



## The Preservative Effects of *Aframomum melegueta* and *Piper guineensis* Extracts on Microbial Load of *Hibiscus sabdariffa* and *Sorghum bicolor* Liquors

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### Authors' contributions

This work was carried out in collaboration between both authors. Author IBO designed the study, supervised it and corrected the manuscript while author TOA did the work and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/JSRR/2014/9935

#### Editor(s):

(1) Surapong Pinitglang, Department of Food Science and Technology, School of Science and Technology, University of the Thai Chamber of Commerce, Thailand.

#### Reviewers:

(1) Aliyu Ibrahim Dabai, Microbiology, Faculty of Science, Usmanu Danfodiyo University Sokoto, Nigeria.  
(2) Anonymous  
(3) Anonymous

Peer review History: <http://www.sciencedomain.org/review-history.php?iid=632&id=22&aid=5924>

Original Research Article

Received 8<sup>th</sup> March 2014  
Accepted 24<sup>th</sup> July 2014  
Published 1<sup>st</sup> September 2014

### ABSTRACT

**Aims:** The preservative effects of hot sterile water extracts of *Aframomum melegueta* and *Piper guineensis* on the shelf life of *Hibiscus sabdariffa* (*H. sabdariffa*) and *Sorghum bicolor* (*S. bicolor*) liquors were investigated.

**Study Design:** Factorial design was used for this study.

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**Place and Duration of Study:** The study was carried out in Chemistry, Food Processing, Sensory and Microbiology Laboratories of the Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria, between September, 2012 and December, 2013.

**Methodology:** Dried *H. sabdariffa* calyx and *S. bicolor* stem sheath were cleaned by removing extraneous materials, milled and sieved separately. Extracts of the two spices, namely *A. melegueta* and *P. guineensis* were prepared in 0.5%, 1.0%, 1.5% and 2.0% concentrations respectively making a total of 5 samples with the control for each liquor. *H. sabdariffa* calyx and *S. bicolor* stem liquors were treated respectively with the different concentrations (0.5, 1.0, 1.5 and 2.0%) of spice extracts in 1000 mls (1 litre) of each liquor respectively and stored at ambient temperature ( $28\pm 2^{\circ}\text{C}$ ) for 4 days. The microbial loads were determined daily until the samples were adjudged spoiled through offensive smell and characteristic odour with observable changes in colour from bright red to black.

**Results:** On the first day, there was no observable bacteria growth and no Coliform count throughout the period of storage. This is an indication that the liquors were prepared under hygienic condition. Microbes were however observed for samples treated with *A. melegueta* and *P. guineensis* extracts on the second day. At 0.5% inclusion, *A. melegueta* extract in *H. sabdariffa* calyx and *Sorghum bicolor* liquors had microbial loads of  $3.70 \times 10^2$  and  $4.42 \times 10^2$  cfu/mL respectively while 0.5% *P. guineensis* extract in *H. sabdariffa* calyx and *Sorghum bicolor* liquors recorded  $3.20$  and  $3.54 \times 10^2$  cfu/mL. At 1.5%, *A. melegueta* extract in *H. sabdariffa* calyx and *S. bicolor* liquors had microbial loads of  $3.20$  and  $4.00 \times 10^2$  cfu/mL respectively. At 1.5%, *P. guineensis* extract in *H. sabdariffa* calyx and *S. bicolor* liquors had microbial loads of  $1.70$  and  $3.18 \times 10^2$  cfu/mL respectively. On the fourth day, the microbial load for 1.5% *A. melegueta* extract in *H. sabdariffa* calyx and *S. bicolor* liquors were  $3.50$  and  $3.80 \times 10^2$  cfu/mL respectively. The 1.5% *P. guineensis* extract in *H. sabdariffa* calyx and *S. bicolor* liquors recorded  $2.82$  and  $3.20 \times 10^2$  cfu/mL respectively. Results showed that the Control samples had higher microbial loads than the spiced (treated) samples indicating some preservative effects of the spices on both liquors. Furthermore, the result showed that *P. guineensis* had a better preservative action than *A. melegueta*. Likewise, the preservatives were found to have better control on microbial growth in *H. sabdariffa* than in *S. bicolor*.

