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Effect of Packaging Materials and Storage Temperatures on the Microbiological Quality of *Hibiscus sabdarifa* Drinks during Ambient and Refrigeration Storage

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Authors' contributions

This work was carried out in collaboration between both authors. Author PCOE designed the study, performed the statistical analysis, wrote the protocol, wrote the final draft of the manuscript and managed the literature searches. Author FIC managed the analyses of the study and part of the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the effect of packaging materials and storage temperatures on the microbiological quality of *Hibiscus sabdarifa* drinks produced with: 1) commercial pineapple flavour (HCPF) and; 2) *Phoenix dactylifera* (38%) and pineapple extract (2%) (HPPE).
Methodology: Pasteurized drinks packaged in polyethylene sachets, plastic and glass bottles were stored at refrigeration (4.4±2^{o}C) and ambient (25±2^{o}C) temperatures for 27 and 9 days respectively.
Results: There was significant (P≤0.05) decrease in total bacterial count in HCPF (≤4.51-≥2.14 Log_{10}CFU/ml) with higher death rate (0.06) in plastic bottles at 4.4±2^{o}C while at 25±2^{o}C it increased significantly (P≤0.05) in HPPE samples (4.00-≤4.95 Log_{10}CFU/ml) with least growth rate in plastic bottles (0.02). Yeast count at 25±2^{o}C (1.28 – 2.15 Log_{10}CFU/ml) was significantly (P≤0.05) higher than at 4.4±2^{o}C (1.00 – 1.60 Log_{10}CFU/ml) and drinks in plastic bottles had the least growth rates (≤0.03). Coliform (2.04 – 2.59 Log_{10}CFU/ml), *Escherichia coli* (2.00 – 2.93 Log_{10}CFU/ml) and *Staphylococcus* (2.00 – 2.50 Log_{10}CFU/ml) sparingly detected, were unable to grow in the drinks with greater inhibition at 25±2^{o}C in all packaging materials. No growth of *Salmonella* was observed in the drinks. Glass bottles favoured more microbial growth but the

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levels were satisfactory for all packaging which is indicative of microbiological safety.
Conclusion: Any of the packaging material can be used for packaging of *Hibiscus sabdarifa* drinks with storage at refrigeration temperature for ≤ 21 days. It is informative to both consumers and producers that the then wasted pineapple peels can serve as an ingredient in *Hibiscus sabdarifa* drink production.

Keywords: Hibiscus sabdarifa; Phoenix dactylifera; pineapple peel extract; packaging materials; microbiological qualities; refrigeration; ambient temperatures.

1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* Linn.) is widely grown in the North Eastern and middle belt regions mostly for its stem, leaves, and calyces [1]. The stems are primarily used for the production of fiber, which can be used as a substitute for jute in making burlap [2]. The calyces have brilliant red color and distinctive flavor that is cherished in the production of a most popular refreshing drink called *zobo* in Nigeria*,* other beverages such as herbal tea, wine, and preserves like syrup, jams, jellies, and sauces [3]. It is used as a natural food colorant and flavoring agent in ice cream, cake, pudding, and rum [4,5]. The juice from the calyces is rich in malic acid, anthocyanins, ascorbic acid and minerals [6,7]. an is cherished for its claimed therapeutic effects [2,8,9].

Dates (*Phoenix dactylifera* L.) are consumed throughout the world for their pleasant flavour, smell and biting texture in addition to their use for flavoring foods, beverages and medication [10]. Dates are rich in natural fibres, vitamins and minerals; and have many health benefits [11,12]. Date fruit are a unique natural treasure marked by high sugar content and are used as a natural sweetener alternative to sucrose in food sectors for baked products, beverage, confectionery, dairy and sugar industries [13- 15].

Pineapple (*Ananas comosus* L.) is a tropical fruit widely consumed in many countries due to its exotic aroma and pleasant flavour, in addition to its health-promoting properties [16]. The peel which is often a waste from traditional pineapple processing has found various uses. It is rich in natural valuable compounds such as antioxidants, vitamins (A, B, B1, B2, B6, B12 and C), minerals (Calcium), enzymes, fibre, ferulic acid and phenolic compounds [16,17]. Pineapple peel extract have been reported to be of various therapeutic uses. Its phenolic compounds support the human antioxidative defense. Other important medicinal properties of the aqueous extract of pineapple peels include antithrombotic, antiedema, immunomodulatory and anticancer effects [18]. The anti-cancer effect of pineapple extract according to Raeisi et al. [19] can be through direct effect on cancer cells or cancer environment or indirect effect involving immune modulator or hematopoietic system. The trend in researched has been the inclusion of pineapple juice to *Hibiscus sabdariffa* drinks. But this present study in addition to the antimicrobial activities of the extract chose its inclusion for the claimed health benefits.

The matured calyces of the roselle flower after harvest are usually sundried in the open air along major roads. A traditional method that exposes the calyces to dust and contamination with different microorganism. The manner of display of the same calyces in the market for sale further leads to contamination. Heat process employed during the production of the calyx extract for a refreshing drink should be inhibitory to microbial contaminants. However, the traditional production process in a poor sanitary condition and lack of adequate personal hygiene practices has led to isolation of diverse microorganisms from the drink and the different spices added to the drink may add their own flora [20]. The risk of *Hibiscus sabdariffa* drink to the health of consumers have been reported based on the isolation and identification of some pathogen along with other microorganisms such as *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Bacillus* spp, *Klebsiella*, *Shigella, Salmonella* sp., Enterobacter spp, *Micrococcus* spp., *Aspergillus niger*, *Rhizopus* spp and *Penicillium* spp [21- 23].

