Physicochemical Properties and Shelf life Stability of 'Tybo' Drink Preserved with Acetic acid and Sodium Benzoate

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Abstract: Freshly prepared tybo drink has a short shelf life which could be extended using chemical preservative. In this study, 0.1 % and 0.3 % concentration of sodium benzoate and acetic acid were incorporated into tybo drink which constitute zobo drink and tigernut milk mix together in the ratio 1:1, 3:1 and 1:3. Tybo drinks without preservative were the control samples. All the products were stored at room temperature (28±2 ^{o}C) for fifteen (15) Days. The total titratable acidity (TTA), pH and microbiological quality of the stored drinks were monitored using standard methods. Our results showed that during storage of the drinks, there was reduction in TTA (0.12 - 0.08 %) but increase in pH (3.16 - 5.63), total bacterial count (4.49 - 7.48 $\log_{10}$CFU/ml) and total fungal count (4.87 - 5.55 $\log_{10}$CFU/ml). Microbial counts of each control sample were higher than other tybo drinks of the same tigernut-zobo drink ratio treated with chemical preservatives with few exceptions. Bacterial genera isolated from the drinks during storage were *Bacillus*, *Micrococcus*, *Shigella*, *Enterococcus, Proteus*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Serratia,* and *Pseudomonas* sp, whereas fungi genera were *Coccidioides*, *Aspergillus, Penicillium*, *Microsporium*, *Trichophyton*, *Cryptococcus*, *Saccharomyces*, *Aphanoascus*, *Candida*, *Chrysosporium*, *Mucor*, and *Rhizopus*. Based on total aerobic plate counts of foods recommended by International Commission on the Microbiological Specifications for Food (ICMSF), the stored tybo drinks was fit for consumption within nine (9) days. Hurdle technology and Hazard Analysis and Critical Control Points (HACCP) during production of the drinks is recommended in order to eliminate pathogenic microbes identified in the product during storage which could be of public health concern.

Keywords: Tybo drink, Physicochemical properties, Acetic acid, Sodium benzoate, Shelf life

INTRODUCTION

Tybo is a nutritious drink prepared by blending tigernut milk and zobo drink. It is a non-alcoholic local beverage (Ezeh, 2017). The two products that result in tybo drink are among indigenous popular drinks consumed in Nigeria (Bristone *et al*., 2018a; Gbadegesin and Gbadamosi, 2017). However, tybo drink is less popular than tigernut milk and zobo drink. So far, limited studies have been carried out on tybo drink in terms of physicochemical properties and shelf life stability.

The name 'Zobo' is derived from 'Zoborodo' which is a word in Hausa language largely spoken in Northern part of Nigeria. It is called Sorrel in English (Ezekiel *et al*., 2016). Zobo is a non-alcoholic locally prepared beverage obtained from acid succulent aqueous extract of different varieties of dried petals of *Hibscus sabdariffa* (Obi, 2015). Physicochemical properties of zobo drink were determined by Gbadegesin and Gbadamosi (2017).

Tigernut drink is a popular imitation milk known as chufa de horchata in Spain prepared using tigernut tubers (Udeozor, 2012; Bristone *et al*., 2015). It is a nutritious non alcoholic drink recommended for everyone including diabetics and lactose intolerance individuals (Okudu and Ogbuike, 2016; Gambo and Dáu, 2014). Tigernut milk could be used in developing yoghurt-like product and ice cream (Wakil *et al.,* 2014; Umelo *et al*., 2014). Improved products have also been developed by blending tigernut-milk either with kunu, soymilk or coconut milk (Belewu and Abodunrin, 2006; Okorie *et al*., 2014; Belewu *et al.,* 2010). Local production and retailing of zobo drink and tigernut milk is not standardized.

It is characterized by unhygienic practices by mostly rural dwellers who engage in the activity as their source of income. Zobo and tigernut milk are retailed using polythene sachets and plastic containers often the ones previously used to bottle other liquid products (Gbadegesin and Odunlade, 2016). Therefore, chance of microbial contamination of both products is high (Obi, 2015; Musa and Hamza, 2013).

