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Full Length Research Paper

Changes in the quality of zobo beverages produced from *Hibiscus sabdarifa* (Linn roscelle) and the effects of extract of ginger alone or in combination with refrigeration

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Quality changes in zobo beverage produced from *Hibiscus sabdarifa* during storage and the effects of extracts of ginger (*Zingiber officinale*) alone or in combination with refrigeration at 6°C were evaluated for six (6) weeks duration. Results shows that the total viable bacterial count increased from 0.9 ± 0.01 log_{10} cfu/ml to 8.42 ± 0.1 Log_{10} cfu/ml at the 9th day of storage and thereafter decrease steadily till the end of the storage period (21) days. While the total viable fungi count increased steadily from no growth after boiling and filtration to 6.14 ± 0.1 log_{10} cfu/ml at the 14th day of storage and thereafter decrease steadily till the end of the storage period. Treatment with 0.2% extract of ginger at ambient laboratory temperature (30.5 ± 2°C) retarded the total viable bacterial and fungi count for 10 days with minimal growth from the 14th day which increase sluggishly. However treatment with 0.2% extract of ginger and refrigeration at 6°C further retarded the total viable bacteria and fungi count for 21 days with insignificant growth from the 28th day which was not sustained. The pH decreased gradually from 5.10 ± 0.01 to 2.98 ± 0.00 while the titratable acidity (TA) increased from 0.021 to 0.060 during storage for 21 days. Slight changes were observed in samples treated with 0.2% extract of ginger whereas the pH and TA were fairly stable in samples treated with 0.2% extract of ginger refrigerated at 6°C for 42 days. Gradual increase was recorded in the total soluble solids (TSS), vitamin C, protein and carbohydrate contents up to the 6th and 9th day of storage and thereafter decreased sharply till the end of the storage period for freshly prepared samples. However minimal changes were noted and recorded in samples treated with 0.2% extract of ginger all through the storage period. Whereas, sample treated with 0.2% extract of ginger and refrigerated at 6°C were fairly stable for 42 days of storage. Overall sensory evaluation shows that acceptability was in the order 0.2% ginger extract + 6°C > 0.2% extract of ginger > freshly prepared samples.

Key words: Zobo beverage, extract of ginger, refrigeration.

INTRODUCTION

Zobo drink, a non alcoholic local beverage is produced from the dried petals of *Hibiscus sabdarifa* (Linn Roscelle) by boiling and filtration. It is gaining wide acceptance, being consumed by several millions of people from different socio-economic classes and background in the West Africa sub-region, especially amongst the youth, who sees zobo drink as an alternative source of cheap and relaxing non alcoholic drink in social gathering (Ogiehor and Nwafor, 2004). With recent government policy emphasizing the need to be less depended on foreign food items and drinks and the outright band on the importation of formulated soft drink and fruit juices, zobo beverage appear to be promising economically and socially.

Zobo beverage has been shown to be a good source of natural carbohydrate, protein and vitamin C which consti-

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tute the major reason(s) for consuming soft drink and fruit juiced (Okoro, 2003; Ogiehor and Nwafor, 2004). These components tend to increase with increase in storage period, possibly potentiated by the activities of the associated microorganisms. Several groups of microorganisms (*Bacillus, Steptococcus, Staphylococcus, Leuconostoc, Lactobacillus, Aspergillus, Penicillium, Geotrichum, Fusanum and Alternana*) have been reported to be associated with zobo beverage during storage (Akinyosoye and Akinyele, 2000; Ogiehor and Nwafor, 2004). The proliferation of the associated microorganism potentiates spoilage and the short shelf life (24 – 48 h) associated with this relish beverage. Considering the increasing acceptance, socio-economic potentials, and ready source of protein and vitamin C, the need to enhance and extend the shelf life by gentle and effective means becomes imperative.

The use of local spices to control the activities of microorganisms in food has been reported (Akpomedaye and Ejechi, 1998; Nwafor and Ogiehor, 2003). Apart from the antimicrobial properties, spices are believed to have medicinal values (especially in African settings) and have desirable determinative influences on the overall organoleptic quality of food when used. In addition, the use of low temperature storage to retard and stabilize microbial growth in food is well documented (Jay, 1978; Ogiehor et al., 1998; Ogiehor et al., 2004). Thus, the application of extracts of spices alone or in combination with low temperature storage could possibly control microbial activities associated with zobo drink while retaining the nutritive and economic quality. Hence, this study could form basis for developing basic data and indices for the production, processing and handling zobo beverage.