Packaging is primarily, an essential part of any preservation process for the protection offered by the package [24]. It prevents food contamination with undesirable organisms and foreign matter [25] in addition to reduction of losses, value addition, extension of shelf-life, maintenance of quality and wholesomeness of products, etc. [25, 26]. Various packaging materials including glass bottles, plastic bottles and polyethylene sachets are currently used for packaging and

storage of *Hibiscus sabdariffa* drink in Nigeria. In most cases, there is no consideration on their suitability. Use of inadequate packaging materials which may not be adequately sterilized renders the product susceptible to microbial contamination thereby reducing the shelf life of the product [27,28]. The storage conditions also influence the quality and safety of the packaged product. A storage stability study at 29^{o}C revealed the presence of different bacteria and yeast sp. [23]. This study therefore, evaluated the effect of packaging materials and storage temperatures on the microbiological quality of *Hibiscus sabdarifa* drink sweetened and flavoured with *Phoenix dactylifera* and pineapple peel extract.

2. MATERIALS AND METHODS

2.1 Raw Materials and Chemicals

Dried *Hibiscus sabdariffa* calyces, pineapple, dates and premixed commercial pineapple sweetener were purchased from fruit garden market, Port Harcourt, Rivers State Nigeria. Chemicals and equipment of analytical grade were obtained from the analytical laboratory, Department of Food Science and Technology, Rivers State University, Port Harcourt.

2.2 Preparation of Pineapple Peel Oil Extract

Cold extraction technique described by Orodu and Akpedi, [29] was employed. The peels were sun dried for 3 days, oven dried for 24 h at 80^{o}C, ground to powder and soaked in n-hexane for 3 days. The mixture was decanted and the oil was obtained after evaporation in a water bath to remove the excess solvent from the oil.

2.3 Laboratory Production of *Hibiscus sabdariffa* Drink

The method of Chibueze et al. [30] was used. Sorted and washed *Hibiscus sabdariffa* calyces (600 g) were boiled for 30-45 min in a pot containing 10 liters of water. After boiling, it was set aside to cool to room temperature and liquid extract filtered using a clean sterile muslin cloth.

2.4 Preparation of Date Slurry

Dried dates were sorted, manually pitted by cutting open one side of the fruit and removing the pit, washed, conditioned by soaking in water for 5 h and then wet milled into slurry using blender.

2.5 Preparation of *Hibiscus sabdariffa* Drink

The test sample was prepared by adding 38% of *Phoenix dactylifera* slurry and 2% of pineapple peel extract to the *Hibiscus sabdariffa* drink (HPPE). *Hibiscus sabdariffa* drink with the commonly used commercial pineapple flavour served as control (HCPF). The mixture was homogenized and sieved using a muslin cloth and thereafter pasteurized at 72ºC for 10 min and allowed to cool before aseptically dispensing 100 ml quantities into the various packaging materials: polythene sachet plastic and glass bottles. A set of the drinks was stored at refrigeration temperature (4.4±2^{o}C) for 27 days and another set at ambient temperature (25±2^{o}C) for 9 days.

2.6 Sampling for Analysis

Sample was aseptically withdrawn after packaging before storage for day 1 and then on every other day for 27 days at refrigeration temperature (4.4±2^{o}C) and 9 days at ambient temperature (25±2^{o}C). The packaging materials for each day were removed from storage and samples withdrawn aseptically for microbiological analysis

2.7 Microbiological Analysis

Microbial media was prepared following the manufacturers instruction by weighing appropriate amounts of the respective media into the required volume of water. The agar was properly dissolved on hot plate stirrer and autoclaved at 121^{o}C for 15 min. The sterile medium was allowed to cool to 45^{o}C in a water bath before dispensing about 10 ml into sterile petri dishes to solidify. One millilitre (1 ml) of the aseptically withdrawn samples was pipetted into 9 mL of sterile peptone water in a sterile 20 mL tubes, vortexed for 3-5 s and serially diluted to 10^{-5}. Aliquots (100 µL) of the appropriate dilutions: 10^{-5} for nutrient agar (NA), 10^{-2} for Eosin methylene blue agar EMB, Salmonella Shigella agar (SSA), MacConkey agar (MCA) and Mannitol salt agar (MSA), and 10^{-1} for potato dextrose agar (PDA) were spread-plated on appropriate microbial media for each microorganism. Total *Bacteria, Coliform, Escherichia coli*, *Salmonella* and *Staphylococcus* were respectively, enumerated on NA, MCA, EMB, SSA and MSA incubated at 37ºC for 24 h, while *Yeast* was enumerated on PDA at 25ºC for

48 h. Plates with countable colonies were counted and calculated in colony forming units per ml (CFU/ml) as (number of colonies x dilution factor)/volume plated. The values were converted to Log_{10} CFU/ml.

2.8 Statistical Analysis

Data collected were statistically analyzed using Minitab (Release 18.1) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences among variables were evaluated by analysis of variance (ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level. Graphs were made using Microsoft Excel (Office 2016).

