinks is the rapid deterioration of the drink. Its shelf-life is approximately 24 hours after production, if not refrigerated. The effect of lime juice on the microorganisms associated with zobo drink was investigated. Zobo drink was prepared by maintaining the appropriate hygienic standards. Microbiological analysis was carried out on the prepared sample, before treatment with lime juice, to determine its effect on the microbial quality of the drink. The mean total coliform count from the prepared sample was 0.1 x 10^{-4} cfu/mL, aerobic bacterial count was 0.2 x10^{-4} cfu/mL and the fungal count was 0.4 x 10^{-4} cfu/mL. Microorganisms isolated from the prepared sample include *Staphylococcus* spp, *Lactobacillus* spp, *Bacillus* spp and *Penicillium* spp. The antimicrobial effect of the lime juice on the different isolates showed significant zone of inhibition even at 6.25% concentration. The total coliform and aerobic bacterial count generally decreased in value and showed no growth at 50-100% concentrations of lime juice for 3 days and 75-100% concentrations for 5 days. The findings from this study showed that lime juice

**Corresponding author: E-mail: obyezeigbotxt1@yahoo.com;*

can be used as natural preservative to prolong the shelf- life of zobo drink for a period 5 days under normal room temperature.

Keywords: Hibiscus sabdariffa; lime juice; antimicrobial quality; zobo drink.

1. INTRODUCTION

Zobo drinks are non-alcoholic local beverages made from differet varieties of dried petals, acid-succulent aqueous extract of calyx of rosette, *Hibiscus sabdariffa,* which is an annual herb that is widely cultivated in India and Africa [1]. Zobo is the local name for *Hibiscus sabdariffa* plant in Hausa (northern Nigeria**)**. There is increase in demand for zobo drinks due to its low price, nutritional and medicinal properties [2]. The non-alcoholic drink (zobo) is quite popular especially in northern Nigeria and is usually served chilled at various social gathering [3]. This drink have been found to be rich in anthocyannins, vitamins, natural carbohydrate, protein and other antioxidants [4,5] and also minerals [6]. The drink is used in curing minor stomach ailments, sore throat and strengthening the heart among other uses [1,5]. The leaves of the plant are also used as vegetables and the seeds are source of oil [7]. Zobo drink if well prepared and packaged will compete favourably with most of the non-alcoholic beverages available in the country, considering the increasing acceptance, socio-economic potentials and ready source of protein, vitamin C and other minerals.

The greatest limitation for large-scale production of zobo drinks is the rapid deterioration of the drink. Its shelf-life is approximately 24 hours following production, if not refrigerated. Microorganisms associated with the dried calyces, the production processes and other factors may contribute to its spoilage. Apart from the fact that most chemical preservatives may have adverse effect on humans, they are expensive and usually not affordable by the local people that produce this drink. Moreover, refrigeration may not be available. There is the need for alternative source of preservative that is natural and human-friendly, affordable and readily available. Fresh lime contains flavonoids, ascorbic acid and hydrocarbons that give it the antimicrobial properties [8]. Therefore, lime juice can be used to improve the shelf-life of zobo drink. This work was aimed at investigating the effect of lime juice on the microbial quality of zobo drink with a view to improving the shelf-life of the drink.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Zobo drink was prepared in Abia State Polytechnic Microbiology Laboratory under proper hygienic conditions. The dried calyses were boiled with distilled water for about 10 – 15 minutes from which the embedded pigments were extracted. After extraction, the filtrate was allowed to cool and sweetened with granulated sugar and spiced with ginger.

