THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Comparative Evaluation of the Microbiological Quality of *Hibiscus Sabdariffa* Drink (Zobo) Produced Using Different Methods

Adeoye, B. K.
Lecturer, Department of Nutrition and Dietetics, Babcock University, Ogun State, Nigeria
Ani, I. F.
Lecturer, Department of Nutrition and Dietetics, Babcock University, Ogun State, Nigeria
Ajuzie, N. C.
Lecturer, Department of Nutrition and Dietetics, Babcock University, Ogun State, Nigeria
Akinlade, A. R.
Lecturer, Department of Nutrition and Dietetics, Babcock University, Ogun State, Nigeria

Abstract:
Hibiscus sabdariffa drink (Zobo) was produced using different methods of production (boiling method, hot soaking method and, maceration and pasteurization method) and different concentrations (1.5%, 5% and 8.5%) of Hibiscus sabdariffa calyx. Hibiscus sabdariffa drink, thus produced was stored at ambient (28± 2oC) and refrigeration (4 ± 2oC) temperatures. pH, titratable acidity, bacterial counts and fungal counts were determined every 48 h. There was an inverse relationship in the pH and total titratable acidity of the samples. Zobo produced by maceration and pasteurization method had the best microbiological quality, 1.1x102, 1.6x102 and 1.9x102. While Hibiscus sabdariffa drink produced by boiling method was least in microbiological quality, 4.8x104, 6.3x104 and 7.1x104 for 1.5%, 5% and 8.5% calyx concentration respectively. At refrigeration condition, growth was slowed down in the Hibiscus sabdariffa drink produced by the different methods.

Keywords: *Calyx, Extraction method Concentration, Ambient, Refrigeration*

1. Introduction

Hibiscus sabdariffa drink (Zobo) is a non-alcoholic local beverage made from different varieties of dried, acid-succulent calyces of the flower *Hibiscus sabdariffa* by boiling and filtration (Ogiebor *et al*., 2008; Kolawole and Okeniyi, 2007). *Hibiscus sabdariffa* L. is a herbaceous plant, cultivated largely in tropical and subtropical areas of both hemispheres. It belongs to the malvaceae family (Oguntona, 1998; Bola and Aboaba, 2004; Morton, 1987) and is known by different names in different part of the world (Morton 1987; ASNAPP, 2003 ; Morton and Roselle, 1987; Glew *et al.,* 1997; Lorenzo *et al.,* 2000 ; McClintock.and El Tahir, 2004 ; Babalola *et al.,* 2001 ; Nyarko *et al.,* 2006 ; Cisse *et al.,* 2009a; Cisse *et al.*, 2009b).

Hibiscus sabdariffa L is a plant that has been used in traditional medicine as an antiseptic and various medicinal uses of infusions of the calyces have been reported such as being a diuretic, cholerectic, febrifugal, hypertensive, anti-helminthic, antimicrobial, decreasing viscosity of the blood and stimulating intestinal peristalsis (Sharaf 1962; Kerharo 1972; Morton 1987; Delgado-Vargas and Paredes-López 2003).

In production of *Hibiscus sabdariffa* drink (Zobo), commonly the extraction operation is carried out at temperatures between 25 and 100°C followed by removal of the calyx through filtration. After filtration, sweetener and other ingredients, such as other fruit juices, flavourants and fruit pieces are added (Cissé *et al.* 2009a). The sweetening agent is preferably a natural sweetening agent, but may be an artificial sweetening agent or a combination of natural and artificial sweetening agents (Appel, 2003).

Hibiscus sabdariffa drink (zobo) is well known in the tropical areas such as the West Indies, Taiwan and Malaysia as a fruity refreshing beverage (Francis, 1989). In the West Indies, sorrel is a prized drink during the Christmas holidays (McCaleb, 1996). Also, zobo is a common drink in Nigeria as it is been drunk in the different parts of the country.

However, there is no standard method for production of *Hibiscus sabdariffa* drink (zobo), the method employed varies from one locality to another and procedures depend on the producers own knowledge and experience which consequently result in zobo of heterogenous quality. The aim of this work is to determine the method of zobo production that will give a product of high microbiological quality which is an important contributory factor to the keeping quality of the drink.