MATERIALS AND METHODS

Source of sample and processing

Matured dried reddish-purple petals of *H. sabdariffa* used for this study were obtained from the open market in Benin City, Edo State, Nigeria and processed according to the method described by Ogiehor and Nwafor (2004). Briefly, weighed 400 g of the matured and dried petals of *H. sabdarifa* Linn was measured into a clean sterile five (5) litres beaker and boiled over a Bunsen flame with four (4) litres of water for 10 - 15 min. This was allowed to cool and thereafter filtered with the aid of a clean sterile muslin cloth. The filtrate was collected in pre-sterilized wide mouth glass bottles and thereafter dispensed into fifty (50 cl) centilitre reinforced plastic bottles (poly product Nig. Ltd) and held at 29 ± 1.5°C in the laboratory (Figure 1).

Treatment

The zobo beverage produced was divided into three (3) sub groups and then treated with extract of ginger (*Zingiber officinale*) according to the scheme developed by Akpomedaye and Ejechi (1998) and Ogiehor et al. (1999) as follows:

Group A: Ginger extracts (0.2%, w/v)
Group B: Ginger extracts (0.2%, w/v + 6°C)
Group C: Control (no treatment)

After the various treatments, the various samples were stored at ambient (laboratory) Temperature (30.5 + 2°C) for 42 days (six weeks)

Microbiological analysis

Bottles were aseptically opened and one millilitre (1 ml) aliquot of each samples for zero (0 h) and ambient stored samples were transferred into 9 ml of 0.1% (w/v). Sterile peptone water was used as diluent. Ten-fold serial dilution was carried out and appropriate dilution was aseptically plated using pour plate technique for total viable aerobic bacteria count on nutrient agar (Biotech) and total fungi count on potade dextrose agar (Biotech) supplemented with chloramphenicol (antibiotics). The media used were prepared and incubated according to the manufacturer's instructions. The numbers of viable microorganisms were counted, calculated and expressed as colonies forming units per mililitre (cfu/ml).

Biochemical and physicochemical analyses

A pH meter (JENWAY, model 3020) was used to determine the pH of the samples. The titrable acidity (% lactic acid), carbohydrate content (%), protein content (%) and the vitamin C content (%) were determined according to the methods of AOAC (1990). While the total soluble solids (TSS) was measured with the aid of a hand refractometer (Atago, Japan) after shacking and stirring with a clean sterile glass rod and expressed as (%Brix).

RESULTS

Results of the changes associated with the quality of zobo beverage (drink) produced form *H. sabdariffa* Linn and the effects of extracts of ginger (*Z. officinale*) alone or in combination with refrigeration at 6°C are shown in Tables 1 - 4. It shows that the total viable bacteria count decreased for 6.20 ± 0.2 $\log_{10}$ cfu/ml to 0.90 $\log_{10}$ cfu/ml after boiling. There after gradual increase was observed up till the 9th day of storage (8.42 ± 0.1 $\log_{10}$ cfu/ml) with subsequent decrease to 3.91 ± 0.2 $\log_{10}$ cfu/ml at the end of the storage period (21 days; Table 1). Similar trend of changes was observed and recorded for total viable fungi count (Table 1). The pH decreased gradually through out the storage period from 5.10 ± 0.01 to 2.98 ± 0.02. Conversely, the titratable acidity (TA%) increased form 0.02 ± 0.01 to 0.06 ± 0.00. Gradual and steady increase up to the 6th - 9th day of storage was recorded in the total soluble solids, vitamin C, protein and carbohydrate contents respectively. However, subsequent decrease was observed till the end of the storage period.

Treatment with 0.2% (w/v) extract of ginger drastically reduced the total viable count. No growth was detected till the 14th day of storage with minimal growth which increase slowly (Table 2). The pH decreased from 5.10 ± 0.01 to 3.96 ± 0.01 at the end of the storage period (42 days). While the TA increased form 0.02 ± 0.001 to 0.04 ± 0.001 where as 33.3, 11.76, 11.53 and 11.11% decrease were recorded for total soluble solids, vitamin

Table 1. Changes in the quality of zobo beverage during storage.

