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Effect of some chemical preservatives on the shelf-life of sobo drink

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The effect of some commonly used chemical preservatives (benzoic acid, sodium benzoate and sodium sulfite) on the shelf-life of *Sobo* drink was investigated by isolation of microorganisms before and during its ambient storage for 14 days. Three bacterial species, i.e. *Lactobacillus acidophilus*, *Bacillus subtilis* and *Bacillus cereus* were isolated. *L. acidophilus* was found only in the samples without preservatives, while *B. subtilis* and *B. cereus* were found in the samples with preservatives. Four fungal species, i.e. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Trichoderma* sp. were isolated. *Trichoderma* sp. was isolated only in the samples without preservatives, while *A. niger, A. flavus, A. fumigatus* were isolated in samples with preservatives. Antimicrobial activities of the chemical preservatives revealed that benzoic acid was the most effective against both bacterial and fungal species, followed by sodium benzoate and sodium sulfite. Of all the samples, only those treated with benzoic acid as preservative remained organoleptically attractive after preservation for 14 days.

Key words: Antimicrobial activity, chemical preservatives, *Sobo*, microorganisms.

INTRODUCTION

Beverages are consumed for their nutritional value, thirst quenching properties, stimulating effect or for their medicinal values (Adegunloye et al., 1996; Elmahmood and Doughari, 2007). *Sobo* drink is a local beverage quite popular in northern Nigeria and is enjoyed at various social gatherings. Its popularity has recently spread across the entire country because of its purported medicinal value, as well as the increasing cost of other available soft drinks (Adegunloye et al., 1996).

Sobo drink is prepared from the calyx of the roselle hemp plant *Hibiscus sabdariffa* (Malvaceae). Roselle, also known as Jamaican sorrel, is probably a native of West Africa and has been cultivated throughout India and part of Asia for centuries. Currently, it is grown throughout the tropics especially in Indonesia, Central Eastern Java, India-Bangladesh, Sri Lanka, the Philippines and the West Indies. Different ethnic groups call the vegetable plant various names. The Yorubas call the leaves 'Amukan', while the flower is called 'Isapa'. The Igbos call it names like 'Ojo', 'Akwaroazo', while the Hausas call the fleshy calyx names like 'Yakuwa', 'Sure' or 'Gurguzu' (Arthney and Dennis, 1991). In spite of the increasing popularity of *Sobo* juice, one of its limitations for large-scale production is that it deteriorates rapidly. In fact, its shelf-life is approximately 24 h, following production if not refrigerated.

The shelf-life of a product is defined as the expected time duration that a product will remain organoleptically acceptable. It is a function of holding temperature and the number of microorganisms remaining in it after processing. It is possible to improve the shelf-life of *Sobo* by slowing down the fermentation process, thus inhibiting discolouration of the *Sobo* drink and possibly permitting large-scale production and preservation for longer period with maximum retention of its nutritive values. Prescott et al. (2002) defined preservatives as a group of chemical compounds deliberately added to food or that appears in food as a result of pre-processing treatment, processing or storage. These include simple organic acids (such as propionic acid, sorbic acid, benzoic acid) *p*-hydroxyl benzoate alkylester (parabens), ethylene/propylene oxides, sulfides, ethylene oxide (as a gas sterilant), ethyl formate and sodium nitrates. The sulphites inhibit yeasts, moulds and bacteria and are most effective as inhibitors of browning in foods. Sulphur dioxide and sulphites are metabolized to sulphate and are excreted in the urine

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without any obvious pathological result. Benzoic acid has been widely employed as an antimicrobial agent in foods and it occurs naturally in cranberries, prunes, cinnamon and cloves. It is well suited for acid foods such as fruit juices, carbonated beverages, pickles and sauerkraut. Benzoic acid has been found to cause no deleterious effect when used in small amounts. It is, however, readily eliminated from the body after conjugation with glycine to form hippuric acid (Ihekoronye and Ngoddy, 1995). This work was therefore aimed at determining the effect of some chemical preservatives on the shelf-life of *Sobo* drink.