2. Materials and Methods

2.1. Preparation of Samples

Hibiscus sabdariffa drink was produced using three different methods of calyx extraction. First method was according to Kimura *et al.*, 1996 (boiling method). Dry calyces of *H. sabdariffa* was boiled in water for 5 min, allowed to cool and filtered with sterilized sieve cloth. Granulated sugar was added to the cooled and filtered drink and was bottled. The second method was hot soaking method where dry calyces of *H. sabdariffa* was added to boiled water at 100^{o}C and allowed to stand for 1h. The calyces were removed by filtration using a sterilized sieve cloth and granulated sugar was added before bottling *(*Ilondu and Iloh*,* 2007). The third method was cold maceration and pasteurization method, dry calyces of *H. sabdariffa* was macerated in cold water(25^{o}C) for 4 h and filtered with sterile cloth (Suliman *et al.*, 2011). Granulated sugar was added before being bottled and was pasteurized in a batch process at 72^{o}C for 5 min (Perry and Staley, 1997; Ukwuru and Uzodinma, 2010; Braide *et al.,* 2012). Three concentrations of the drink were produced using each of the methods. The concentrations of the calyx of *Hibiscus sabdariffa* used were 1.5% as in commercial practice (Bolade, *et al.*, 2009), 5.0% (Sáyago-Ayerdi *et al.*,2007) and 8.5% (Appel,2003).

The different samples were dispensed into previously sterilized plastic bottles and were stored at ambient temperature of 28± 2^{o}C and refrigeration temperature of 4 ± 2^{o}C. pH, total titratable acidity (physic – chemical properties)**,** fungal and bacterial growth were detected every 48 h for 8 days.

2.2. Isolation and Enumeration of Microorganisms

Pour plate method was used for microbiological analysis. (Osuntogun and Aboaba, 2004). The total numbers of viable microorganisms (colonies) developed were enumerated and expressed as colony forming unit per milliliters (cfu/ml) by the methods of Harrigan and McCance (1990).

2.3. pH and Total Titratable Acidity

pH was determined with a pH meter which was standardized with standard buffer solution 4.0 and 7.0. The pH was measured by inserting directly the electrodes into 10 ml beaker containing the sample. The total titratable acidity (TTA) of the drink (expressed as percentages of citric acid) was determined by titrating 10ml of the samples against 0.1 N NaOH (AOAC, 2004).

3. Results

Storage Condition	Days of Storage	pH Calyx Concentration			TTA Calyx Concentration		
		1.5%	5.0%	8.5%	1.5%	5.0%	8.5%
Ambient	0	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{b}
	2	3.10^{a}	3.00^{a}	2.90^{a}	0.07^{a}	0.09^{b}	0.11^{b}
	4	2.90^{a}	2.80^{a}	2.70^{a}	0.07^{a}	0.11^{ab}	0.11^{b}
	6	2.90^{a}	2.70^{a}	2.70^{a}	0.11^{a}	0.14^{a}	0.14^{ab}
	8	2.90^{a}	2.70^{a}	2.70^{a}	0.11^{a}	0.14^{a}	0.19^{a}
Refrigeration	0	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{b}
	2	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{b}
	4	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{b}
	6	3.00^{a}	3.00^{a}	2.90^{a}	0.07^{a}	0.11^{ab}	0.14^{ab}
	8	3.00^{a}	2.90^{a}	2.90^{a}	0.07^{a}	0.11^{ab}	0.14^{ab}

Table 1: Physio- chemical properties of stored H.sabdariffa drink produed using boiling method

Means with the same superscript within the same column are not significantly different ($p< 0.05$)

Storage Condition	Days of Storage	pH Calyx Concentration			TTA Calyx Concentration		
		1.5%	5.0%	8.5%	1.5%	5.0%	8.5%
Ambient	0	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	2	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	4	2.90^{a}	2.90^{a}	2.90^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	6	2.90^{a}	2.80^{a}	2.80^{a}	0.11^{a}	0.11^{ab}	0.14^{a}
	8	2.90^{a}	2.80^{a}	2.80^{a}	0.11^{a}	0.14^{a}	0.14^{a}
Refrigeration	0	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	2	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	4	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.11^{a}
	6	3.10^{a}	3.00^{a}	3.00^{a}	0.07^{a}	0.09^{b}	0.14^{a}
	8	3.10^{a}	3.00^{a}	2.90^{a}	0.07^{a}	0.09^{b}	0.14^{a}

Table 2: Physico – chemical properties of stored H. sabdaffa drink produced using hot soaking method.