2.2 Microbiological Analysis

One milliliter of zobo drink sample was put in 9 mL of sterile distilled water in sterile test tube, shaken and then serially diluted. From the appropriate dilution, 0.1 mL was inoculated separately on nutrient agar, MacConkey agar and Potato dextrose agar plates and spread evenly using sterile bent glass rod. Each experiment was carried out in duplicates to get a mean standard value of the colony forming units (cfu/mL) on the plates. The inoculated nutrient agar and MacConkey plates were incubated at 37ºC for 24 hours while the Potato dextrose agar plates were incubated at room temperature for 5 – 7 days. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per milliliter (cfu/mL) and coliform respectively [9]. Each of the bacterial colonies on both Nutrient agar and MacConkey agar plates was sub-cultured and pure culture obtained. Isolates were identified by carrying out tests which include Gram staining, spore staining and biochemical test which include catalase, coagulase, oxidase, citrate utilization, indole, urease, methyl red, Voges-Proskauer and sugar fermentation [10].

Fungal isolates observed on Potato dextrose agar plates were identified by their cultural characteristics such as color, morphology and growth pattern of mycelia [9].

2.3 Antimicrobial Analysis of Lime Juice

The lime fruits were washed with sterile water and cut open with a sterile knife and the juice pressed out aseptically. The crude extracts were obtained by filtration [11]. Sterile cork borer of

about 6mm was used to make wells on prepared sterile molten Nutrient agar plates in triplicates. 1.0 mL of lime juice at different concentrations of 100%, 75%, 50%, 25%, 12.5% and 6.25% were aseptically introduced into each well. The setup was allowed to stand for about 5 minutes before incubation. The antimicrobial activities of the lime juice in each of the wells was determined by measuring the diameter of the zones of inhibition in millimeter [12].

2.4 Treatment of Zobo Samples with Lime Juice

Some lime fruits were surface- sterilized with 70% ethanol and peeled using a pre-sterilized knife. The fruits were squeezed aseptically into a 100ml sterile conical flask [13]. Ten-fold dilutions of the zobo drink were obtained and 0.1, 0.5, 1.0, 1.5 and 2.0 mL of lime juice concentration were added respectively to each test tube of the ten-fold dilution. The mixtures were allowed for 6hours and then for 24 hours at room temperature. From each treatment, 0.1 mL was inoculated into duplicate plates of Nutrient agar and MacConkey agar at 37ºC and Potato Dextrose agar at room temperature. The colonies were counted and recorded as colony forming units per milliliter (cfu/mL^{-1}) after 24 hrs for a period of 5 days [14].

2.5 Study Questionnaire

Twenty individuals were randomly selected to evaluate the taste and appearance of the drink after treatment with lime juice for the period of 5 days.

3. RESULTS

Table 1 shows the microbial counts of zobo drink prepared under proper hygienic conditions. The result showed an average aerobic bacterial count of 0.2×10^4 cfu/mL, average coliform count of 0.1×10^4 cfu/mL and average fungal count of 0.4×10^4 cfu/mL.

The morphological and biochemical characteristics of the bacterial isolates are shown in Table 2. A total of three bacterial isolates were identified which include *Bacillus* spp*, Staphylococcus* spp and *Lactobacillus* spp, *Penicillium* spp was the only fungal isolate identified (Table 3).

Table 4 shows the antimicrobial effect of lime juice on the different isolates. *Staphylococcus* spp was the most susceptible isolate with 22.0 mm as the mean zone of inhibition while *Penicillium* spp was the least susceptible with 14.2 mm zone of inhibition at 100% concentration respectively. At 12.5% concentration of lime juice, *Penicillium* spp was resistant.

Table 5 shows the total microbial counts of Zobo drink after treatment with lime juice. The result showed that lime juice at 50- 100% concentration had no growth at room temperature for a period of 3 days and a period of 5 days at 75-100% concentration.

Table 6 shows the result of the questionnaire on the taste of Zobo drink treated with lime juice after 5 days. A total of 17(94.4%) claimed the drink tasted “good” while 1(5.6%) claimed the drink tasted normal at 100% concentration respectively. In all concentration, none reported “sour” taste.

Table 7 shows the result of the questionnaire on the appearance of Zobo drink treated with Lime Juice after 5 days. Out of 18 individuals that reported on the appearance, 4(22.2%) claimed the appearance was good, 13(77.1%) attested that appearance was normal at 100% concentration respectively. In all the concentrations, none reported “poor in appearance”.