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa, Aspergillus* spp., *Penicillium* spp., *Geotrichum* spp. *Alternaria* spp., and *Fusarium* spp. are microorganisms which have been found in zobo drink which could cause food spoilage (Ezekiel *et al.*, 2016; Braide *et al.*, 2012). High population of lactic acid bacteria, coliforms, *Bacillus subtilis*, *B. cereus*, *S. aureus, Rhizopus* sp., *Saccharomyces* sp., and *Mucor* sp. are associated with tigernut milk and implicated in spoilage of the product (Bristone *et al*., 2018b). Zobo drink and tigernut milk has a short shelf life of less than 2 days if left under ambient temperature without any preservative added to it (Obi, 2015; Musa and Hamza, 2013). Tybo drink also has a short shelf life. However, zobo drink could remain organoleptically attractive up to 2 weeks if it is treated with benzoic acid (Adesokan *et al.,* 2013). Addition of natural tropical preservatives and chemical preservatives could also extend the shelf life of tigernut milk up to 8 days (Nwobosi *et al*., 2013).

Ezeh (2017) prepared tybo drink and reported that its nutritional composition to be 40.8 mg of ascorbic acid, 6.6 mg of calcium, 2.4 g of potassium, 4.6 g of phosphorus, 2.1 g of sodium, 5.4 g of protein and 2.7 g of fat. Available information on tybo drink in terms of carbohydrate content is lacking. In a related study, Eke-Ejiofor and Nnodim (2019) fermented fresh and dried tigernut milk extract enriched with zobo juice which resulted in wine comparable with grape wine. Although studies on zobo drink and tigernut milk drink have extensively been reported, there is limited published works on tybo drink which is a blend of both drinks in different ratios. Therefore, this study seeks to determine the shelf life stability and physicochemical properties of tybo drinks prepared using different ratios of tigernut milk and zobo drink which was preserved with 0.1 % and 0.3 % concentration of sodium benzoate and acetic acid.

MATERIALS AND METHODS

The following items namely *Hibiscus sabdariffa* Calyx, fresh tigernut tubers, fresh ginger and ripe pineapple used to prepare tybo drink were purchased from Mile 3 market, Port Harcourt. All the items were transported to the Food Processing Laboratory, Department of Microbiology, University of Port Harcourt. The procedure described by Ogbonna *et al.* (2013) and Obi (2015) with slight modification was followed in preparing tigernut milk and zobo drink, respectively. During production of zobo drink, an aliquot portion of pineapple was added as sweetener as well as ginger in order to add flavour to the drink. A blend of the tigernut milk and zobo drink known as tybo drink was prepared (Ezeh, 2017).

Tybo drink formulations

Three formulations of tybo drink which comprises zobo drink and tigernut milk in the ratio 1:1, 3:1 and 1:3 was prepared and poured in sterile air tight containers labeled Sample A, B and C, respectively. Sample A was a combination of 50 ml of zobo drink and 50 ml of tigernut milk. Sample B was a combination of 75 ml of zobo drink and 25 ml of tigernut milk. Sample C was a combination of 25 ml of zobo drink and 75 ml of tigernut milk. 0.1 % concentration of acetic acid was separately added to Sample A, B and C which was subsequently labeled sample ATA1, BTA1 and CTA1 whereas that of 0.3 % acetic acid was labeled ATA2, BTA2 and CTA2. Similarly, 0.1 % concentration of sodium benzoate was separately incorporated into another set of tybo drink formulation labeled A, B and C which was subsequently labeled sample

ATS1, BTS1 and CTS1 whereas that of 0.3 % sodium benzoate was labeled ATS2, BTS2 and CTS2. Three formulations of tybo drinks without preservatives labeled AT, BT and CT were the control samples.

Physicochemical analysis of tybo drinks

The pH and total titratable acidity of the tybo drinks stored at room temperature (28±2 ^{o}C) were monitored at 3 Days interval for a period of 15 Days.

Determination of pH

pH meter (PHS-25) pec medical USA was used for the test using the procedure described by Adelekan *et al.* (2014). Buffer 5 and buffer 7 solution were first used to calibrate the pH meter to ensure sensitivity and accuracy. Each sample to be tested was poured inside 10 ml measuring cylinder and then transferred into beaker. The pH meter electrode was dipped into the sample. The reading on display unit was recorded when the pH value was stable. The pH meter electrode was thoroughly rinsed with distilled water before testing other samples

Determination of total titratable acidity

The method described by AOAC (2010) was adopted. Ten milliliter (10 ml) of the various formulations of tybo drinks were separately transferred into conical flasks and 25 ml of distilled water was added to each of the flask. Fifty milliliter (50 ml) of 0.1 M NaOH was filled into the burette and titrated against the samples using 3 drops of phenolphthalein as indicator. The end point of the titration was indicated by a sharp appearance of pink colour, after which the corresponding burette reading was taken. The total titratable acidity was calculated using the formula:

$$\text{Total titratable acidity (\%)} = \frac{\text{titre x blank x normality of base x ml equivalent of citric acid}}{\text{Weight of sample}}$$

Microbiological analysis of tybo drinks

One milliliter (1 ml) of each sample of tybo drink formulation was diluted using ten-fold serial dilution. One milliliter (1 ml) of diluted sample from 10^{-4} were pour plated on nutrient agar (NA) and Sabouraud dextrose agar (SDA) plates for isolation of bacteria and fungi, respectively. The inoculated plates for isolation of bacteria were incubated at 37 ^{o}C for 48 h while that of fungi were at room temperature (28±2 ^{o}C) for 72 h. The colony forming units were counted and then recorded.