Means with the same superscript within the same column are not significantly different ($p< 0.05$)

Storage Condition	Days of Storage	pH Calyx Concentration			TTA Calyx Concentration		
		1.5%	5.0%	8.5%	1.5%	5.0%	8.5%
Ambient	0	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.09^a
	2	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.09^a
	4	3.20^a	3.00^a	2.90^a	0.07^a	0.09^a	0.11^a
	6	3.20^a	2.90^a	2.90^a	0.09^a	0.11^a	0.11^a
	8	3.10^a	2.90^a	2.90^a	0.09^a	0.11^a	0.11^a
Refrigeration	0	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.11^a
	2	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.11^a
	4	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.11^a
	6	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.11^a
	8	3.20^a	3.00^a	3.00^a	0.07^a	0.09^a	0.11^a

Table 3: Physico – chemical properties of stored zobo produced using cold maceration and pasteurization method

Means with the same superscript within the same column are not significantly different ($p< 0.05$)

Storage Condition	Days of Storage	Total Bacterial Counts (cfu/ml)			Fungal counts (cfu/ml)		
		Calyx 1.5%	Conc. 5.0%	8.5%	Calyx 1.5%	Conc. 5.0%	8.5%
Ambient							
	0	-	$0.3x10^1$	$0.5x10^1$	-	-	-
	2	$1.1x10^1$	$1.5x10^1$	$3.8x10^1$	$0.2x10^1$	$0.6x10^1$	$1.1x10^1$
	4	$2.4x10^2$	$3.2x10^2$	$3.7x10^2$	$1.0x10^1$	$1.3x10^1$	$1.4x10^1$
	6	$3.5x10^3$	$4.8x10^3$	$5.6x10^3$	$0.4x10^2$	$1.2x10^2$	$1.8x10^2$
	8	$4.8x10^4$	$6.3x10^4$	$7.1x10^4$	$7.0x10^2$	$1.0x10^3$	$1.3x10^3$
Refrigeration							
	0	-	$0.3x10^1$	$0.5x10^1$	-	-	-
	2	-	$0.3x10^1$	$0.7x10^1$	-	-	-
	4	-	$0.5x10^1$	$1.0x10^1$	-	-	-
	6	$0.2x10^1$	$0.7x10^1$	$1.4x10^1$	-	-	-
	8	$0.2x10^1$	$1.0x10^1$	$1.7x10^1$	-	-	-

- = not detected

Table 4: Microbiological quality of stored zobo produced using boiling method

Storage Condition	Days of Storage	Total bacterial counts (cfu/ml)			Fungal counts (cfu/ml)		
		Calyx 1.5%	Conc. 5%	8.5%	Calyx 1.5%	Conc. 5%	8.5%
Ambient							
	0	$0.2x10^1$	$0.4x10^1$	$0.4x10^1$	-	-	-
	2	$0.6x10^1$	$1.4x10^1$	$3.1x10^1$	$0.1x10^1$	$0.7x10^1$	$1.0x10^1$
	4	$1.3x10^1$	$2.5x10^2$	$1.3x10^2$	$0.3x10^1$	$1.1x10^1$	$1.3x10^1$
	6	$2.0x10^2$	$6.0x10^2$	$5.6x10^3$	$7.0x10^1$	$1.0x10^2$	$1.7x10^2$
	8	$7.0x10^3$	$1.4x10^4$	$4.7x10^4$	$2.0x10^2$	$4.0x10^2$	$7.0x10^2$
Refrigeration							
	0	-	$0.4x10^1$	$0.4x10^1$	-	-	-
	2	-	$0.5x10^1$	$0.6x10^1$	-	-	-
	4	-	$0.7x10^1$	$1.0x10^1$	-	-	-
	6	$0.2x10^1$	$0.8x10^1$	$1.4x10^1$	-	-	-
	8	$0.2x10^1$	$1.0x10^1$	$1.7x10^1$	-	-	-

- = not detected

Table 5: Microbiological quality of stored zobo produced using hot soaking method