3. RESULTS AND DISCUSSION

3.1 Total Bacterial Count of *Hibiscus sabdarifa* Drinks in Different Packaging Materials during Refrigeration (4.4±2°C) and Ambient (25±2°C) Temperature Storage

The total bacteria count (TBC) of the samples are shown in Fig. 1, with the exponential law corresponding to the equation for representation of exponential growth indicating the specific growth rate given as $X = X_0e^{\mu t}$; where X is the final biomass corresponding to Y, X_0 is the biomass at time zero, μ is the specific growth rate with the time t. Generally, there were significant (P≤0.05) variations in the bacteria count over time, between the two types of drinks, amongst the packaging materials and at the different temperatures. At refrigeration temperature, in the HCPF drink (Fig. 1a), plastic had significantly (P≤0.05) the highest count on day 7, 13 and the least counts on day 9, 15 - 25 but did not differ (P>0.05) from others on day 27. Glass had significantly (P≤0.05) the least count on 1 - 13 and did not vary from sachets on day 15 - 27. The trend in count indicated decrease in viability of the bacteria with specific death rate significantly (P≤0.05) highest in plastic bottles (0.058). Samples in glass bottles had an increase in count with specific growth rate of 0.002, which is more of a stationary phase. In the HPPE drinks (Fig. 1b), on day 1, bacteria were below detection limit in all the samples, plastic had no growth on day 3 while samples in sachet and glass had counts of 4 Log_{10}cfu/ml. The trend in count of bacteria in the plastic and sachet was that of growth with specific growth rate 0.003 and 0.0001 respectively while samples in glass bottles had decrease in count. Due to the inconsequential increases or decreases, the count could be said to be in the stationary stage over the storage period. At ambient temperature, the HCPF drink samples (Fig. 1c), in plastic had significantly (P≤0.05) the least bacteria count on day 3 and 7, and the highest count on day 5 and 9. No growth of bacteria was observed on day 1. The trend was that of decrease in count with time and samples in glass bottles had significantly (P≤0.05) the highest specific death rate of 0.222. In HPPE samples (Fig. 1d), there was no significant (P>0.05) difference in counts of total bacteria on day 1 and 3 while on day 5 and 7 samples in plastic had significantly (P≤0.05) the highest count and the least on day 9. The trend was that of increase in count with time and samples in plastic bottles had significantly (P≤0.05) the least specific growth rate of 0.020. Between the two drinks, the trend was a decrease in the HCPF while in HPPE it was an increase in count.

Total bacteria count of the drinks during storage was within the acceptable level of $<10^3$-10^4 [31]. The trend of a decrease in the HCPF drink samples and an increase in the HPPE point to effect of date and pineapple extract in providing a more favourable growth medium for the bacteria in HPPE drink samples. Only fermentative lactic acid bacteria can survive and strive to grow in an acid medium such as *Hibiscus sabdarifa* drinks. This is in line with the finding of Nwafor and Ikenebomeh, [23] who isolated some fermentative bacteria from *Hibiscus sabdarifa* stored in ambient temperature. The temperature of storage had significant (P≤0.05) effect on the bacteria count during the storage period. At refrigeration temperature the specific death rate in HCPF drink samples was significantly (P≤0.05) higher than at ambient temperature, while the growth rate in HPPE drinks was significantly (P≤0.05) lower than ambient temperature. This was contrary to expectation as more growth was expected at ambient temperatures than at lower refrigeration temperature. However, the synergy in hurdle technology could be at play as samples at ambient temperature had greater decrease in pH. Storage at refrigeration temperature is therefore better than at ambient and such refreshing drinks are better served chilled. At ambient temperature, glass had the highest death rate in HCPF drinks and plastic the least growth rate in HPPE drinks, while at refrigeration temperature, HCPF drink samples in plastic had highest death rate and glass the least growth in HPPE drinks. These variations amongst the packaging materials placed plastic as a better

packaging material for HCPF at refrigeration temperature and HPPE drink at ambient temperatures. While glass would be better for HCPF at ambient temperature and HPPE drinks at refrigeration temperature.

3.2 Total Yeast Count of *Hibiscus sabdarifa* Drinks in Different Packaging Materials during Refrigeration (4.4±2^{o}C) and Ambient (25±2^{o}C) Temperature Storage

Fig. 2, presented the total yeast count of the *Hibiscus sabdarifa* drinks. Generally, significant (P≤0.05) increase was observed in the yeast count with storage time, between the packaging materials, the two drink types and the storage temperatures. At refrigeration temperature, the HCPF samples (Fig. 2a) had significant (P≤0.05) difference in counts on day 9, 17, 21 and 27. Plastic had significantly (P≤0.05) the highest growth on day 9 and sachet had the least on day 9, 17, 21 and 27. The trend in the yeast count was an indication of a stationary stage with counts of >1.00 – <1.16 Log_{10}CFU/ml. The specific growth rate in samples packaged in glass bottles (0.039) was significantly (P≤0.05) higher and those in plastic (0.031) was the least. In the HPPE samples (Fig. 2b), there was no growth on day 1-11 for glass, day 1 for plastic and day 1-3 for sachet. The trend in the yeast count was an indication of a stationary stage with count of >1.03 – <1.59 Log_{10}CFU/ml. Samples in glass bottles had specific growth rate (0.056) that was higher than those in sachet (0.036) and plastic (0.025). At ambient temperature, in HCPF samples (Fig. 2c) the yeast count ranged from >1.51 - <1.75 Log_{10}CFU/ml. There was no significant (P>0.05) difference in the yeast count over time but the specific growth rates for samples in glass, plastic and sachet are 0.049, 0.015 and 0.026 respectively. The rate of growth in the plastic bottle was significantly (P≤0.05) lower than the other packaging materials. In the HPPE drink samples (Fig. 2d), the values ranged from >1.28 - <2.20 Log_{10}cfu/ml. Drinks packaged in glass bottles had significantly (P≤0.05) the least yeast count during the storage period while the counts between plastic and sachet did not vary. There was no significant variation in the yeast count over time but the trend in growth was sachet > plastic > glass with specific growth rates of 0.063, 0.036 and 0.021 respectively.