Purification of isolates

Discrete colonies were picked using a sterilized wire loop and streaked aseptically on freshly prepared nutrient agar and Sabouraud dextrose agar plates, accordingly and repeatedly up to three times to obtain pure colonies. The pure colonies were stored on agar slants for further analysis.

Identification of isolates

The bacterial isolates were observed for their colonial morphology followed by Gram staining using the method described by Isu and Onyeagba (2002). Biochemical tests namely oxidase, catalase, indole, urease, hydrogen sulphide production, Triple Sugar Iron Agar (TSI), methyl red/Voges-Proskauer, citrate and carbohydrate utilization were also carried out using the methods described by Cheesbrough (2002) in order to identify the bacterial isolates. Lactophenol cotton blue staining of the fungal isolates using the procedure described by Samuel and Frederick (2018) was done and their morphological characteristics were recorded.

RESULTS

Figures 1-3 shows the pH of tybo drinks stored at room temperature (28±2 ^{o}C) at 3 Days interval. Also shown in Figures 4-6 is the total titratable acidity of the drinks stored at room temperature (28±2 ^{o}C). Figures 7 and 8 shows the total bacterial and fungal count, respectively of tybo drinks during storage at room temperature (28±2 ^{o}C). Average bacterial and fungal count of tybo drinks stored for 15 Days is presented in Figure 9. Cultural and morphological characteristics of bacterial isolates from tybo drinks stored at room temperature (28±2 ^{o}C) is depicted in Table 1.

Biochemical characterization of bacteria isolated from tybo drinks stored at room temperature (28±2 °C) is depicted in Table 2. Shown in Table 3 is the morphology of fungi isolated from the tybo drinks stored at room temperature (28±2 °C). Tables 4 and 5 shows the probable genera of fungi and bacteria, respectively isolated from the tybo drinks stored at room temperature (28±2 °C). Figures 10-12 shows the frequency of occurrence of fungi genera isolated from different formulations of tybo drink during storage at room temperature (28±2 °C). The frequency of occurrence of bacteria genera isolated from different formulations of tybo drink during storage at room temperature (28±2 °C) is depicted in Figures 13 – 15.

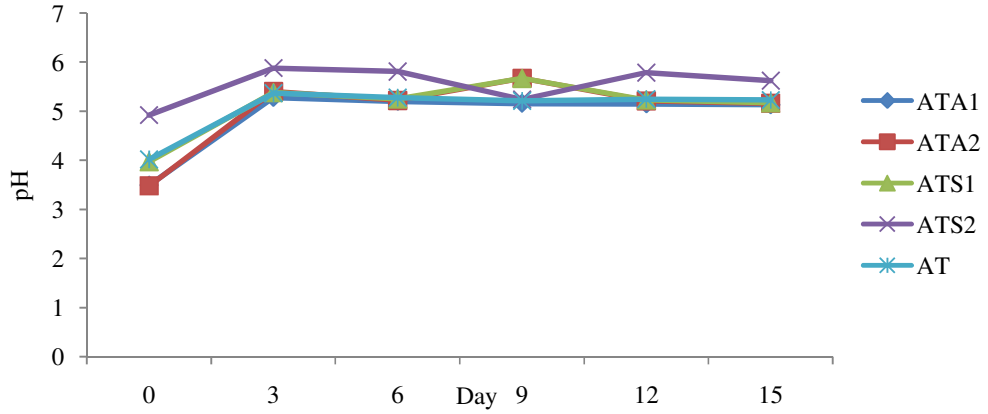

Figure 1. pH of tybo drinks stored at room temperature (28±2 °C)