Storage Condition	Days of Storage	Total bacterial counts (cfu/ml)			Fungal counts (cfu/ml)		
		Calyx 1.5%	Conc. 5%	8.5%	Calyx 1.5%	Conc. 5%	8.5%
Ambient							
	0	-	-	-	-	-	-
	2	-	$0.1x10^1$	$0.3x10^1$	-	-	-
	4	$0.2x10^1$	$0.3x10^1$	$1.1x10^1$	-	$0.3x10^1$	$0.8x10^1$
	6	$2.0x10^1$	$1.3x10^2$	$1.7x10^2$	$0.2x10^1$	$1.2x10^2$	$1.8x10^2$
	8	$1.1x10^2$	$1.6x10^2$	$1.9x10^2$	$1.0x10^1$	$1.5x10^2$	$2.1x10^1$
Refrigeration							
	0	-	$0.4x10^1$	$0.4x10^1$	-	-	-
	2	-	$0.5x10^1$	$0.6x10^1$	-	-	-
	4	-	$0.7x10^1$	$1.0x10^1$	-	-	-
	6	$0.2x10^1$	$0.8x10^1$	$1.4x10^1$	-	-	-
	8	$0.2x10^1$	$1.0x10^1$	$1.7x10^1$	-	-	-

- = not detected.

Table 6: Microbiological quality of stored zobo produced using cold maceration and pasteurization method

4. Discussion

Processing method, calyx concentration and storage condition are very important determinant of the quality and shelf- life of *H. sabdariffa* drink (Omemu *et al.,* 2006; Cisse *et al.,* 2009). Three methods of production of *H. sabdariffa* drink were studied; boiling, hot soaking and, cold maceration and pasteurization methods. *Hibiscus sabdariffa* drink of three different concentrations (1.5%, 5% and 8.5%) of *H. sabdariffa* calyx was produced using each of the methods.

The pH of the freshly produced samples was between 3.0 – 3.1 and TTA, 0.07 – 0.11 for *Hibiscus sabdariffa* drink produced by boiling(Table 1). For *Hibiscus sabdariffa* drink produced by hot soaking method, the pH of the freshly produced samples was between 3.0 – 3.1 and TTA, 0.07 – 0.11(Table 2). While the pH of the freshly produced samples of *Hibiscus sabdariffa* drink produced by cold maceration and pasteurization method was between 3.0 – 3.2 and TTA, 0.07 – 0.09(Table 3). These observations are similar to 3.2 reported by Egbere *et al.*,(2007) for *Hibiscus sabdariffa* drink of 10% concentration of *H. sabdariffa* calyx. The pH of *Hibiscus sabdariffa* drink with lowest concentration of *H. sabdariffa* calyx(1.5%) was high compared to pH of higher concentrations of *H. sabdariffa* calyx (i.e 5% and 8.5%). There was no detected difference in the pH of *Hibiscus sabdariffa* drink with 5% and 8.5% concentrations of *H. sabdariffa* calyx.

The TTA recorded is comparable to 0.02 - 0.08 reported by Omemu *et al.,* (2006), 0.06 reported by Amoo (2000) and Bolade *et al.*, 2000 who reported between 0.15 – 0.22 for concentrations used in commercial production. The *Hibiscus sabdariffa* drink samples stored at 28±2^{o}C and 4±2^{o}C were observed for 8 days. There was no significant change in the pH during storage in all the samples. The TTA of zobo samples containing 5% and 8.5% concentration of *H. sabdariffa* calyx produced by boiling and hot soaking method changed significantly ($p< 0.05$) during storage.

The increase in TTA of *Hibiscus sabdariffa* drink samples at ambient and refrigeration temperatures during storage, is similar to what was reported by Omemu *et al.,* (2006). TTA increased with increase in the period of storage Ashaye and Adeleke (2009) and Nwokocha *et al.*, (2012).The TTA recorded for the *Hibiscus sabdariffa* drink samples during storage were similar to 0.15 and 0.23% reported by Nwafor and Ikenebomeh (2009) for *Hibiscus sabdariffa* drink from different ratio of dried red colour calyces and water ratio, and boiling durations. The inverse relationship observed in TTA and pH agrees with the report of Egbere *et al.,* (2007).