Table 1. Total microbial counts of Zobo drink

Sample	Total aerobic bacterial count (cfu/mL)	Total coliform count (cfu/mL)	Total fungal count (cfu/mL)
A	0.1×10^4	0.2×10^4	0.3×10^4
B	0.3×10^4	0.1×10^4	0.4×10^4
C	0.2×10^4	0.1×10^4	0.5×10^4
Average microbial count	0.2×10^4	0.1×10^4	0.4×10^4

Key: A-C Represent different analyses from the prepared samples

Table 2. Morphological and biochemical characteristics of isolates

Test	*Bacillus* spp	*Staphylococcus* spp	*Lactobacillus* spp
Morphology	Rod	Cocci	Rod
Gram reaction	+	+	+
Spore	+	-	-
Catalase	+	+	+
Coagulase	-	+	-
Citrate	-	+	-
Oxidase	-	-	+
Indole	-	-	+
Urease	-	+	-
Methyl red	+	+	-
Voges- Proskauer	-	-	+
Glucose	A/-	A/G	A/-
Lactose	A/G	A/-	A/G
Sucrose	A/-	A/-	A/G
Manitol	A/G	A/-	A/-

Key: A = Acid production; A/G = Acid and gas production; + = positive; - =negative

Table 3. Cultural and Morphological Characteristics of the Fungi Isolate

Morphology	Identification
Thick walled, no septation, oval spores, occurs in chain and some in singles.	*Penicillium* spp

Table 4. The Antimicrobial effect of lime juice on the different isolates

Organisms	Concentration (%)/ zone of inhibition (mm)					
	100%	75%	50%	25%	12.5%	6.25%
Bacillus spp	19.4	17.1	13.0	10.8	7.1	6.2
Staphylococcus spp	22.0	19.4	15.2	14.0	9.8	7.0
Lactobacillus spp	20.1	18.0	14.9	12.8	9.1	6.2
Penicillium spp	14.2	11.0	9.0	6.2	0	0

4. DISCUSSION

In the quest toward zero import dependence and food security in Nigeria, the food drink industry has come under scrutiny, as many imported drinks (especially “energy drinks”) have almost no food value, contain harmful or even carcinogenic chemicals and have been shown to aggravate certain diseases such as diabetes and high blood pressure [15]. This has precipitated research into locally produced drinks such as zobo drink. Zobo drink has been shown to possess medicinal/pharmaceutical properties including anti-diabetic, antihypertensive and anti-inflammatory properties [16].

Despite these obvious advantages, the leap from locally marketed product to large scale production is still improbable due to its poor shelf life [17]. This is due to high microbial proliferation that is presently associated with zobo drink sold in Nigeria. Zobo drink when left for more than 24 hours at room temperature turn sour. This may be as a result of microbial fermentation. This fermentation process has been found to cause loss of taste and nutritional value, increased rate of browning, offensive odour and presence of cloudy materials at the bottom of the containers [18].

In the present study, the antimicrobial effect of lime juice on the shelf-life of zobo drink was investigated. The result obtained revealed that lime juice has significant effect in prolonging the shelf-life of zobo. This observation is supported by the findings of [19] and Ibukun et al. [20] on the antimicrobial effect of lime juice on different microbial genera. The result obtained from the treatment of zobo drink with lime at 50- 100% concentration showed no growth at room temperature for a period of 3 days and a period of 5 days at 75-100% concentration. The result obtained from the questionnaire carried out on the treatment of zobo drink with lime juice showed that lime juice had no negative effect on the taste and appearance of zobo drink after

5days. This is also supported by the work carried out by Nwachukwu et al. [21] who observed that lime juice reduced the bacterial load of Zobo drink. Oboh et al. [22] had earlier reported that the bioactive component of lime juice was P-hydroxybenzoate with citric acid (pH 2.54). The antibacterial activity of P-hydroxybenzoic acid esteri (Parabens) highlighted by Hugo and Russell [23] showed a wide range of activities against microorganisms. Thus, they serve as preservatives in foods, toiletries and in some pharmaceutical preparations such as emulsions, creams and lotions. This finding from the study therefore, recommends the addition of lime juice at appropriate concentration to help prolong the shelf life of the drink, where refrigeration is not available.