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T

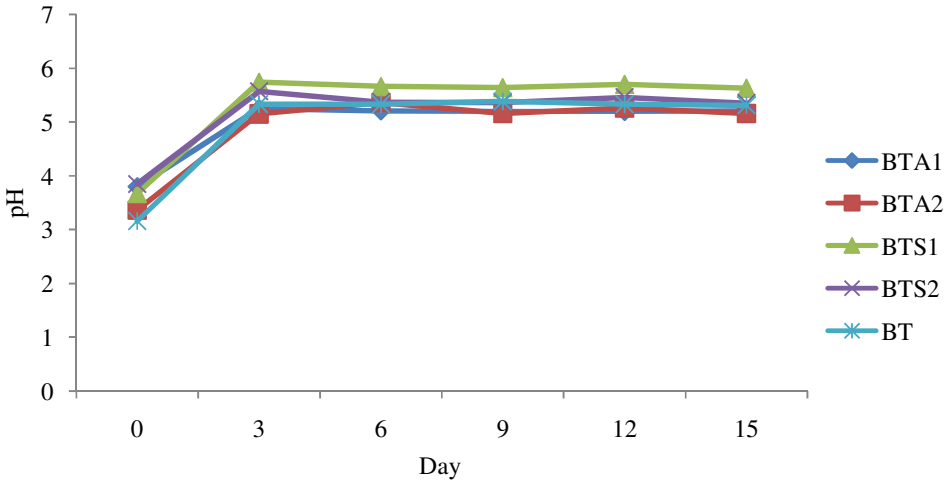

Figure 2. pH of tybo drinks stored at room temperature (28±2 °C)
T = tigernut milk drink; Z = zobo drink; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T

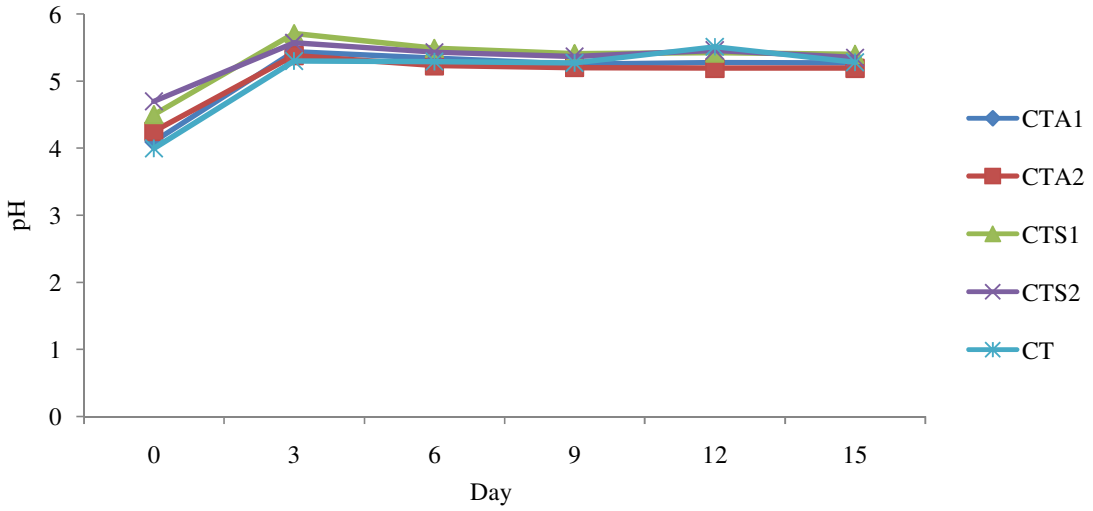

Figure 3. pH of tybo drinks stored at room temperature (28±2 °C)
T = tigernut milk drink; Z = zobo drink; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

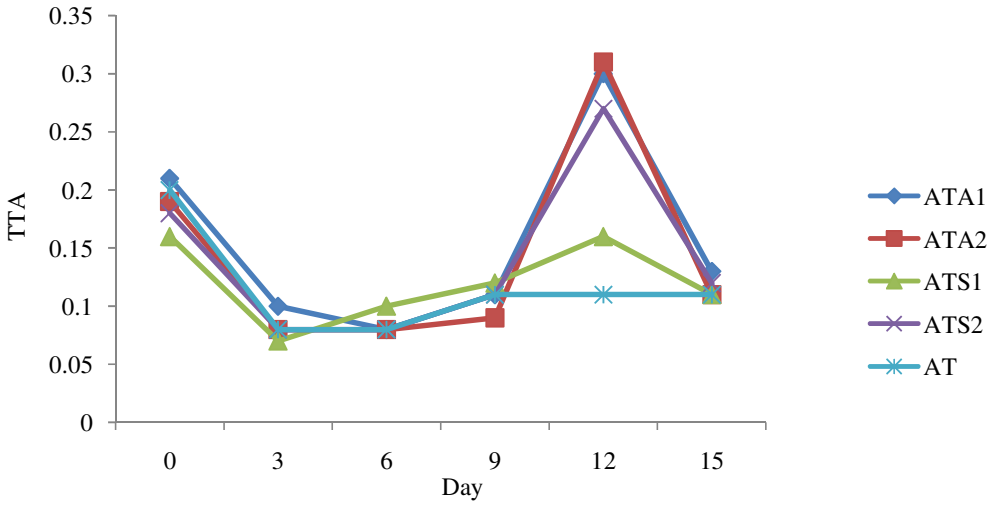

Figure 4. Total titratable acidity of tybo drinks stored at room temperature (28±2 °C)
T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T;