The total bacterial counts for *Hibiscus sabdariffa* drink produced by boiling method ranged from NIL - 0.5×10^1 (Table 4), *Hibiscus sabdariffa* drink produced by hot soaking method ranged from 0.2×10^1 – 0.4×10^1(Table 5) and total bacterial count for *Hibiscus sabdariffa* drink produced by cold maceration and pasteurization method was NIL(Table 6). There was no fungal growth in the fresh *Hibiscus sabdariffa* drink samples produced by either of the methods. (Ukwuru and Uzodinma, 2010).

Progressive bacterial and fungi growth was observed during storage but the rate of growth differ under different storage conditions. At 48 h following production under ambient condition, there was an increase in the microbial growth in all the *Hibiscus sabdariffa* drink samples produced by the three methods except in *Hibiscus sabdariffa* drink with 1.5% concentration of *H. sabdariffa* calyx produced by cold maceration and pasteurization method. This agrees with the earlier reports of Osuntogun and Aboaba (2004) and Omemu *et al.,*(2006) that *Hibiscus sabdariffa* drink (zobo) has a short shelf- life of about 24 h when not refrigerated. Increase in the concentration of the *H. sabdariffa* calyx, seemed to increase the microbial load as the calyx of *H. sabdariffa* naturally carries microorganism (Omemu *et al.,* 2006)

At the end of the storage period *Hibiscus sabdariffa* drink produced by boiling method (Table 4) had the highest total bacteria count of 4.8×10^4cfu/ml for 1.5% *H. sabdariffa* calyx concentration, 6.3×10^4 cfu/ml for 5% *H. sabdariffa* calyx concentration and 7.1×10^4 cfu/ml for 8.5% *H. sabdariffa* calyx concentration. The reason for this observation could have been as a result of the effect of heat on natural antimicrobial compound in the *H. sabdariffa* calyx(Mazza and Brouillard, 1987; Baranac *et al.* 1997a; Kopjar *et al.*, 2009). *Hibiscus sabdariffa* drink produced by hot soaking method (Table 5) had the total bacteria count of 7.0×10^3cfu/ml for 1.5% *H. sabdariffa* calyx concentration, 1.4×10^4 cfu/ml for 5% *H. sabdariffa* calyx concentration and 4.7×10^4 cfu/ml for 8.5% *H. sabdariffa* calyx concentration. While *Hibiscus sabdariffa* drink produced by cold maceration and pasteurization method had very low total

bacterial count of 1.1 $\times 10^2$ cfu/ml for 1.5% *H. sabdariffa* calyx concentration, 1.6 $\times$ 10^2cfu/ml for 5% *H. sabdariffa* calyx concentration and 1.9 $\times$ 10^2 cfu/ml for 8.5% *H. sabdariffa* calyx concentration. The low bacteria count recorded for cold maceration and pasteurization method is an indication of elongation of zobo shelf life by the method (Egbere *et al.,* 2007).

The fungi count of *Hibiscus sabdariffa* drink produced by boiling method was the highest at the end of the storage period. The fungi count of the *Hibiscus sabdariffa* drink produced using this method being $7.0x10^2$cfu/ml for 1.5% concentration of *H. sabdariffa* calyx, $1.0x10^3$cfu/ml for 5% concentration of *H. sabdariffa* calyx and $1.3x10^3$cfu/ml for 8.5% concentration of *H. sabdariffa* calyx this is followed by *Hibiscus sabdariffa* drink produced by hot soaking method which had $0.2x10^3$cfu/ml for 1.5% concentration of *H. sabdariffa* calyx, $0.4x10^3$cfu/ml for 5% concentration of *H. sabdariffa* calyx and $0.7x10^3$cfu/ml for 8.5% concentration of *H. sabdariffa* calyx. *Hibiscus sabdariffa* drink produced by maceration and pasteurization method had the lowest fungi count of $1.0x10^1$cfu/ml for 1.5% concentration of *H. sabdariffa* calyx, $1.5x10^1$cfu/ml for 5% concentration of *H. sabdariffa* calyx and $2.1x10^1$cfu/ml for 8.5% concentration of *H. sabdariffa* calyx (Egbere *et al.,* 2007; Mazza and Brouillard, 1987; Baranac *et al.* 1997a; Kopjar *et al.*, 2009).

5. Conclusion

Cold maceration and pasteurization method produced *H. sabdariffa* drink with better microbiological quality followed by hot soaking method.