Period of storage (days)										
Parameter	**fu**	**0**	**2**	**4**	**6**	**9**	**12**	**15**	**18**	**21**
TVC bacteria	6.20±0.1	0.90±0.1	3.25±0.1	6.39±0.3	7.13±0.2	8.42±0.10.1	6.32±0.2	4.14±0.1	4.09±0.3	6.20±
Log_{10}cfu/ml)										
TVC Fungi	3.12±0.1	NG	1.07±0.1	3.78±0.1	5.55±0.2	5.63±0.2	6.14±0.1	5.39±0.3	4.25±0.2	2.92±
Log_{10}cfu/ml)										
PH		5.10±0.01	4.95±0.02	3.86±0.03	3.64±0.02	3.41±0.01	3.36±0.01	3.24±0.01	3.00±0.00	2.98±
TA(%)		0.20±0.00	0.20±0.00	0.30±0.001	0.03±0.001	0.04±0.001	0.04±0.00	0.04±0.00	0.05±0.00	0.06±
TSS(%prix)		0.09±0.001	0.09±0.001	0.16±0.001	0.24±0.001	0.18±0.001	0.14	0.0020.09±0.001	0.04±0.001	0.02±
Vit C		0.34±0.01	0.38±0.01	0.45±0.1	0.48±0.01	0.36±0.01	0.6±0.01	0.44±0.1	0.44±0.1	0.14±
Protein		0.20±0.01	0.055	0.068	0.10	0.15	0.18	0.13	0.06	0.00
CHO		0.45	0.59	0.68	0.76	0.84	0.73	0.53	0.46	0.26

TVC = Total Viable Count; TA Titratable Acidity; TSS = Total Soluble Solids; CHO= Cabohydrate and Vit C = Vitamin C, NG= No Growth

Table 2. Changes in the quality of zobo beverage treated with 0.2% extract of ginger during storage 30.5±2^0 C

Period of Storage											
Parameter	**fu**	**0**	**2**	**4**	**6**	**10**	**14**	**21**	**28**	**35**	**42**
TVC Bacteria		NG	NG	NG	NG	NG	$0.8x10^1$	0.90±0.01	2.70±0.2	2.95±0.1	3.63±
TVC Fungi		NG	NG	NG	NG	NG	$0.6x10^1$	1.23±0.1	1.41±0.1	3.58±0.2	3.74±
PH		5.10±0.01	5.05±0.01	4.92±0.01	4.83±0.01	4.6±0.01	4.4±0.01	4.31±0.01	4.20±0.02	4.05±0.02	3.96±
TA(%)		0.02±0.001	0.021±0.02	0.02±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.04±
TSS(% Brix)		0.09±0.001	ND				ND				0.06±
Vit C		0.34±0.01	ND				ND				0.30±
Protein		0.02±0.001	ND				ND				0.023±
CHO		0.45±0.02	ND				ND				0.40±

TVC = Total Viable Count; TA Titratable Acidity; TSS = Total Soluble Solids; CHO= Cabohydrate and Vit C = Vitamin C, NG= No Growth

C, protein and carbohydrates contents at the end of the storage period (42 days) Table 2.

Table 3, shows the effects of 0.2% extract of ginger treatment in combination with refrigeration at 6°C on the quality changes of zobo beverage during storage. No viable microbial growth was detected till the 21st day of storage. However minimal growth (1.04 ± 0.01 log_{10} ml) which was not sustained was recorded on the 28th day. The pH decreased minimally from 5.10 ± 0.01 to 4.64 ± 0.01 at the end of the storage period, while titratable acidity was fairly stable all through the storage period. However insignificant decease of 16.66, 5.88, 7.69 and 4.44% were recorded for total soluble solids, vitamin C, protein and carbohydrates content at the end of the storage period (42 days).

Sensory evaluation of the various quality attributes at the end of the storage period is shown on Table 4. Statistical analysis of the data obtained indicates that the various quality attributes were

Table 3. Changes in the quality of zobo beverage treated with 0.02% extract of ginger in combination with refrigeration at 6^0 during stc

Period of Strage (days)											
Parameter	**fu**	**0**	**2**	**4**	**6**	**10**	**14**	**21**	**28**	**35**	**42**
TVC Bacteria		NG	NG	NG	NG	NG	NG	NG	1.04±0.01	1.43±0.01	1.55±
TVC Fungi		NG	NG	NG	NG	NG	NG	NG	$0.5x10^1$	$0.9x10^1$	1.8x
PH		5.10	5.05±0.01	4.92±0.01	4.83±0.01	4.6±0.01	4.4±0.01	4.31±0.01	4.20±0.02	4.05±0.02	3.96±
TA(%)		0.02	0.021±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.04±
TSS(Brix)		0.09				ND					0.075
Vit C		0.34				ND					0.32
Protein		0.026				ND					0.024
CHO		0.45				ND					0.43

TVC = Total Viable Count; TA Titratable Acidity; TSS = Total Soluble Solids; CHO= Cabohydrate and Vit C = Vitamin C, NG= No Growth.