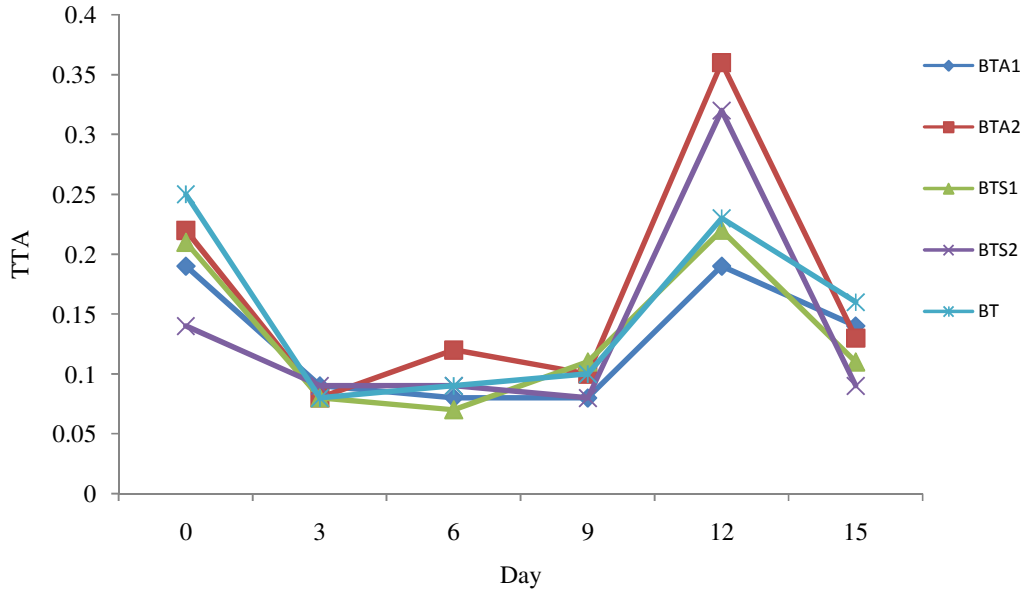

Figure 5. Total titratable acidity of tybo drinks stored at room temperature (28±2 ^{o}C)
T = tigernut milk drink; Z = zobo drink; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T;

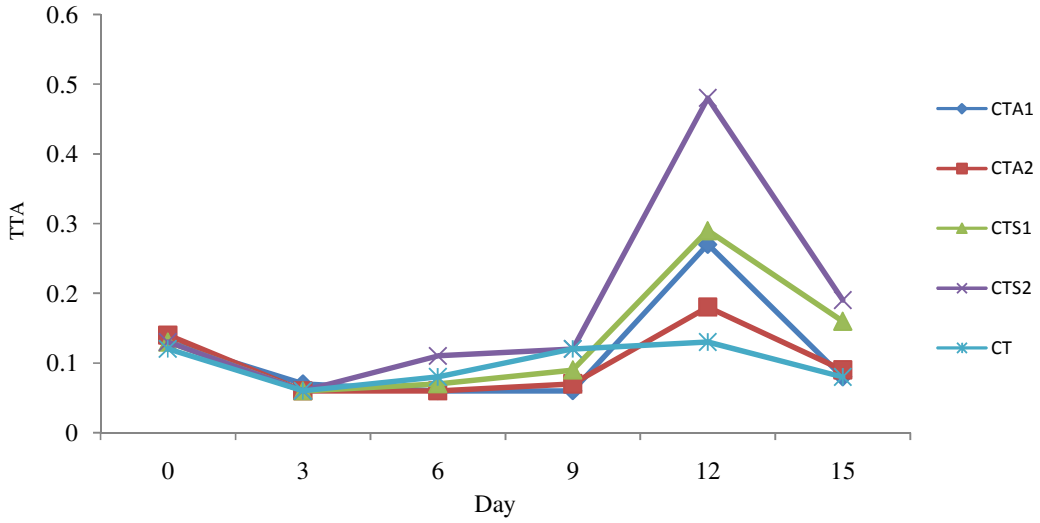

Figure 6. Total titratable acidity of tybo drinks stored at room temperature (28±2 ^{o}C)
T = tigernut milk drink; Z = zobo drink; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