6. References

i. AOAC, 2004. Official methods of analysis of the Association of Official Analytical Chemists.18.Edn. Association of Analytic Chemists, Washington,D.C.
ii. Appel, S.D. 2003. Red sorrel, Hibiscus sabdariffa.The other cranberry implants and gardens. Volume 18, Number 2
iii. Ashaye, O.A. and Adeleke, T.O. 2009. Quality attributes of stored Roselle jam International Food Research Journal 16: 363-371.
iv. ASNAPP, 2003. Market Survey: Hibiscus sabdariffa. Agribusiness Sustainable Natural African Plant Products. Available at:http://www.herbs.org/africa/hibiscus.html (accessed 22 April 2003).
v. Baranac, J.M., Petranović, N.A. and Dimitrić-Marković, J. M. 1997a. Spectrophotometric study of anthocyan copigmentation reactions 2.Malvin and the nonglycosidized flavone quercetin, J. Agric. FoodChem.45 (5), 1694-1697.
vi. Bola, O. and Aboaba, O.O. 2004. Micrological and Physico – Chemical Evaluation of some Non-alcoholic beverages. Pak. J. Nut., 3: 188- 192.
vii. Bolade, M.K., Oluwanala, I.B. and Ojo, O. 2009. Commercial Practice of Roselle (Hibiscus sabdariffa), beverageproduction: optimization of hot water extraction and sweetness level. World Journal of Agricultural Science, 5 (1): 126-131
viii. Babalola, S.O., Babalola, A.O. and Aworh, O.C. 2001. Compositional attributes of roselle (Hibiscus sabdariffa L.). J. Food. Technol. Africa. 6, 133-134.
ix. Braide1, W., Oranusi, S. and Peter-Ikechukwu, A.I. 2012. Perspectives in the hurdle techniques in the preservation of a non alcoholic beverage, zobo. African Journal of Food Science and Technology Vol. 3(2) pp. 46-52,
x. Cisse, M., Dornier, M., Sakho, M., Diop, C. M., Reyne, S.M. and Sock, O. 2009a. La production de bissap (Hibiscus sabdariffa) au Sénégal Fruits, 64, p. 111–124.
xi. Cisse, M., Dornier, M., Sakho, M., Ndiaye, A., Reynes, M. and Sock, O. 2009b. Le bissap (Hibiscus sabdariffa L.) : composition et principales utilisations Fruits, 64, (3) p.179– 193.
xii. Delgado-Vargas, F. and Paredes-López, O. 2003..Natural Colourants for Food and Nutraceutical Uses. CRC Press, LLC:Boca Raton, FL.
xiii. Egbere, O.J., Anuonye, J.C., Chollom, P. F and Okpara, P. V. 2007. Effects of some preservation techniques on the quality and storage stability of zobo drink (A Nigerian, non-alcoholic beverage from (Hibiscus sabdariffa) Journal of Food TechnologyVolume 5 No.3 pp 225 – 228.
xiv. Francis, F. J. 1989. Food colourant: anthocyanins. Critical Reviews in Food Science and Nutrition 28:273– 278
xv. Glew, R.H., VanderJagt, D. J., Lockett, C., Grivetti, L.E., Smith, G.C, Pastuszyn, A. and Millson, M. 1997. Amino acid, fatty acid, and mineral composition of 24 indigenous plants of BurkinaFaso. J. Food Compos. Anal.10, 205–217.
xvi. Harrigan, W.F. and McCance, M. 1976. Laboratory methods in food and diary microbiology.Academic Press, London. pp. 225–231.
xvii. Ilondu, E.M. and Iloh, A.C. 2007. Inhibition of Three Fungal Isolates from Sorrel Drink (Zobo)Using HurdleTechnique. World Journal of Agricultural Sciences 3 (3): 339-343.
xviii. Kerharo, J. 1972. Sensegal Bisap (H. sabdariffa) or Guinea sorrel or red sorrel. Plant Medicine Pytotherapy 5:272–81.
xix. Kopjar, M., Piližota, V., Šubarić, D. and Babić, J. 2009. Prevention of thermal degradation of red currant juiceanthocyanins by phenolic compoundsaddition. Croat. J. Food Sci. Technol. 1 (1) 24-30
xx. Lorenzo, D., Atti-Serafini, L., Santos, A., Frizzo, C.D., Paroul, N., Paz, D., Dellacassa E and Moyna, P. 2000. Achyrocline satureioides essential oils from southern Brazil and Uruguay. Planta Medica 66, 476–477.
xxi. Mazza, G. and Brouillard, R.1987. Recent developments in the stabilization of anthocyanins in food products, Food Chem. 25 (3), 207-225.