MATERIALS AND METHODS

Collection of samples

Dried calyx of *H. sabdariffa* was purchased from Jimeta market in Yola, Adamawa State, Nigeria and was taken to the laboratory in a clean cellophane bag. The chemical preservatives, i.e. sodium sulfite, sodium benzoate and benzoic acid, in concentrations of 0.1% (w/v) each were obtained from the Laboratories of the Department of Chemistry, Department of Food Science and Technology, Federal University of Technology, Yola and the Department of Food Science and Technology, Federal Polytechnic, Bauchi, Bauchi State, Nigeria.

Identification of the plant

The plant was identified and authenticated by Mr. D.F. Jatau of the Department of Forestry and Wildlife Management, School of Agriculture and Agricultural Technology, Federal University of Technology, Yola Adamawa State, Nigeria where the specimen voucher was deposited.

Preparation of *Sobo* drink

Dried calyx of *H. sabdariffa* (15 g) was sorted and then washed with tap water. They were then boiled for 10 min and allowed to cool. The juice was extracted by squeezing the boiled calyxes and then sieving the thick red suspension using a clean sterile sieve. 50 g of granulated white sugar and 100 ml of pineapple juice were added to enhance the taste and flavour. The S*obo* drink was then divided into four 100 ml samples and each of three different preservatives, sodium sulfite (sample A), sodium benzoate (sample B) and benzoic acid (Sample C), respectively, were added to three of the 100 ml samples. No preservative was added to the remaining 100 ml volume (sample D) (control) (Fasoyiro et al., 2005; Morton, 1987; Adenipekun, 1998).

Determination of pH

20 ml of each *Sobo* sample containing preservatives (A, B and C) and one without preservative (D) (control) was dispensed into two separate clean sterile beakers and the pH was determined using a pH meter (Jenway 3020) (Cheesbrough, 2002).

Isolation and enumeration of bacteria

The pour plate method was used for the isolation and enumeration of bacteria in the *Sobo* samples. Ten-fold serial dilutions of homogenized suspension were prepared aseptically by transferring 1 ml of the *Sobo* sample into 9 ml of sterile distilled water. 1 ml of the 10^{-6} dilution of the *Sobo* samples (A, B, C and D) was aseptically pipetted into a sterile Petri dish and cool sterile molten Nutrient agar was poured into the plates, swirled gently to mix the contents and then allowed to solidify. The plates were incubated at 37ºC for 24 h. The procedure was repeated on the 2nd, 3rd, 6th, 8th, 10th, 12th and 14th day of preservation of the drink (at room temperature) and the number of colonies that developed was enumerated using the colony counter (Prescott et al., 2002).

Isolation of fungi

The pour plate method was also used for the isolation of fungi. Ten-fold serial dilutions of the *Sobo* samples (A, B, C and D) was aseptically prepared by transferring of the sample into 9 ml of sterile distilled water. 1 ml of the 10^{-6} dilution was aseptically pipetted into a Petri dish, allowed to cool and 18 ml of sterile molten Sabouraud Dextrose Agar was dispensed into the plate and then swirled gently to mix the contents. The agar was allowed to solidify and then incubated at room temperature for 5 days (Cheesbrough, 2002).

Identification of isolates

The characterization and identification of bacteria and fungal isolates were based on the colonial morphology, staining reactions (including spore staining) and standard biochemical tests, as described by Kregervan (1984), Sneath et al. (1986), Barnett et al. (1990), Claus (1991) and Cheesbrough (2002).