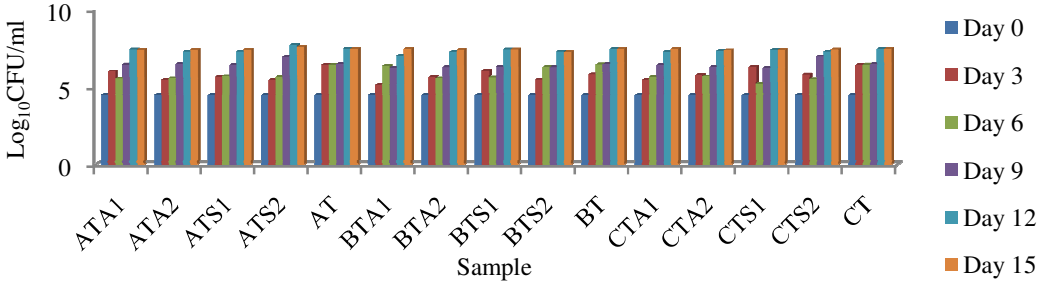

Figure 7. Total bacterial count of tybo drinks monitored during storage at room temperature (28±2 °C)

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

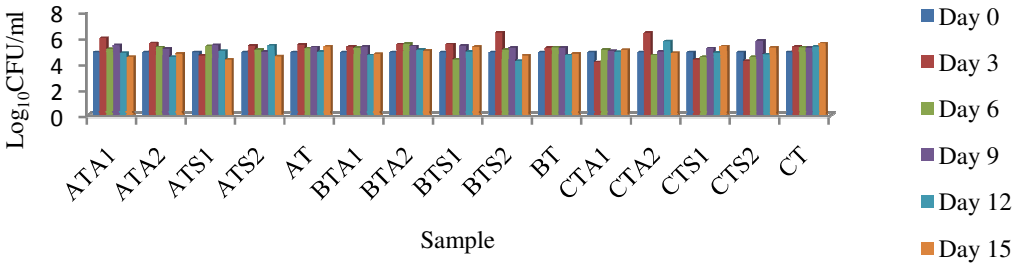

Figure 8. Total fungal count of tybo drinks monitored during storage at room temperature (28±2 °C)

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

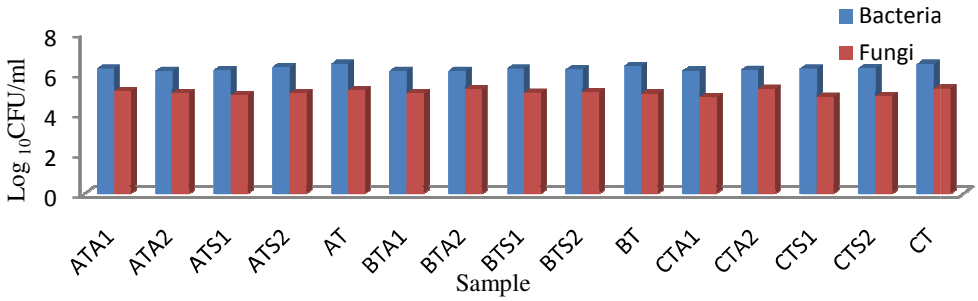

Figure 9. Average bacterial and fungal count of tybo drinks stored at room temperature (28±2 °C) for 15 Days

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid, ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

Table 1. Cultural and Morphological Characteristics of Bacterial Isolates from Tybo Drinks Stored at Room Temperature (28±2 °C)

Code	Size	Colour	Elevation	Surface appearance	Shape/form	Edge type/margin
B_1	1 mm	Cream	Raised	Smooth and mucoid colonies	Circular	Undulate
B_2	2 mm	Bright yellow	Convex	Smooth and shiny colonies	Punctiform	Entire
B_3	3 mm	White	Convex	Shiny-mucoid colonies	Circular	Entire
B_4	2 mm	Orange	Convex	Rough, shiny colonies	Punctiform	Entire
B_5	2 mm	White	Raised	Smooth, glistering colonies	Irregular	Entire
B_6	2 mm	Golden yellow	Convex	Smooth, glistering colonies	Punctiform	Entire
B_7	3 mm	White	Convex	Smooth-shiny colonies	Circular	Entire
B_8	1 mm	Yellow	Raised	Rough-dry surface	Circular	Entire
B_9	4 mm	Yellowish green	Raised	Smooth colonies	Irregular	Undulate
B_{10}	3 mm	Pinkish red	Umbonate	Mucoid colonies	Circular	Entire