xxii. McCaleb, R. 1996. Roselle Production Manual (Hibiscus sabdariffa). Herb Research Foundation, USA. Available at: http://www.herbs.org/.africa/hibiscus production manual. html (accessed 18 March 2003).

xxiii. McClintock, N.C. and El Tahir, I. M. 2004. Hibiscus sabdariffa L., in : Grubben, G.J.H., Denton, O.A. (Eds.), PROTA 2 (Plant Resources of Tropical Africa): vegetables[CD-Rom], PROTA Wagening., Neth.

xxiv. Morton, J.F. 1987. Roselle in Fruits of Warm Climates (ed.CF Dowling, Jr), pp. 281–6.Media Incorporated: Greensborough, NC.

xxv. Morton, J.F. and Roselle, M. J. 1987. Fruits of warm climates, in: Dowling C.F. (Ed.), Media,Inc., Greensboro, NC, USA, pp. 281–286.

xxvi. Nwafor, O.E. and Ikenebomeh, M. J. 2009. Effect of sodium benzoate on the growth and enzyme activity of Aspergillus niger and Penicillium citrinum in zobo drink during storage at 30±2^{o}C. African Journal of Food Science and Technology Vol. 3(3) pp. 66-72.

xxvii. Nwokocha, J.V., Okoronkwo, N.E., Eze, S.O. and Nwokocha, N.J. 2012. Comparism of the preservative activity of alligator pepper and ginger extracts on zobo liquor during storage at ambient temperature. Academic Research International vol 2, no. 3.

xxviii. Nyarko, G., Bayor, H., Craigon, J, and Suleimana, I. A. 2006. The effect of container types, seed dressings and desiccants on the viability and vigour of roselle (Hibiscus sabdariffa L var.sabdariffa) seeds. Pak. J. Biol. Sci. 9 (4), 593–597.

xxix. Ogiehor, I. S., Nwafor, O. E. and Owhe-Ureghe, U. B. 2008. Changes in the quality of zobo beverages produced from Hibiscus sabdarifa (Linn roscelle) and the effectsof extract of ginger alone or in combination with refrigeration. African Journal of Biotechnology Vol. 7 (8), pp. 1176-1180.

xxx. Oguntona, T. 1998. Green leafy Vegetables. In:Osagie, A.U. and Eka, O.U. Nutritional Quality of Plant Foods. Ambik Press Benin City, Nigeria, pp: 120- 133.

xxxi. Okeniyi, S.O. and Kolawole, J.A. 2007. Quantitative mineral ion content of a Nigerian local refreshing drink zobo (Water extract of hisbiscus sabdariffa calyx). Research J. Pharmacol., 1: 23-26.

xxxii. 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 Vol. 5 (3), pp. 254-259

xxxiii. Osuntogun, B. and Aboaba, O. O. 2004. Microbiological and physico-chemical evaluation of some non-alcoholic beverages. Pakistan Journal of Nutrition, 3: 188-192.

xxxiv. Perry, J.P. and Staley, J.T. 1997. Microbiol.: Dynamics and Diversity. Harcourt Brace College Publishers, New York, USA.pp 430- 502

xxxv. Sáyago-ayerdi, S.G. Arranz, S., Serrano, J. and Goñi, J. 2007. Dietary Fiber Content and Associated Antioxidant Compounds in Roselle Flower (Hibiscus sabdariffa L.)Beverage. Universidad Complutense de Madrid, 28040 Madrid, Spain,

xxxvi. Sharaf, A.1962. The pharmacological characteristics of Hibiscus sabdariffa L. Plant Medicine 10: 48–52.

xxxvii. Suliman, A.M., Ali, A.O., Idriss, S. A. and Abdualrahman, M. A. 2011. A Comparative Study on Red and White Karkade (Hibiscus sabdariffa L.) Calyces, Extracts and their Products. Pakistan Journal of Nutrition 10 (7):680- 683, 201

xxxviii. Ukwuru, M.U. and Uzodinma, C. C. 2010. Preservative effect of spices and their flavor acceptability in zobo drink. Nigerian Food Journal, vol 28, no 2.