

Full Length Research Paper

# Effects of different packaging materials on microbiological, physio-chemical and organoleptic quality of zobo drink storage at room temperature

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The effects of different packaging materials on microbiological, physiochemical and organoleptic quality of zobo drink prepared from *Hibiscus sabdariffa* were investigated. Freshly prepared zobo drink at 45 °C was aseptically dispensed into glass bottles, plastic bottles and polythene sachets and stored at 29 ± 2 °C for 6 months. Microbiological and physico-chemical (carbohydrate, protein, pH, vitamin C and total soluble solids content) quality changes were assessed on a monthly basis. Organoleptic quality was evaluated soon after preparation and at the end of 6 months storage period. Results indicated various degrees of changes in quality of the samples in different packaging materials; changes were more remarkable in the polythene sachet packaged samples. Microbial species isolated were *Bacillus subtilis*, *Lactobacillus planetarium*, *Streptococcus lactis*, *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Geotrichum candidum*. Lower pH and total soluble solids (TSS) values were recorded in the packaged samples as compared with fresh sample, whereas carbohydrate, protein and vitamin C contents showed higher values in the packaged samples. Glass bottle proved to be the best packaging materials for zobo drink storage at ambient temperature (29 ± 2 °C).

**Key words:** Packaging, Microbiological, physico-chemical, organoleptic, zobo, temperature.

## INTRODUCTION

Zobo drink is obtained from flowers of *Hibiscus sabdariffa*. It is a refreshing drink produced in a home and industry, and is now becoming very popular among people of various socioeconomic classes in West Africa and Nigeria in particular.

The drink as currently produced is not shelf stable due to microbial spoilage. The spoilage is manifested as losses in physico-chemical and organoleptic quality. Proper packaging has been proved to be a means of preventing loss of quality of products in storage (Efiuvwevwe and Uwanogho, 1990; Ogiehor et al., 2004). Improper packaging has also been implicated as a source of microbial contamination in food processing operations (Paine, 1992; Leistner, 1997). Various pack-

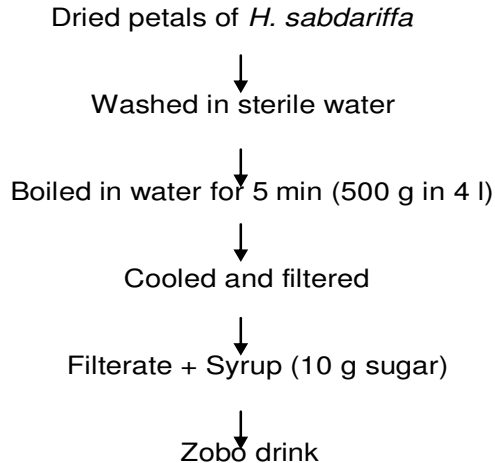
aging materials including glass bottles, plastic bottles and polythene sachets are currently used for zobo drink storage in Nigeria. In most cases, there is no consideration for their suitability. There are no reports on the effect these packaging materials will have on the quality of zobo drink during storage particularly at ambient temperature. Elsewhere, the effects of packaging films on tomato fruit storage life have been earlier reported (Geeson et al., 1985).

The objective of the present study therefore, is to evaluate the effects of these commonly used packaging materials on the microbiological physico-chemical and organoleptic quality of zobo drink during storage at ambient temperature (29 ± 2 °C).

## MATERIALS AND METHODS

Dried reddish flowers (petals) of *H. sabdariffa* were obtained from

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**Figure 1.** Steps in the preparation of zobo drink.

an open market in Warri, Delta State. They were collected in sterile polythene bags and transported to Microbiology laboratory at Delta State University, Abraka for processing. The packaging materials (glass bottles, plastic bottles and polythene sachets) were also bought in Warri Main Market. They are fresh and unused ones.

#### Preparation of zobo drink

Zobo drink was prepared according to the recipe in Maggi menu cook book (1996) (Figure 1).

#### Packaging

Freshly prepared zobo drink was aseptically dispensed into 25 cl of the 3 different sterile packaging materials, glass bottles, plastic bottles and polythene sachets and stored at room temperature ( $29 \pm 2^\circ\text{C}$ ) for a period of 6 months. Analyses were carried out every month for microbiological and physico-chemical changes. Organoleptic evaluation was carried out soon after preparation and at the end of the 6 months storage period.