Table 2. Biochemical Characterization of Bacteria Isolated from Tybo Drinks Stored at Room Temperature (28±2 °C)

										Sugar utilization		
Code	Gram stain	Oxidase	Catalase	Indole	Urease	H_2S/Gas	Slant/butt	MR/VP	Citrate	Glucose	Lactose	Probable organisms
B_1	+ Rods	+	-	-	-	-/-	A/A	+/-	+	+	-	*Bacillus* sp.
B_2	+ Cocci	+	+	-	-	+/-	B/A	+/-	+	+	+	*Micrococcus* sp.
B_3	-Rods	-	+	-	-	-/-	B/A	+/-	-	+	-	*Shigella* sp.
B_4	+Cocci	+	+	-	-	-/-	B/A	-/+	-	+	+	*Enterococcus* sp.
B_5	-Rods	-	+	-	-	+/+	A/A	+/-	-	+	-	*Proteus* sp.
B_6	+Cocci	-	+	-	-	-/-	A/A	-/+	+	+	+	*Staphylococcus* sp.
B_7	+Cocci	-	-	-	-	-/-	A/A	+/-	-	-	+	*Streptococcus* sp.
B_8	+Rods	-	-	-	-	-/+	A/A	-/-	-	+	+	*Lactobacillus* sp.
B_9	-Rods	+	+	-	-	-/+	B/B	-/-	+	-	-	*Pseudomonas* sp.
B_{10}	-Rods	-	+	-	-	-/-	B/A	-/+	+	+	-	*Serratia* sp.

*A = Acid *B = Base

Table 3. Morphology of Fungi Isolated from Tybo Drinks Stored at Room Temperature (28±2 ^{o}C)

Macroscopy	Microscopy	Probable organisms
Colony with greenish center, texture appears velvety to powder, white on surface and reverse is yellowish.	Coloured septate, hyphae, presence of phialides and long conidiophores.	*Penicillium* sp.
Black colour colony, texture is velvety and cottony, reverse is white.	Septate hyphae and hyaline, branched conidiosphores.	*Aspergillus* sp.
Colony appears moist, globorous, and membranous producing white and cottony aerial mycelium, reverse is pale.	Hyphae are septate and hyaline, conidiophores absent.	*Coccidioides* sp.
Colony appears white, coarsely fluffy, spreading colony with a distinctive hairy or feathery texture.	Presence of macroconidia that are asymmetrically shaped and have thick cell wall and coarsely roughened.	*Microsporium* sp.
Colony appears flat, white to cream, has a rough appearance and a deep white red reverse pigment.	Reflexive hyphal branching and endothrix invasion of hair.	*Trichophyton* sp.
Colonies appears encapsulated, mucoid, globose to elongated yeast-like cells.	Presence of hyphae, basidiospores, cells produce a polysaccharide capsule.	*Cryptococcus* sp.
Colonies were flat, smooth, moist, glistening and have creamy colour.	Septate hyphae, globose ascospores.	*Saccharomyces* sp.
Colonies appeared yellow, creamy with unbranched shape.	Buing, spherical to elongated cells forming pseudomycelium.	*Candida* sp.
White cottony colony, mucoid, reverse is whitish.	Single and branching sporangiosphores filled with sporangiospores at the tip.	*Mucor* sp.
White wooly colony with orange spot rapidly filling the plate.	Non septate hyphae, sporangiospheres are ovoid in shape and are directly opposite the branched rhizoid.	*Rhizopus* sp.
Colonies are flat, powdery texture, white to cream in colour.	Large club-shaped conidia containing fruiting bodies or ascomata at their centers.	*Aphanoascus* sp.
Colonies are flat, or folded, dry, powdery, or velvety with a white or cream coloured center, reverse is cream colour.	Large conidia, septate hyphae.	*Chrysosporium* sp.

Table 4: Fungi Isolated from Tybo Drinks Stored at Room Temperature (28±2 ^{o}C)