**Conclusion:** The preservative effects of *Aframomum melegueta* and *Piper guineensis* extracts on microbial load of *Hibiscus sabdariffa* and *Sorghum bicolor* liquors showed that *A. melegueta* had lower antimicrobial activity than *P. guineensis*, thus *P. guineensis* may be preferred as a natural antimicrobial (preservative) to extend the shelf life of foods.

**Keywords:** *Aframomum melegueta*; *Piper guineensis*; *Hibiscus sabdariffa*; *Sorghum bicolor*; preservatives; liquor.

## 1. INTRODUCTION

Nigeria is faced with so many challenges in the area of storage and preservation of foods [1]. According to [1], about 70% of agricultural products get wasted yearly as a result of crude and manual methods of processing, unsanitary conditions of food processing environments, insufficient storage equipment and facilities and instability in the electric power supply. Many of the indigenously prepared foods have limited shelf life of about 3-5 days after which they are susceptible to microbial deterioration by yeast, moulds and bacteria. The food and 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 e.g. diabetes and high blood pressure [2]. This has necessitated research into local drinks which include *H. sabdariffa* calyx and *Sorghum bicolor* liquors.

*Hibiscus sabdariffa* liquor is a non-alcoholic local beverage produced from the dried petals/calyx (*Linn roselle*) by boiling and filtration. *Hibiscus sabdariffa* is a tropical plant of considerable economic potential. *Hibiscus sabdariffa* calyx has been shown to possess medicinal or therapeutic properties including anti-diabetic, antihypertensive and anti-inflammatory [3]. Its calyces have been suggested as food colorants for food industries, emulsifier for carbonated drinks, jam manufacture, juices and natural food colorants [4]. The calyces are rich in anthocyanin, ascorbic acid and hibiscus acid. The calyces are water soluble with brilliant and attractive red colour and with sour and agreeable acidic taste which aid digestion [5]. It enjoys wide acceptance from different socio economic classes in Nigeria and some West Africa sub-regions as an alternative source of cheap and relaxing non-alcoholic drink. *Hibiscus sabdariffa liquor* can be sweetened with sugar and served chilled or hot as tea. The drink serves as a cheaper alternative to the industrially produced carbonated soft drinks and is also available in every nook and cranny of the country [6].

*Sorghum bicolor* is a mature black-purple sheath which is used as colour additive in cooking meals, taken as beverages (infusion drink) when steeped or boiled in water as folklore for the management of anaemia and some other diseases in many Nigerian homes. It is used to extend food shelf life and found to be a nutrient dense commodity as reported by [7,8]. *Sorghum bicolor* has the highest known concentration of apigenin which corresponds to its anthocyanin quantity thereby making it of great commercial value as natural colorant in Nigeria [9]. *Sorghum bicolor* liquor is also administered to help raise blood levels in treatment of anaemia, stop pain and inflammation, prevent cell damage (antioxidant) and increase cellular immunity in persons living with HIV/AIDS [10]. In South Western Nigeria, the extract has been used for many generations to treat sickle cell anaemia, leukaemia, multiple myeloma, headaches, heart and other blood-related disorders. The stem of *Sorghum bicolor* has also been reported to be used as an anti-malaria and antihelmintic [11].