Yeasts are mostly associated with fruit surfaces and flower nectarines. They are favoured by acid pH and play important role in fruit fermentation. Yeasts are part of the normal microflora of the *Hibiscus sabdarifa* calyces and their presence in the *Hibiscus sabdarifa* drinks agreed with the study where some yeast species such as *Aspergillus niger*, *Saccharomyces cerevisiae* and *Geotrichum candidum* were isolated from zobo drinks under ambient storage [23]. The yeast count (1 – 2 Log_{10}CFU/ml) obtained in this present study from two *Hibiscus sabdarifa* drink samples were lower than the fungi count in zobo drinks (4 – 6 Log_{10}CFU/ml) preserved with sent leave and allowed to stand [22]. This further explains the presence of yeast in *Hibiscus sabdarifa* drink while the difference in count can be attributed to the different processing methods particularly the pasteurization after packaging of the drinks. The increase in yeast count with storage time implies their ability to grow under the storage conditions irrespective of the acidity of the drinks. The growth rate was significantly (P≤0.05) higher at ambient temperature which is about the optimum temperature for the growth of most yeast. The more nutrient supplied by the addition of *Phoenix dactylifera* to the HPPE samples may account for the higher growth rate in HPPE drink samples against the artificially flavoured HCPF drink. The condition in glass bottles favoured more growth than plastic and sachet packaging. Plastic with the least growth rates would be a preference in packaging this *Hibiscus sabdarifa* drinks.

3.3 *Escherichia coli* Count of *Hibiscus sabdarifa* Drinks in Different Packaging Materials during Refrigeration (4.4±2^{o}C) and Ambient (25±2^{o}C) Temperature Storage

Escherichia coli was sparingly detected in the samples as shown in Fig. 3. At refrigeration temperature, in the artificially flavoured drink (HCPF) samples (Fig. 3a), there was counts of 2.00 and 2.15 Log_{10}CFU/ml in samples packaged in glass and plastic bottles respectively on day 15 while count of 2.15 Log_{10}CFU/ml was detected in glass and sachet samples on day 27. In the *Phoenix dactylifera* and pineapple extract drink (HPPE) samples (Fig. 3b), there was no growth of *E. coli* in the sachet samples throughout the period of storage while glass had counts of 2.93, 2.80 and 2.39 Log_{10}CFU/ml on day 7, 15 and 27 respectively. Plastic had counts of 2.93 and 2.00 Log_{10}CFU/ml on day 7 and 27 respectively. At ambient temperature, the only growth of *E. coli* observed in the samples, was for HCPF samples in plastic bottles with a count

of 2.11 Log_{10}CFU/ml on day 7 (Fig. 3c). and; HPPE samples in plastic bottles and sachet with counts of 2.65 and 2.04 Log_{10} CFU/ml respectively on day 7 (Fig. 3d).

E. coli is a gram-negative, facultative anaerobic rod shaped bacteria that are common part of the normal intestinal flora of humans and other warm-blooded animals [32]. They are the most widely used indicator of faecal contamination and microbiological safety. The number of *E. coli* on the days detected were within the recommended acceptable microbiological level ≤10^2 CFU/ml [31,33]. The sparing number of *E. coli* and the absence of growth on some days signified the inability of *E. coli* to proliferate in the drinks under the different storage conditions though they may be presence in the sample but was below detection limit. It also pointed to the hygienic condition of the prepared drinks. The inhibition of growth was more at ambient temperature where the drinks were characterized with lower pH.

3.4. Coliform Count of *Hibiscus sabdarifa* Drinks in Different Packaging Materials during Refrigeration (4.4±2^{o}C) and Ambient (25±2^{o}C) Temperature Storage

The number of Coliform enumerated in the samples at refrigeration temperature are shown in Fig. 4. In HCPF samples (Fig. 4a), the growth of coliform was observed in samples packaged in glass bottles on day 11 and 19 with counts of 2.45 and 2.3 Log_{10}CFU/ml respectively. In plastic samples, it was observed on day 11, 19 and 21 with counts of 2.39, 2.59 and 2.24 Log_{10}CFU/ml, while sachet had counts of 2.15 and 2.54 Log_{10}CFU/ml on day 11 and 13 respectively. In HPPE samples (Fig. 4b), there was no growth of coliform except on day 13 for all samples with count of 2.04, 2.15 and 2.24 Log_{10} CFU/ml for samples in glass, plastic and sachets respectively and on day 11 and 19 for samples in plastic only with count of 2.30 Log_{10} CFU/ml. At ambient temperature, there was no coliform growth observed in the sample except for HPPE sample packaged in plastic bottle with a count of 2.10 Log_{10}CFU/ml.