#### Microbiological analysis

Fungal growths were determined using pour plate method in acidified potato dextrose agar (oxid) following incubation at  $28^\circ\text{C}$  for 48 h. Growths were calculated and expressed as colony forming units per millimeter (cfu/ml). Discrete colonies were thereafter aseptically picked and stained with lactophenol cotton blue solution on a microscope slide and examined (Harrigan and McCance, 1976) and then identified (Barnett and Hunter, 1976).

Bacteria growths were estimated using spread plate technique (using 0.10 ml of appropriate dilution in 0.10% peptone water) on duplicate nutrient agar (oxid) plates incubated at  $30^\circ\text{C}$  for 24 h. Bacterial isolates of discrete characteristics were picked and identified using standard methods (Buchanan and Gibbons, 1974; Harrigan and McCance, 1976).

#### Physico-chemical analysis

pH of zobo drink was determined using Jenway model 302 pH meter after standardizing with phosphate buffer at pH 4 (AOAC,

1980). Titratable acidity (TA) was determined by titrating 0.10 M sodium hydroxide (NaOH) against 10 ml of zobo drink using phenolphthalein as indicator (AOAC, 1980). Titratable acidity was expressed as percentage lactic acid. Total carbohydrate content of zobo drink sample was determined according to Plummer (1978) and Odibo et al. (1990). Protein content was estimated by the Lowry et al. (1951) and Plummer (1978) methods. Vitamin C content was estimated by titrating 2,6-dichlorophenolindophenol against 5 ml of zobo drink sample treated with glacial acetic acid (Plummer, 1978; AOAC, 1980). Total soluble solids content was determined at  $29 \pm 2^\circ\text{C}$  using Abbe hand refractometer (Atago Co. Ltd, Japan). Percentage total soluble solids content was calculated as sucrose, using sucrose conversion Table corrected to  $20^\circ\text{C}$  (Pearson, 1976).

#### Organoleptic (sensory) quality evaluation

Organoleptic quality evaluation was carried out using the parameters of taste, colour (appearance), aroma and overall acceptability with a 9 point hedonic scale (1 - 9) according to Larmond (1982). A 10 member panel was used to score the various parameters.

#### Statistical analysis

All experimental data were subjected to statistical analysis of mean, standard deviation and analysis of variance (ANOVA) (Ogbeibu, 2005). The significant value was determined by the t-distribution test using appropriate computer software.

## RESULTS

#### Microbiological quality of zobo drink

The microbiological quality assessment showed that no microbial growth was detected at the time of packaging (Table 1). Growth appearances were however recorded in all the packages at the end of the 1<sup>st</sup> month. This was higher in fungi than bacteria. Maximum growth appearances were observed in the 3<sup>rd</sup> month. Thereafter growth dropped steadily, till the last month of storage. Microorganisms isolated were *Bacillus subtilis*, *Lactobacillus plantarum*, *Streptococcus lactis*, *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Geotrichum candidum*. The effectiveness of the packaging materials in preventing microbial growth is in the order, glass bottle > plastic bottle > polythene sachet.

#### Physico-chemical quality

There were various degrees of variations in physico-chemical quality of zobo drink stored in different packaging materials (Table 2). The changes were significant in polythene sachet as when compared with fresh product. There was no significant difference ( $P < 0.01, 0.05$ ) between the product in glass bottle and the fresh product. At the end of the storage period, the order of stability of the parameters in the packaging materials was glass bottle > plastic bottle > polythene sachet.

**Table 1.** Effects of packaging materials on the microbial quality (total viable count; log<sub>10</sub> cfu/ml) of zobo drink during storage at 29 ± 2°C.

Period of storage (months)	Glass bottle		Plastic bottle		Polythene sachet	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
0	ND	ND	ND	ND	ND	ND
1	1.07±0.005	2.50±0.10	1.17±0.03	2.45±0.03	2.36±0.04	2.50±0.05
2	1.30±0.01	2.63±0.01	2.36±0.05	2.52±0.01	2.37±0.01	3.24±0.06
3	2.36±0.04	2.75±0.5	2.44±0.05	2.70±0.07	3.49±0.015	3.40±0.09
4	2.36±0.00	2.35±0.50	2.53±0.04	2.48±0.03	2.38±0.06	3.54±0.00
5	1.00±0.10	1.55±0.08	1.29±0.05	2.49±0.02	1.36±0.06	3.58±0.02
6	1.00±0.10	1.49±0.015	1.07±0.01	2.45±0.05	1.20±0.05	3.60±0.08

Each value is the mean ± standard deviation of triplicate determinations  
 ND = Not detected.