Determination of antimicrobial activity of preservatives

Each of the preservatives was constituted to a concentration of 200 mg/ml in three different test tubes. To determine the antibacterial activity, 2 ml of each of the preservatives was dispensed into a sterile Petri dish and 18 ml of previously molten Mueller- Hinton agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) was added and allowed to solidify. The *Sobo* sample was then inoculated onto the agar by using a swab. The swab was dipped into each of the *Sobo* samples (A, B, C and D) in four different test tubes, withdrawn and slightly pressed against the wall of the test tube to reduce the excess liquid. The swab was then smeared onto the plate to inoculate the samples. The culture plate was then incubated at 37ºC for 24 h (for bacteria) and at room temperature for three days for fungi (Prescott et al., 2002). After the period of incubation, all the plates were examined for the presence or absence of microbial growth. Absence of growth indicated the inhibitory action of the preservatives (Prescott et al., 2002).

Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of chemical preservatives

The minimum inhibitory concentration (MIC) of the chemical preservatives was determined for each of the isolates at varying concentrations of 300, 250, 200, 150, 100.0, 50.0, 25.0 and 12.5 mg/ml, as described by Doughari and Okafor (2007) (with slight modifications). The different concentrations were dispensed in series of test tubes, 1 ml of Nutrient broth (NB) (Oxoid) (for bacterial isolates) and Potato Dextrose broth (PDB) (Oxoid) (for fungal isolates) was added and then a loopful of each of the bacterial and fungal isolates previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. Tubes containing NB

Table 1. pH and Temperature values of *Sobo* samples before and during storage.

Sample	pH*	pH**	Temperature (ºC)	*Colour/taste	**Colour/taste
A	3.16	3.16	25	Red/Sweet	Red/Sweet
B	3.82	3.62	25	Red/Sweet	Dark Red/Sour
C	3.18	3.0	25	Red/Sweet	Dark Red/Sour
D	3.2	3.0	25	Red/Sweet	Dark Red/Sour

* = before storage; ** = during storage; A = sample with benzoic acid; B = sample with sodium sulfite; C = sample with sodium benzoate; D = sample without preservative (control).

Table 2. Bacterial and fungal isolates obtained from *Sobo* during storage at ambient temperature for 14 days.

Sample	Bacteria	Fungi
A	*Bacillus subtilis,*	*Aspergillus niger,*
	Bacillus cereus	*Aspergillus fumigatus*
B	*Bacillus subtilis, Bacillus cereus*	*Aspergillus niger,*
		Aspergillus fumigatus,
		Aspergillus flavus
C	*Bacillus subtilis,*	*Aspergillus niger,*
	Bacillus cereus	*Aspergillus fumigatus*
D	*Bacillus subtilis,*	*Aspergillus niger,*
	Bacillus cereus,	*Aspergillus fumigatus,*
	Lactobacillus acidophilus	*Aspergillus flavus, Trichoderma* sp.

A = sample with benzoic acid; B = sample with sodium sulfite; C = sample with sodium benzoate; D = sample without preservative (control).

only (for bacteria) and PDB only (for fungi) were seeded with the isolates to serve as control. All the tubes were then incubated at 37^{o}C for 24 h. After incubation, the tubes were then examined for microbial growth by observing for turbidity, and concentrations without growth were taken as the MIC.

To determine the MMC, a loopful of broth was collected from those MIC tubes that did not show any growth and were inoculated on sterile Nutrient agar (Oxoid) (for bacterial isolates) and on Potato Dextrose agar (PDA) (Oxoid) (for fungal isolates) by streaking. Each of the agar plates (NA and SDA) were streaked with the isolates only to serve as control. The plates were then incubated at 37^{o}C for 24 h (for bacterial isolates) and at ambient temperature for three days (for fungal isolates). After incubation the concentration at which no visible growth was seen was noted as the MMC.