The sparse number of coliform in the drinks signified an unfavourable condition for their grow in the drinks. The ambient condition of storage was harsher on *coliform* than the refrigeration temperature as indicated by the absence of growth at ambient temperature. Coliforms are Gram negative, non-spore forming, short aerobic or facultative anaerobic rods that are mostly indicative of microbiological quality [32]. The presence of coliform in many foods does not necessarily indicate unsatisfactory hygiene measure as they are part of the normal flora of many raw foods. Also their presence in food does not necessarily indicate faecal contamination as they can survive and grow in food processing environment where other pathogenic Enterobacteriaceae may not. Faecal coliform are indicative of microbiological safety. The test for coliforms and faecal coliform can give false reassurance of safety when lactose-negative microorganisms such as *Salmonella*, *Shigella* and enteroinvasive strains of *E. coli* predominate. Fortunately, *Salmonella* was not found in the drinks which is a positive assurance of the safety of the two different drinks irrespective of packaging materials and the storage temperatures.

3.5 Total *Staphylococcus* and *Salmonella* Count of *Hibiscus sabdarifa* Drinks in Different Packaging Materials during Refrigeration and Ambient Temperature Storage

Fig. 5 showed the total *Staphylococcus* count in the *Hibiscus sabdarifa* drink samples. In HCPF samples (Fig. 5a), the growth of *Staphylococcus* in glass samples was observed on day 7 alone (2.00 Log_{10}CFU/ml), samples in plastic bottles on day 7 and day 13 had counts of 2.15 and 2.00 Log_{10}CFU/ml while samples in sachet had counts of 2.30 and 2.00 Log_{10}CFU/ml on day 3, 7 and 13. In HPPE samples (Fig. 5b), *Staphylococcus* growth was observed on day 1, 7 and 13 with a count of 2.50. 2.00, and 2.30 Log_{10}CFU/ml in samples packaged in glass bottles. Samples in plastic bottles on day 7 and 13 had counts of 2.24 and 2.30 Log_{10}CFU/ml and samples in sachet had a count of 2.00 Log_{10}CFU/ml on day 13 alone. Drinks in the different packaging materials at ambient temperature had no growth of *Staphylococcus.* Storage of the *Hibiscus sabdarifa* drinks packaged in different packaging materials at both refrigeration and ambient temperatures had no growth of *Salmonella.*