**Table 2.** Effect of packaging materials on the physico-chemical quality of zobo drink at the end of six months storage period at 29 ± 2°C.

Parameter	Fresh	Packaging materials		
		Glass bottle	Plastic bottle	Polythene sachet
pH	3.50 ± 0.10	3.40 ± 0.03	3.00 ± 0.10	2.75 ± 0.03
Titrateable acidity (%)	0.084 ± 0.002	0.079 ± 0.001	0.096 ± 0.001	0.80 ± 0.03
Carbohydrate (%)	0.76 ± 0.003	0.78 ± 0.01	0.82 ± 0.01	0.80 ± 0.01
Protein (%)	0.33 ± 0.001	0.35 ± 0.005	0.39 ± 0.005	0.40 ± 0.02
Vitamin C (mg/ml)	0.35 ± 0.02	0.36 ± 0.005	0.39 ± 0.01	0.44 ± 0.04
Total soluble solids (% brix)	10.80 ± 0.05	10.60 ± 0.05	10.20 ± 0.5	9.50 ± 0.01

Each value is the mean ± standard deviation of triplicate determinations

### Organoleptic quality

Effect of packaging materials of the organoleptic quality such as colour, taste, and aroma at the end of 6 months storage period is shown in Figure 2. The various quality attributes were different in the different packaging materials. The variation was however more remarkable in polythene sachet. Overall acceptability score was in the order: glass bottle > plastic bottle > polythene sachet.