RESULTS

Results of the investigation showed that the pH of the *Sobo* sample was generally acidic irrespective of the type of preservative added (Table 1). The pHs were 3.16 for the sample with benzoic acid (A), 3.82 for the sample with sodium sulfite (B) and 3.18 for the sample with sodium benzoate (C) . The pH of the control sample (D) without preservative was 3.20. The acidity increased slightly for all the samples during preservation, except for those with benzoic acid with the respective pH values as 3.16 (samples with benzoic acid), 3.62 (samples with sodium sulfite) and 3.0 (samples with sodium benzoate and the control samples). Results indicated no variation in temperature (25ºC) for all the samples. Results also showed that organoleptically, the taste (sweet) and colour (red) of samples with benzoic acid were not altered after preservation, but there was a marked change in colour (red to dark red) and taste (sweet to sour) for all the other samples (Table 1).

Table 2 shows the bacteria and fungal species identified. Results showed the presence of three bacterial species namely: *B. subtilis, Bacillus cereus* and *Lactobacillus acidophilus. B. subtilis* and *Bacillus cereus* were found in all the samples investigated, while *L. acidophilus* was found only in the *Sobo* samples that had no preservative (D) (control). Results also showed that *A. niger* and *A. fumigatus* were found in all the samples, while *A. flavus* and *Trichoderma* sp. was isolated from samples containing sodium sulfite (B) as preservative and the control sample (D) (without preservative).

Table 3 shows the bacterial counts of samples investtigated. Results showed that the *Sobo* samples had an initial bacterial count of 1.13×10^{7} cfu/ml, but the count of the samples with preservatives (A, B and C) was observed to progressively drop, while that of the samples without preservatives (D) (control) increased progres-

Table 3. Bacterial counts of *Sobo* samples prepared in the laboratory and stored at ambient temperature for 14 days.

Sample	Bacterial count 1 x 10^7 (cfu/ml)/Days							
	1	2	3	6	8	10	12	14
A	0.82	0.78	0.69	0.57	0.46	0.39	0.37	0.36
B	0.92	0.87	0.85	0.81	0.78	0.76	0.73	0.71
C	0.86	0.81	0.78	0.74	0.70	0.69	0.67	0.69
D	1.13	1.07	1.18	1.24	1.27	1.29	1.32	1.34

A = sample with benzoic acid; B = sample with sodium sulfite; C = sample with sodium benzoate; D = sample without preservative (control).

Table 4. Antimicrobial activity of chemical preservatives used for investigation.

Preservative (200 mg/ml)	Antifungal activity				Antibacterial activity		
	A. niger	*A. fumigatus*	*A. flavus*	*Trichoderma* sp	*B. subtilis*	*B. cereus*	*L. acidophillus*
benzoic acid	-	-	-	+	-	+	+
sodium sulfite	+	+	+	+	+	+	+
sodium benzoate	-	+	+	+	+	+	+

- = no growth; + = presence of growth

Table 5. Minimum inhibitory concentration (MIC) (mg/ml) and minimum microbicidal concentration (MMC) (mg/ml) values of the chemical preservatives.

	Benzoic acid		Sodium sulfite		Sodium benzoate	
	MIC	MMC	MIC	MMC	MIC	MMC
A. niger	50	100	300	300	300	300
A. fumigatus	100	100	300	300	300	300
A. flavus	100	200	300	300	300	300
Trichoderma sp.	200	300	300	300	300	300
B. subtilis	25	50	300	300	300	300
B. cereus	250	300	300	300	300	300
L. acidophilus	250	300	300	300	300	300

sively. Results of antimicrobial activity of the chemical preservatives used, showed that the fungal species isolated were not inhibited by sodium sulfite. Sodium benzoate only inhibited the growth of *A. niger*, while benzoic acid inhibited the growth of *A. flavus* , *A. fumigatus* and *A. niger* at 200 mg/ml. None of the preservatives had any effect on *Trichoderma* sp. For antibacterial activity, only samples with benzoic acid inhibited the growth of *B. subtilis* (MIC 25 mg/ml, MMC 50mg/ml). All other preservatives (MIC and MBC values of 300 mg/ml) showed no antibacterial activity (Table 4 and 5).