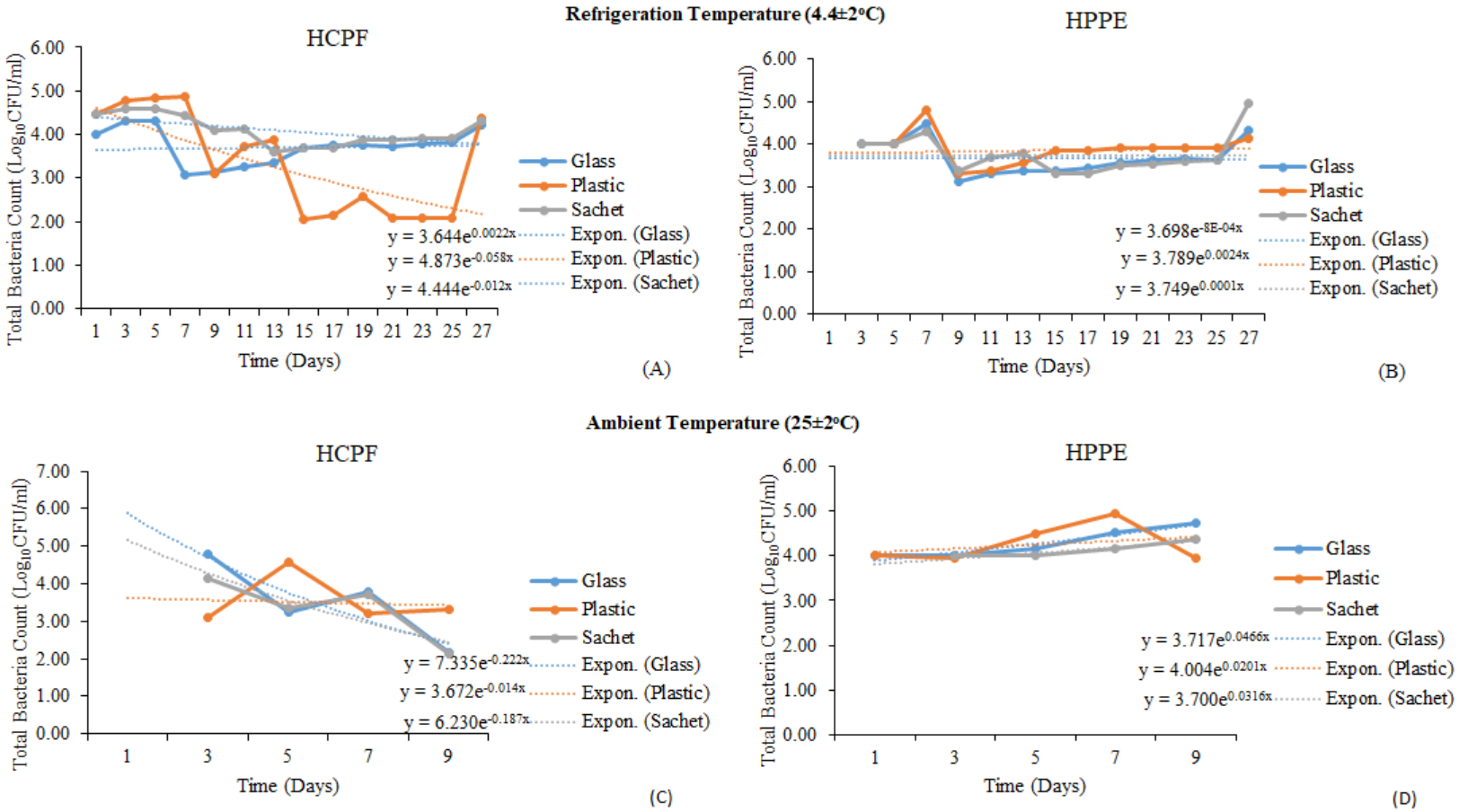

Fig. 1. The total bacteria count (TBC) of *Hibiscus sabdarifa* drinks with artificial commercial pineapple flavour (HCPF) and; *Phoenix dactylifera* with natural pineapple peel extract (HPPE) packaged in different packaging materials and stored at refrigeration and ambient temperatures

Points on graph are means of duplicate samples
HCPF - Hibiscus sabdarifa + commercial artificial pineapple flavour
HPPE - Hibiscus sabdarifa +Phoenix dactylifera + pineapple peel extract

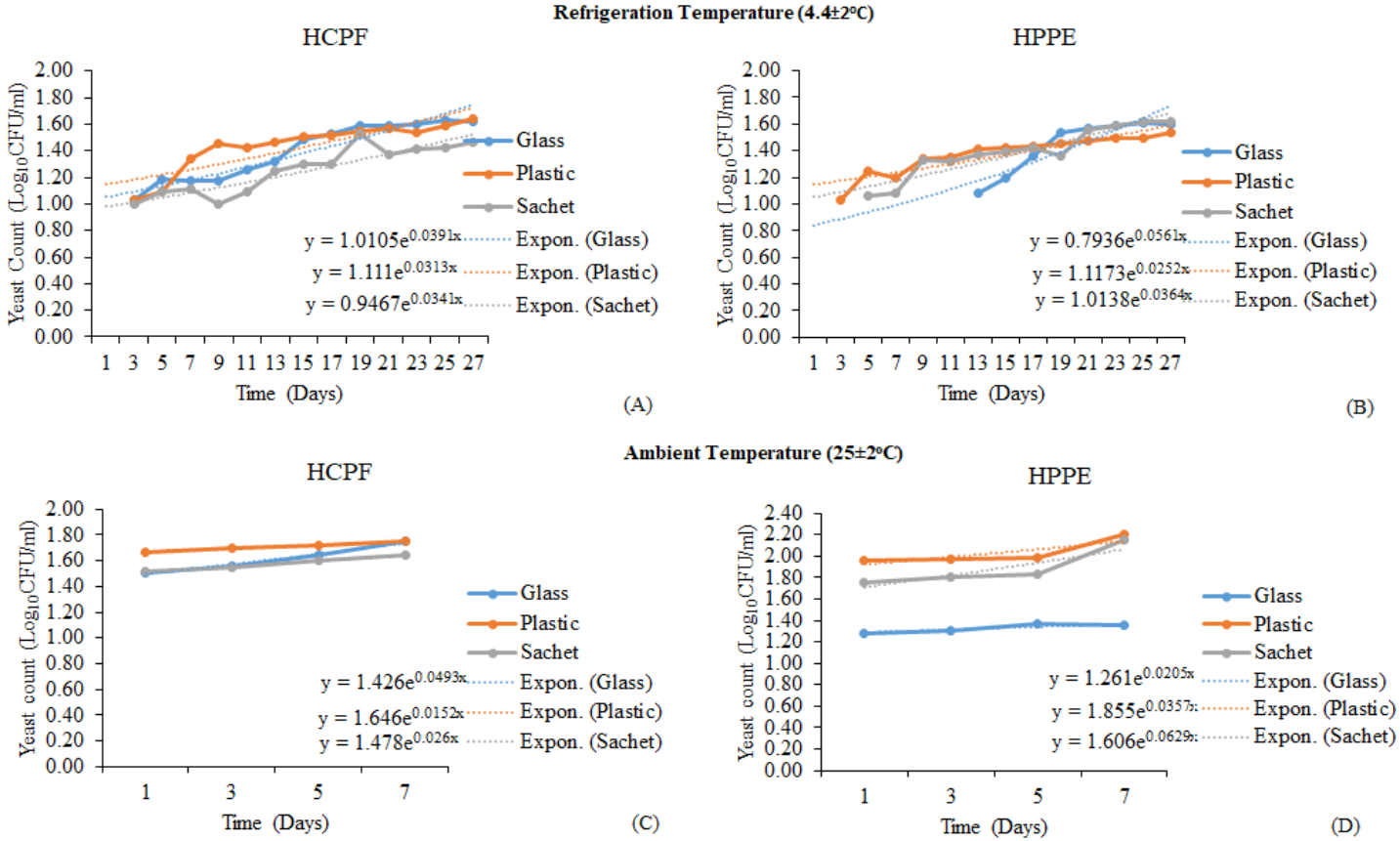

Fig. 2. The yeast count (Log_{10}CFU/ml) of *Hibiscus sabdarifa* drinks with artificial commercial pineapple flavour (HCPF) and; *Phoenix dactylifera* with natural pineapple peel extract (HPPE) packaged in different packaging materials and stored at refrigeration and ambient temperatures