Table 5. Total microbial counts of Zobo drink after treatment with lime juice

Concentration of lime (%)/ days	TAC	TCC	TFC
Day 1			
100	0	0	0
75	0	0	0
50	0	0	0
25	0	0	0
12.5	0.1×10^4	0	0.2×10^4
6.25	0.1×10^4	0	0.3×10^4
Day 2			
100	0	0	0
75	0	0	0
50	0	0	0
25	0	0	0
12.5	0.4×10^4	0.3×10^4	0.4×10^4
6.25	0.8×10^4	0.3×10^4	0.7×10^4
Day 3			
100	0	0	0
75	0	0	0
50	0	0	0
25	0.1×10^4	0	0.1×10^4
12.5	0.4×10^4	0.3×10^4	0.4×10^4
6.25	0.8×10^4	0.3×10^4	0.7×10^4
Day 4			
100	0	0	0
75	0	0	0
50	0.1×10^4	0	0
25	0.4×10^4	0.1×10^4	0.4×10^4
12.5	0.9×10^4	0.6×10^4	0.8×10^4
6.25	1.4×10^4	0.9×10^4	1.0×10^4
Day 5			
100	0	0	0
75	0	0	0
50	0.4×10^4	0.1×10^4	0.4×10^4
25	1.0×10^4	0.8×10^4	1.0×10^4
12.5	1.2×10^4	1.0×10^4	1.3×10^4
6.25	1.9×10^4	1.2×10^4	1.5×10^4
Raw lime without zobo	0	0	0
Raw zobo without lime	2.1×10^4	1.8×10^4	2.0×10^4

Table 6. Result of the questionnaire on the taste of Zobo drink treated with lime juice after 5 days

Lime concentration (%)	Total no. sampled	Total no. that taste good (%)	Total no. that taste normal (%)	Total no. that taste sour (%)
100	18	17 (94.4)	1 (5.6)	0
75	18	16 (88.8)	2 (11.1)	0
50	18	3 (16.6)	15 (83.3)	0
25	18	2 (11.1)	16 (88.8)	0

Table 7. Result of the questionnaire on the appearance of Zobo drink treated with lime juice after 5 days

Lime concentration (%)	Total no. sampled	Total no. that appeared good (%)	Total no. that appeared normal (%)	Total no. for poor appearance
100	18	4 (22.2)	13 (77.1)	0
75	18	2 (11.1)	16 (88.8)	0
50	18	0 (0)	18 (100)	0
25	18	1 (5.6)	17 (94.4)	0

5. CONCLUSION

The greatest limitation for large-scale production of zobo drinks is the rapid deterioration of the drink. Preservation of zobo drink with chemical preservatives may be expensive and unaffordable to the local people that produce this drink and may also have some adverse effect on humans. There is a need for an alternative source of preservative that is natural and human-friendly, affordable and readily available. In the present study, the effect of lime juice in prolonging the shelf-life of zobo drink was investigated. The result revealed that lime juice can preserve zobo drink by reducing the microbial load. However, good environmental and personal hygiene should be maintained when processing. Additives and packaging materials should also be properly sterilized. This study therefore recommends the use of lime juice for the preservation of zobo drink to increase the shelf-life.

ACKNOWLEDGEMENT

This study team appreciates Abia State Polytechnic, Aba, for making the Microbiology Laboratory available for our use.

COMPETING INTERESTS

Authors have declared that no competing interests exit.