Despite these obvious advantages, the leap from locally marketed product to commercial product is still relatively improbable due to its poor shelf life. This is due to high microbial proliferation from unsanitary preparation, harsh storage conditions and improper packaging materials. Ginger extract (hot water) has been shown to elongate the shelf life of *Hibiscus sabdariffa* and *Sorghum bicolor* liquor from two to four weeks [12] while lime has been shown to be active against some bacteria present in *Hibiscus sabdariffa* liquor. According to [7], *Aframomum melegueta* has been shown to possess anti-tumour, anti-proliferative, antihypertensive, bactericidal and nematocidal properties due to its 6-gingerol, 6-paradol, shagaols and zingiberene contents. It is also anti-inflammatory and cancer inhibiting. Although it has been shown to possess antimicrobial activity against food microbes, there are relatively few studies on its use in food preservation. Higher consumption of food of plant origin such as *Hibiscus sabdariffa* and *Sorghum bicolor* stem liquor, rich in natural antioxidants that can scavenge free radicals has been reported to fight against degenerative diseases (cyclophosphamide-induced oxidative stress) thereby improving the body's antioxidant status [13,14].

The use of local spices to control the activities of microorganisms in foods has been reported [15]. The antimicrobial activity varies depending on the type of spices or herbs. The extent to which a material is used as a spice is dictated primarily by its essential oils or

oleoresins [16]. The volatile oils being responsible for the aroma and taste of most spices, as it contains terpenes, sesquiterpenes, alcohols, esters, aldehydes, ketones, and phenols [15]. The addition of extracts of spices could possibly control the microbial activities associated with food samples while retaining the nutritive and economic quality.

Therefore, the aims and objectives of this work is to investigate and compare the preservative effects of *Aframomum melegueta* and *Piper guineensis* extracts on *Hibiscus sabdariffa* and *Sorghum bicolor* liquors.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Mature dried reddish-purple *Sorghum bicolor* stem sheath and *Hibiscus sabdariffa calyx* were purchased from Igbona market in Osogbo, Osun State, Nigeria. The spices used in preserving the liquor include *Piper guineensis* and *Aframomum melegueta* which were also sourced from the same market between July and August, 2012. Food grade sucrose was procured from Borepo supermarket at Osogbo. All reagents used in this study are of analytical grade.

### 2.2 Methods

The stem sheath and calyxes were inspected and sorted to remove sticks, dirt and stones which are commonly associated with them. They were then dried in a cleaned oven at 30°C for 6 hrs for moisture uniformity. *Piper guineensis* and *Aframomum melegueta* were further dried under the same condition for the same period of time. The materials were ground separately into flour and kept in cleaned containers.

#### 2.2.1 Preparation of *Hibiscus sabdariffa calyx liquor*

The drink was prepared according to the method prescribed by [5,7]. About 200g of the calyx powder was added to one litre of boiling water and covered to boil for 10 minutes. The filtrate was obtained via sieving with a clean hand sieve while the residue was discarded. To the filtrate, 100g of granulated sugar was added and then stirred to hasten dissolution. The resulting liquor was then filtered again to remove undissolved sugar crystals and particulate matter which had not been earlier removed. The filtrate was then dispensed into factory sterilized 150 ml labelled plastic bottles before pasteurization at 75°C for 15 mins and then immediately cooled under running tap water (Fig. 1).