Points on graph are means of duplicate samples

HCPF - Hibiscus sabdarifa + commercial artificial pineapple flavour

HPPE - Hibiscus sabdarifa +Phoenix dactylifera + pineapple peel extra

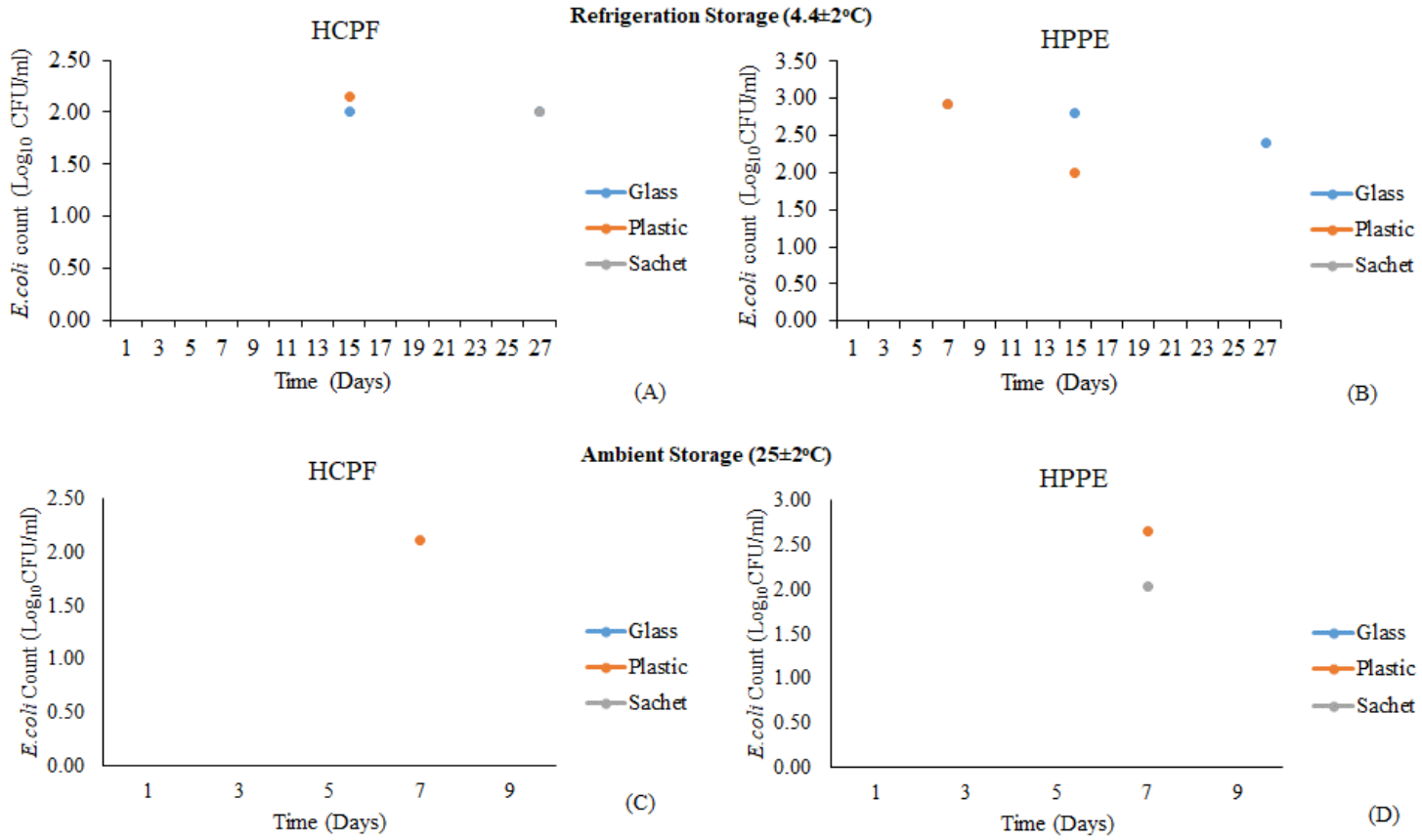

Fig. 3. *Escherichia coli* count (Log_{10}CFU/ml) in *Hibiscus sabdarifa* drinks with artificial commercial pineapple flavour (HCPF) and; *Phoenix dactylifera* with natural pineapple peel extract (HPPE) packaged in different packaging materials and stored at refrigeration

Points on graph are means of duplicate samples
HCPF - Hibiscus sabdarifa + Commercial artificial pineapple flavour
HPPE - Hibiscus sabdarifa +Phoenix dactylifera + Pineapple peel extract