Table 4. Sensory evaluation at the end of storage

Attributes						
Type of Sample	**Mouthfeel**	**Appearance**	**Consistency**	**Taste**	**Aroma**	**Overall Score**
Ginger treated sample	6.82±0.2	6.05±0.5	5.05±0.3	6.24±0.2	5.65±0.4	5.96±0.32
Ginger treated + refrigeration	7.05±0.5	5.83±0.2	5.33±0.3	6.85±0.5	5.94±0.2	6.20±0.34
Freshly Prepared sample	5.57±0.3	6.45±0.3	6.08±0.2	4.95±0.2	5.14±0.3	5.64±0.26

significantly different at various levels amongst the various samples evaluated. Overall acceptability scores showed no significant difference amongst the samples evaluated. However preference was in the order: ginger treated + refrigerated samples > ginger treated samples > freshly prepared samples.

DISCUSSION

The decrease from 6.20 ± 0.01 $\log_{10}$ cfu/ml to 0.90 ± 0.01 $\log_{10}$ cfu/ml for total viable bacteria count and 3.12 ± 0.1 $\log_{10}$ cfu/ml to lack of growth for total viable fungi count after boiling and filtration may be traced to cell death, injuries and homeostatis disturbance potentiated by the boiling effects. The subsequent increase observed up till the 9^{th} day for total viable bacteria count and 12^{th} day for fungi count can be associated with return of favourable micro-environmental conditions which enhanced the recovery of injured cells and repair of homeostatic imbalance. However, the subsequent decrease till end of the storage period (21 days) observed may be associated with slight nutrient depletion and the pooled effects of by-products of metabolism. Similar findings have been documented for related food items (Ogiehor et al., 1998; Ogbulie et al., 1993). However, the lack of growth recorded up till the 14^{th} day of storage in samples treated with 0.2% extract of ginger may be related to the antimicrobial effects of ginger (Table 2). While the longer duration of no growth (21 days) observed and recorded in samples treated with 0.2% ginger extract and held at 6^oC may be closely associated to the multi target effects of boiling, ginger extract and refrigeration on the associated microorganisms and the inability of the microorganisms to muzzle up enough energy to overcome the resultant stress. These findings support previous reports on the use of combination of preservative factors to stabilize the microbial quality of traditional and novel foods (Leistner, 1978, 1992; Gould, 1998; Ogiehor et al., 2003).

The initial increase recorded in the vitamin C, protein, carbohydrates and total soluble solids contents may be related to the extensive microbial

activities which potentiate the release of bound nutrients while the subsequent decrease observed and recorded may be due to possible utilization by the associated microorganisms, bioconversion into organic acids and other organic compound. These may partly explain the continuous and gradual decrease recorded in the pH through out the storage period and partly account for the stepwise increase observed and recorded in the titratable acidity. However, the minimal changes observed and recorded in the vitamin C, protein total soluble solids and carbohydrates contents in samples treated with 0.2% extract of ginger and stored at 30.5 ± 2°C may be due to the antimicrobial activities of ginger which help to stabilize the microbial activities by creating barriers which the microorganisms present were unable to overcome. Furthermore, the higher degree of stability recorded in the sample treated with 0.2% extract of ginger and refrigeration at 6°C demonstrates the benefits of combination of preservative factors in stabilizing the microbial, nutritive and economic quality of foods. These findings support previous reports for related indigenous and novel food items (Ogiehor et al., 1998; Ogiehor, et al., 2003; Ogiehor et al., 2004).

Sensory evaluation of the various quality attributes at the end of the storage period showed that the various quality attributes were significantly different at different levels amongst the samples evaluated. Overall acceptability scores showed that samples treated with extract of ginger and refrigeration at 6°C were most preferred. Further preference test using the ranking scale (Watts et al., 1987) indicated preference to be in the order; samples treated with ginger extract + refrigeration > samples treated with ginger extract alone > freshly prepared samples. This shows that combination of extract of ginger and refrigeration enhanced the organoleptic quality and contributes to the overall acceptability of zobo beverage. This further supports previous findings that local spices enhance the shelf stability and organoleptic quality of foods (Akpomedaye and Ejechi, 1998; Nwafor and Ogiehor, 2003).

In summary, this study have shown that extract of local spices (ginger) alone or in combination with low temperature storage (refrigeration) extended the shelf life of zobo beverage for a minimum of 6 weeks and contributed to the overall quality and acceptability. In addition, findings are useful in developing measurable and reliable indices for the production, processing and handling of zobo beverage.

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