#### 2.2.2 Preparation of *Sorghum bicolor liquor*

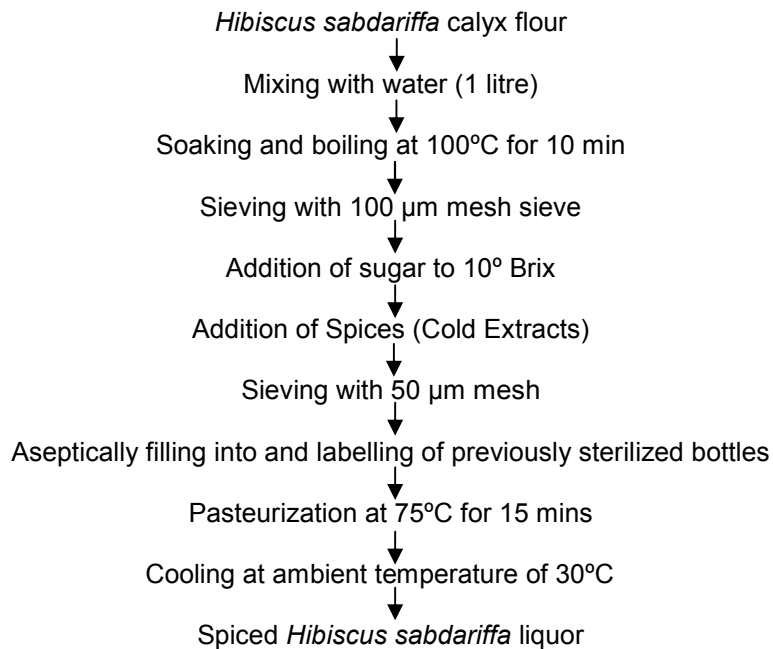
The method described by [7] was adapted with some modifications. Two hundred grams of the stem sheath flour was mixed and soaked in one litre of water for 30 mins at ambient temperature before boiling for 10 mins. The extract was filtered with cleaned muslin cloth to obtain clear supernatant. The filtrate was also sweetened using food grade sucrose to a brix level of 10° and filtered again. The sweetened liquor was then dispensed into previously labelled and sterilized bottles before pasteurization at 75°C for 15 mins and then cooled under running tap water (Fig. 2).

### **2.2.3 Extraction of spices**

The *Aframomum melegueta* and *Piper guineensis* were prepared by the method described by [17]. Five grams of each sample was ground into powder and extracted with 100 mls of hot sterile water (80°C at 5 g/100 ml for 72 hrs and filtered using filter papers as described in the flow charts shown in Figs. 1 and 2. Different concentrations (0.5, 1.0, 1.5 and 2.0) % of the spices was prepared according to the method developed by [16,18].

### **2.2.4 Treatment of the liquors with spices**

About 50 mls of *Sorghum bicolor* and *Hibiscus sabdariffa* calyx liquors were placed in five separate sterile containers respectively and treated with the different concentrations of the spices. After the various treatments, the spiced treated samples were stored at ambient temperature until they were adjudged spoilt (Figs. 1 and 2).



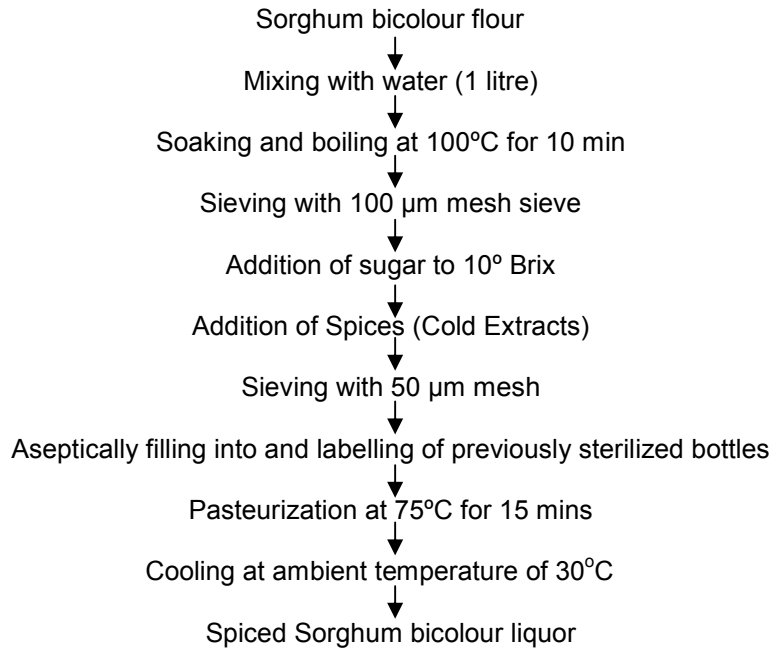
**Fig. 1. Flow chart for the production of Spiced *Hibiscus sabdariffa* liquor**

Source: [6]

## **2.3 Microbiological Analysis**

### **2.3.1 Total viable count (TVC)**

One ml from each of the samples was separately mixed in 9.0 mls of sterile peptone water. The dilution was serially made until  $10^{-5}$  level of the dilution was obtained. Total viable counts of bacteria were determined by enumerating the colony forming units (cfu) by pour plating 1.0mls of  $10^{-5}$  diluents and incubated at 37°C for 3 days. The experiments were carried out in triplicates [17].