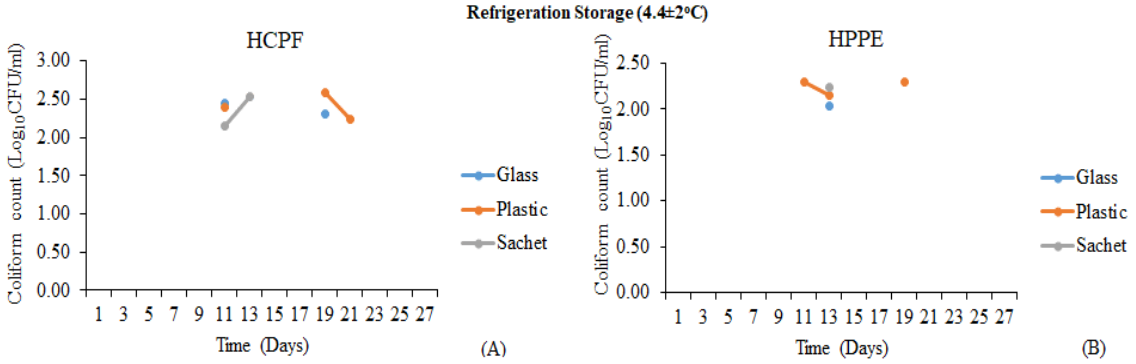

Fig. 4. Coliform count (Log_{10}CFU/ml) in *Hibiscus sabdarifa* drinks with artificial commercial pineapple flavour (HCPF) and; *Phoenix dactylifera* with natural pineapple peel extract (HPPE) packaged in different packaging materials and stored at refrigeration

Points on graph are means of duplicate samples
HCPF - Hibiscus sabdarifa + Commercial artificial pineapple flavour
HPPE - Hibiscus sabdarifa +Phoenix dactylifera + Pineapple peel extract

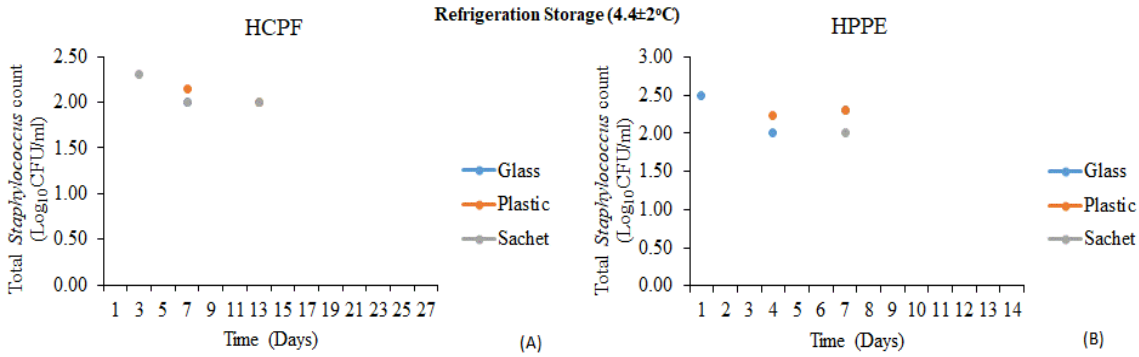

Fig. 5. Total *Staphylococcus* count (Log_{10}CFU/ml) in *Hibiscus sabdarifa* drinks with artificial commercial pineapple flavour (HCPF) and; *Phoenix dactylifera* with natural pineapple peel extract (HPPE) packaged in different packaging materials and stored at refrigeration

Points on graph are means of duplicate samples
HCPF - Hibiscus sabdarifa + Commercial artificial pineapple flavour
HPPE - Hibiscus sabdarifa +Phoenix dactylifera + Pineapple peel extract

Staphyloccous aureus have been identified in *Hibiscus sabdarifa* drinks [21,22]. Though they can grow over a wide range of pH 4 - 9.8 they can't compete well with normal flora of most foods especially those that contain large amounts of lactic acid bacteria [32]. *Staphylococcus* can be found everywhere and are part of the normal flora of humans. Their presence in food is often associated with the food handlers, contaminated surfaces, equipment and temperature control. The presence of *staphylococcus* in low numbers in the drinks in this present study therefore is a good indication of good hygiene and temperature control. A dose of *Staphylococcus* required to produce enterotoxin in food was reported to be 5 – 6 Log_{10}CFU/g by Khanom et al. [34]. *Staphylococcus* counts (2 – 2.5 Log_{10}CFU/ml) in the *Hibiscus sabdarifa* (HCPF and HPPE) drinks in this present study was below the enterotoxin producing dose indicating that the samples were satisfactorily fit for consumption. Raimi, [35] associated the presence of pathogens in *Hibiscus sabdarifa* drink with deterioration and spoilage, others attributed it to poor hygiene practices and poor sanitary conditions during the

drink production [21,22]. The absence of *Salmonella* and the safe levels of *E. coli* and *Staphylococcus* during the storage period and its absence in some days thereafter is a good indication of microbiological safety and imply that the samples are safe for consumption.

4. CONCLUSION

The study revealed that at refrigeration temperature, total bacterial count in HCPF decreased significantly (P≤0.05) with higher death rate in plastic bottles while at ambient temperature, there was increase in HPPE drinks with least growth rate in plastic bottles. Yeast count at ambient temperature was significantly (P≤0.05) higher and the drinks in plastic bottles had the least growth rates (≤0.03). Coliform, *Escherichia coli* and *Staphylococcus* where unable to grow in the drinks with greater inhibition at ambient temperature in all packaging materials. There was no growth of *Salmonella* in the drinks. The satisfactory levels of the organisms found during the storage period is a good indication of microbiological safety. Each of the packaging material would be recommended for packaging of *Hibiscus sabdarifa* drinks with storage at refrigeration temperature not more than 21 days. This study is informative to consumers, and persons who are involved in the production of *Hibiscus sabdarifa* drink in determining the ideal packaging material and storage conditions to maintain its microbiological quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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