DISCUSSION

The *Sobo* juice produced from the calyx of *H. sabdariffa* was very acidic with low pH values. The pH values obtained, when compared with the pH of vegetable juices (which is between 4 a nd 6) is quite low (Frazier and Westhoff, 1986). The high acidity of the juices could account for the low numbers and few types of organisms isolated, although the isolates have been found to be associated with food spoilage (Stainer et al., 1987; Prescott et al., 1999). Results obtained indicated that populations of different types of microorganisms are present in *Sobo* drink. Fasoyiro et al. (2005) suggested that the presence of microorganisms in *Sobo* drink produced with a boiling method is indicative of post-production contamination resulting from the addition of sugar and other additives. The presence of *B. subtilis* and *B. cereus* in the *Sobo* drinks containing preservatives may be due to the presence of endospores. These structures are extraordinarily resistant to environment stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation (Prescott et al., 1999).

Though the counts of viable microorganisms were too high to be acceptable in a drink, the count was generally

low compared with count of microflora in related food materials (Frazier and Westhoff, 1986; Prescott et al., 2002; Elmamood and Doughari, 2007). From the results, it can be deduced that the chemical preservatives used were effective against the microorganisms. This is shown by the reduction in the bacterial counts over time, which contrasted in the case of the samples without preservatives. Preservatives have been used to store food substances and they act by inhibiting, retarding or arresting the growth of microorganisms or of any such deterioration resulting from their presence or of masking the evidence of any such deterioration (Ihekoronye and Ngoddy, 1995). To be in accord with good manufacturing practices, the use of preservatives should not adversely affect the nutritive value of food or should not permit the growth of food poisoning organisms while suppressing the growth of others that would make spoilage evident (Ihekoronye and Ngoddy, 1995). Investigation of the antimicrobial activity of the preservatives revealed that benzoic acid was the most effective on both fungal and bacterial species. At 200 mg/ml, benzoic acid inhibited the growth of *B. subtilis*, *A. flavus*, *A. fumigatus* and *A. niger*, and samples containing this preservative had the lowest bacterial count from day 1 - 14 of preservation at room temperature. Preservatives may be microbicidal and kill the target organisms or they may be microbiostatic in which case they simply prevent them from growing, thus prolonging the shelf-life of the product (Fawole and Oshe, 2002). In this study, even after 14 days of preservation, samples containing benzoic acid (sweet and red throughout preservation period) had more keeping quality than samples with other preservatives, which had lost colour (turned dark red), gone sour and even odorous. The shelf-life can thus be extended by the use of preservatives or storage at conditions that will not favour bacterial multiplication (Pelczar et al*.,* 2002). Samples without preservatives (sample D) (control), however, exhibited the highest bacterial count from day 1 - 14 of preservation and consequently, had the poorest keeping quality as compared to those with preservatives (samples A, B and C). *Sobo* drink when left for two or three days at room temperature turns sour. This may result from fermentation due to microbial action. This fermentation process has been found to lead to loss of taste and nutritional value, increased rate of browning and offensive odour, and perhaps presence of cloudy materials at the bottom of the container, which is an acute state after many days of preparation (Adenipekun, 1998).

Conclusion

Results of this investigation revealed the extension of the shelf-life of the *Sobo* drink between 4 - 14 days especially with samples containing benzoic acid. There-fore, the use of chemical preservatives in the appropriate concentrations in conjunction with proper sanitary practices, the shelf-life of *Sobo* drink can be enhanced such that fermentation is reduced and discolouration is inhibited. Consequently, *Sobo* drink can be produced on a large scale and preserved for longer periods and still retain its nutritive value such that the drink will attract acceptance. The cheapness of the raw materials, its preparation and the finished product makes the drink particularly a worthwhile venture. However, further investigation of the microbial populations associated with the dried calyx of *H. sabdariffa,* and determination of the chemical composition of the plant parts may provide effective and appropriate preservation techniques for the drink.

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