**Fig. 2. Flow chart for the production of Spiced Sorghum bicolor liquor**

Source: [7]

### **2.3.2 Total coliform count (TCC)**

Mac Conkey broth was used for the detection of Coliform bacteria by the multiple tube technique. The medium was distributed in 9.0 ml quantities into standard test tubes with inverted Durham tubes and then autoclaved for 20 mins at 121°C. Well homogenized samples were serially diluted ( $10^1$  and  $10^2$ ) with 0.1% peptone water. One ml from each dilution was aseptically inoculated into triplicate of 9 ml sterile Mac Conkey broth in standard test tubes and incubated for 48 hrs at 37°C. Positive tests are expected to produce gas in the Durham tubes and change the colour of the medium [17].

## **3. RESULTS AND DISCUSSION**

### **3.1 Results**

The results of the effect of *Aframomum melegueta* and *Piper guineensis* extracts on the microbial load of *Hibiscus sabdariffa* (Linn roselle) calyx and *Sorghum bicolor* stem sheath liquors are presented in Tables 1, 2 and 3.

### **3.2 Discussion**

The microbial analysis of the fresh beverage revealed that there was no observable bacteria growth on the first day of production in both samples. No growth was observed in any of the samples until over 48 hours (2 days) of storage. Samples of *H. sabdariffa* calyx liquor and *Sorghum bicolor* treated with 1.0% *Aframomum melegueta* had average microbial loads of ( $3.70$  and  $4.10 \times 10^2$ ) cfuL<sup>-1</sup> respectively while the 1.0% *Piper guineensis* extract in

*H. sabdariffa* calyx and *Sorghum bicolor* liquors recorded 2.80 and 3.30 x10<sup>2</sup> cfumL<sup>-1</sup> respectively. Result obtained have shown a clear trend of microbial growth suppression with increase in the percentage of inclusion of the preservatives (extracts) to the beverage samples up to 2.0% concentration. The microbial load for samples treated with 1.5% *Piper guineensis* were 2.82 and 3.20 x10<sup>2</sup> cfumL<sup>-1</sup> for *H. sabdariffa* and *Sorghum bicolor* respectively at the end of 4 days storage, while those treated with 1.5% *Aframomum melegueta* had a reading of 3.60 and 3.80 x 10<sup>2</sup> cfumL<sup>-1</sup> on *H. sabdariffa* and *Sorghum bicolor* respectively for the same storage period. Similar findings have been documented for related food items by [18,19]. This implies that both preservatives (*Aframomum melegueta* and *Piper guineensis*) have good preservative effects on *H. sabdariffa* than in *Sorghum bicolor*. Moreover, results have shown *Piper guineensis* to be a better preservatives than *Aframomum melegueta*. The drinks can be said to be safe for consumption since the microbial loads did not exceed the safe limit level of 10<sup>5</sup> recommended by [17,18,20]. In summary, samples treated with *Piper guineensis* recorded lower microbial counts, thus indicating more antimicrobial activity than *Aframomum melegueta* and may therefore be preferred to *Aframomum melegueta* as a natural antimicrobial preservative. The active component of the spices is reported to be its essential oil fractions which is claimed to be inhibitory to microbial growth and this is said to vary from one spice to the other [20].