REFERENCES

1. Lawton BP. Hibiscus: hardy and tropical plant for the garden. Tiber Press, Portland. 2004;122.
2. Oboh G, Elusiyan CA. Nutrient composition and antimicrobial properties of sorrel drinks (Zoborodo). J. Med. Food. 2004;7:340-342.
3. Aliyu L. Roselle (*Hibiscus sabdariffa*) production as affected by pruning and sowing date. J. of Applied Agric. Technol. 2000;6:16-20.
4. Wong PK, Yusof S, Ghazali HM, Man YC. Physico-chemical characteristics of roselle (*Hibiscus sabdariffa* L.). Nutr. Food Sci. 2002;32:68-73.
5. Available:http://www.nijb.com, 2004. Retrieved 23/11/2014.
6. Babalola SO, Babalola AO, Aworh OC. Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa* L). J. Food Technol. Afr. 2000;6:133-134.
7. Okoro CE. Production and quality evaluation of wine produced from zobo extract (*Hibiscus sabdariffa*). Prod. Niger. Inst. Food Sci. Technol. 2003;12:26-27.
8. Jay JM. Modern food microbiology. 4th Edn. CBS. Publisher, New Delhi. 1996;701.
9. Baker FJ, Breach MR. Medicinal microbiological techniques. First edition. Butterworth and Co Ltd, London; 1980.
10. Harrigan WF, Mc Cance ME. Laboratory methods. In: Harrigan WF, Mc Cance ME editors. Food and dairy microbiology.

Revised Ed. Academic Press, New York, USA. 1976;12-15.
11. Onyeagba A. Laboratory guide for microbiology. Crystal Publishers, Owerri, Imo State, Nigeria. 2004;95-119.
12. Rahman M, Khatun A, Rahman SM, Rashid MA. Antioxidant, antimicrobial and cytotoxic activities of *Vitis trifolia* Linn. J. Dhaka Intl. Univ. 2010;1(1):181-184.
13. Efiuwevwere BJO, Oyelade. Bio-deteriorative and Physico-chemical changes in modified atmosphere packaged orange and the microbial quality of preserver and unpreserved juice. Trop. Sci. 1991;31:325-333.
14. Fasoyiro SB, Ashaye AA, Samuel FO. Chemical and Storability of flavoured (*Hibiscus sabdariffa*) drink. World. J. Agric. Sci. 2005;1:165-168.
15. Omemu AM, Edema MO, Atayese AO, Obadina AO. A survey of the microflora of *Hibiscus sabdariffa* (Roselle) and the resulting zobo juice. African Journal of Biotechnology. 2006;254-259.
16. Osueke JC, Ehirim FN. Chemical, Nutritional and Sensory analysis of zobo drink (var *sabdariffa*) and selected soft drinks. Journal of Agriculture and Food Science. 2004;2(1):33-37.
17. Paine FA. What is Shelf Life? Food Technology International. 1992;21(2):234-235.
18. Adenipekun IT. Extraction and colour of Roselle (*Hibiscus sabdariffa* Linn) juice. M.Sc. Thesis, submitted to the Department of Chem., University of Ibadan, Ibadan, Nigeria. 1998;10.
19. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakazi O. Studies on the antimicrobial effect of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). African Journal of Biotechnology. 2004;3:552-554.
20. Ibukun A, Tayo A, Toyin A, Tolu O. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (Lime Fruit) As Used Locally. Afr. J. Trad. CAM. 2007;4(2):185-190.
21. Nwachukwu E, Onovo OM, Ezeama CF. Effect of lime juice on the bacterial quality of zobo drinks locally produced in Nigeria. Research Journal of Microbiology. 2007;2:787-791.
22. Oboh PA, Agbonlahor DN, Ekundayo AO, Orhe-Ureghe UB. Antibacterrial activity of *Citur aurantifolia* (lime) juice against some Gram-positive and Gram- negative bacteria. Nigeria Annual Network Science. 1995;2:1-9.
23. Hugo WB, Russell A. Pharmaceutical microbiology 5th ed. Blackwell Scientific Publication, London. 1993;212-250.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=829&id=8&aid=7585