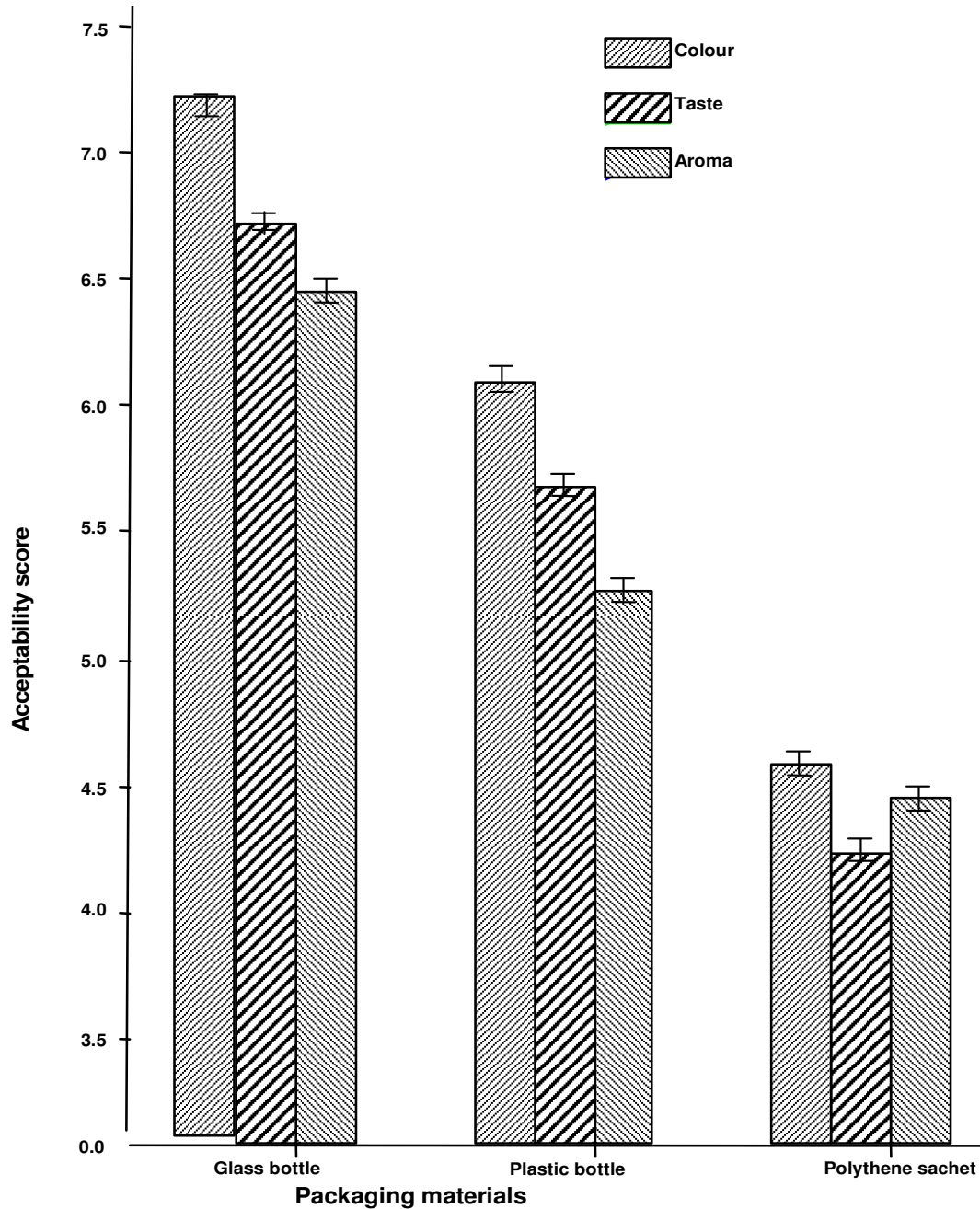
### DISCUSSION

The presence of microorganisms in zobo drink after one month in different packaging materials when non was detected at the time of packaging is an indication that they were not completely destroyed by the initial treatments. The differences in total viable counts (TVC) in zobo drink stored in different packaging materials may be attributed to the nature of the packaging materials themselves. One such nature factor is relative permeability to air. Polythene sachet is more permeable to air than glass and plastic bottles. Permeability to gases such as oxygen, carbon (IV) oxide and water vapour has been reported to affect the growth and survival of microorganisms in packaged foods (Efiuvwevwe and

Uwanogho, 1990; Paine, 1992; Ogiehor et al., 2004). Permeability to air (oxygen) may be aiding the growth of fungi encountered in the present study. This agrees with an earlier report by Geeson et al. (1985).

Low bacteria count in the packages may be due to low pH (acid) and high sugar content which do not favour the growth of most bacteria, since they act as hurdles against them. Similar findings have been reported (Efiuvwevwe and Eka, 1991; Leistner, 1994).

Variations in physico-chemical quality of fresh zobo drink sample as compared with those stored in the packaging materials at the end of the storage period may be linked to the activities of microorganisms in packaged unprocessed samples. Metabolic activities of microorganisms may have caused the release of metabolites such as organic acids resulting in increased titrateable acidity and subsequent lower pH values recorded. Changes in carbohydrate, protein and vitamin C contents may be attributed to fermentative activities of associated microorganisms, which break down various components thereby releasing the nutrients. This corroborates some earlier reports (Efiuvwevwe and Hubson, 1989). On the other hand reduction in total soluble solids (TSS) in the packaged sample may be attributed to utilization of TSS by the microorganisms especially fungi. This is similar to the observed decrease in reducing sugar content of tomatoes



**Figure 2.** Effect of packaging materials on organoleptic quality of zobo drink after 6 months storage at room temperature ( $29 \pm 2^\circ\text{C}$ ).

inoculated with *Alternaria tenuis* (Efiuvwevwe and Eka, 1991).

Variations in organoleptic quality of colour, taste, aroma and overall acceptability of zobo drink in different packaging materials is likely to be due to the level of protection offered by the materials microbial action and environmental influences such as gas permeability. This may be responsible for the more remarkable variations in organoleptic quality recorded in polythene sachet and the least acceptability score recorded therein. It is already

known that permeability to gases is important in the microbiology of packaged foods (Mathlouthi, 1994; Rao, 1996).

This study has shown that although the 3 packaging materials are suitable for the storage of zobo drink at ambient temperature ( $29 \pm 2^\circ\text{C}$ ), glass bottle is most suitable. The findings are useful in developing reliable data and indices for predicting the safety during processing, handling and storage of this new refreshing drink.

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