**Table 1. Bacteria Load of Spiced *Hibiscus sabdariffa* liquor during storage at ambient (28±2°C) temperature**

Spice	Spice conc (%)	Storage time (Days) and microbial counts			
		1 (10 <sup>2</sup> ) Cfu/mL	2 (10 <sup>2</sup> ) Cfu/mL	3 (10 <sup>2</sup> ) Cfu/mL	4 (10 <sup>2</sup> ) Cfu/mL
Control	-	NG	5.63	5.45	5.24
<i>Aframomum melegueta</i>	0.5	NG	3.70	4.00	4.00
	1.0	NG	3.50	3.80	3.92
	1.5	NG	3.20	3.50	3.50
	2.0	NG	3.00	3.40	3.50
	<i>Piper guineensis</i>	0.5	NG	3.20	3.20
<i>Piper guineensis</i>	1.0	NG	2.80	2.60	2.90
	1.5	NG	1.70	2.00	2.82
	2.0	NG	1.80	3.15	2.45

Keywords: NG means "No growth and Cfu means "Colony forming units"

**Table 2. Bacteria Load of Spiced *Sorghum bicolor* liquor during storage at ambient (28±2°C) temperature**

Spice	Spice conc (%)	Storage time (Days) and microbial counts			
		1 (10 <sup>2</sup> ) Cfu/mL	2 (10 <sup>2</sup> ) Cfu/mL	3 (10 <sup>2</sup> ) Cfu/mL	4 (10 <sup>2</sup> ) Cfu/mL
Control	-	NG	5.63	5.45	5.24
<i>Aframomum melegueta</i>	0.5	NG	4.42	4.30	4.60
	1.0	NG	4.20	4.00	4.10
	1.5	NG	4.00	3.85	3.80
	2.0	NG	3.80	3.60	3.70
	<i>Piper guineensis</i>	0.5	NG	3.54	3.55
<i>Piper guineensis</i>	1.0	NG	3.20	3.40	3.40
	1.5	NG	3.18	2.82	3.20
	2.0	NG	2.80	3.70	3.40

Keywords: NG means "No growth and Cfu means "Colony forming units"

**Table 3. Coliform counts of Spiced *Hibiscus sabdariffa* and *Sorghum bicolor* liquors during storage at ambient (28±2°C) temperature**

Spice	Spice conc (%)	Storage time (Days) and coliform counts			
		1 (10 <sup>2</sup> ) Cfu/mL	2 (10 <sup>2</sup> ) Cfu/mL	3 (10 <sup>2</sup> ) Cfu/mL	4 (10 <sup>2</sup> ) Cfu/mL
Control	-	NG	NG	NG	NG
<i>Aframomum melegueta</i>	0.5	NG	NG	NG	NG
	1.0	NG	NG	NG	NG
	1.5	NG	NG	NG	NG
	2.0	NG	NG	NG	NG
<i>Piper guineensis</i>	0.5	NG	NG	NG	NG
	1.0	NG	NG	NG	NG
	1.5	NG	NG	NG	NG
	2.0	NG	NG	NG	NG

Keywords: NG means "No growth and Cfu means "Colony forming units"

## 4. CONCLUSION AND RECOMMENDATION

### 4.1 Conclusion

The results from this study showed that shelf stable, non-alcoholic drinks could be produced from *Hibiscus sabdariffa* and *Sorghum bicolor* with pasteurization and use of spices. It is evident from this study also, that *Aframomum melegueta* and *Piper guineensis* extracts possess antimicrobial properties, since they can extend the shelf life of *Hibiscus sabdariffa* and *Sorghum bicolor* for up to 4 days at tropical ambient storage conditions.

### 4.2 Recommendation

Bacterial growth was detected in all samples after second day of production. It is therefore, recommended that the spices should be used synergistically with other means of preservation. Further research into combination treatments with other means such as carbonation, refrigeration, freezing or high temperature-short time heating is therefore recommended.

### CONSENT

Not applicable.

### ETHICAL APPROVAL

Not applicable.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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