Code	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
ATA1	*Coccidiodes* sp.	*Coccidiodes* sp.	*Saccharomyces* sp.	*Saccharomyces* sp.	*Rhizopus* sp.	*Rhizopus* sp.
ATA2	*Candida* sp.	*Coccidiodes* sp. *Microsporium* sp.	*Coccidiodes* sp. *Microsporium* sp.	*Penicillium* sp.	*Penicillium* sp.	*Penicillium* sp.
ATS1	*Aspergillus* sp	*Aspergillus* sp	*Saccharomyces* sp.	*Saccharomyces* sp.	*Saccharomyces* sp.	*Candida* sp.
ATS2	*Candida* sp.	*Candida* sp. *Aphanoascus* sp.	*Aphanoascus* sp.	*Aspergillus* sp.	*Mucor* sp.	*Mucor* sp.
AT	*Aspergillus* sp. *Penicillium* sp. *Aphanoascus* sp.	*Coccidioides* sp. *Cryptococcus* sp.	*Trichophyton* sp. *Cryptococcus* sp.	*Penicillium* sp.	*Rhizopus* sp.	*Rhizopus* sp.
BTA1	*Coccidiodes* sp.	*Coccidiodes* sp.	*Trichophyton* sp.	*Trichophyton* sp.	*Penicillium* sp.	*Penicillum* sp.
BTA2	*Trichophyton* sp.	*Penicillium* sp.	*Cryptococcus* sp.	*Aphanoascus* sp.	*Trichophyton* sp.	*Mucor* sp.
BTS1	*Aspergillus* sp.	*Aspergillus* sp.	*Penicillium* sp.	*Rhizopus* sp.	*Rhizopus* sp.	*Mucor* sp.
BTS2	*Chrysosporium* sp.	*Chrysosporium* sp.	*Microsporium* sp.	*Mucor* sp.	*Mucor* sp.	*Rhizopus* sp.
BT	*Candida* sp.	*Mucor* sp. *Rhizopus* sp.	*Rhizopus* sp.	*Penicillium* sp.	*Penicillium* sp.	*Rhizopus* sp.
CTA1	*Saccharomyces* sp.	*Saccharomyces* sp.	*Rhizopus* sp.	*Candida* sp.	*Candida* sp.	*Penicillium* sp.
CTA2	*Saccharomyces* sp.	*Saccharomyces* sp.	*Mucor* sp.	*Mucor* sp.	*Penicllium* sp.	*Penicillium* sp.
CTS1	*Chrysosporium* sp.	*Chrysosporium* sp. *Microsporium* sp.	*Microsporium* sp.	*Aspergillus* sp.	*Aspergillus* sp. *Rhizopus* sp.	*Aspergillus* sp. *Mucor* sp.
CTS2	*Microsporium* sp.	*Rhizopus* sp.	*Mucor* sp.	*Mucor* sp.	*Saccharomyces* sp.	*Candida* sp.
CT	*Saccharomyces* sp. *Penicillium* sp.	*Rhizopus* sp. *Candida* sp.	*Mucor* sp. *Rhizopus* sp.	*Rhizopus* sp. *Mucor* sp.	*Mucor* sp. *Trichophyton* sp. *Aphanoascus* sp.	*Aphanoascus* sp.

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

Table 5. Bacteria Isolated from Tybo Drinks Stored at Room Temperature (28±2 ^{o}C)

Code	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
ATA1	*Staphylococcus* sp.	*Staphylococcus* sp	*Lactobacillus* sp.	*Serratia* sp.	*Serratia* sp.	*Serratia* sp.
ATA2	*Staphylococcus* sp.	*Enterococcus* sp.	*Enterococcus* sp. *Lactobacillus* sp. *Pseudomonas* sp.	*Enterococcus* sp.	*Micrococcus* sp.	*Shigella* sp.
ATS1	*Micrococcus* sp.	*Micrococcus* sp.	*Staphylococcus* sp.	*Lactobacillus* sp.	*Shigella* sp.	*Shigella* sp.
ATS2	*Shigella* sp.	*Staphylococcus* sp	*Staphylococcus* sp.	*Staphylococcus* sp.	*Pseudomonas* sp. *Staphylococcus* sp.	*Shigella* sp.
AT	*Staphylococus* sp. *Micrococcus* sp.	*Staphylococus* sp. *Micrococcus* sp.	*Serratia* sp.	*Serratia* sp. *Enterococcus* sp.	*Enterococcus* sp. *Micrococcus* sp. *Proteus* sp.	*Shigella* sp. *Proteus* sp. *Serratia* sp. *Staphylococcus* sp.
BTA1	*Proteus* sp. *Bacillus* sp.	*Staphylococcus* sp. *Proteus* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp. *Serratia* sp. *Lactobacillus* sp.	*Staphylococcus* sp. *Bacillus* sp.
BTA2	*Bacillus* sp.	*Staphylococcus* sp. *Bacillus* sp.	*Pseudomonas* sp.	*Pseudomonas* sp. *Streptococcus* sp.	*Pseudomonas* sp. *Streptococcus* sp.	*Pseudomonas* sp. *Micrococcus* sp. *Bacillus* sp.
BTS1	*Micrococcus* sp. *Staphylococcus* sp.	*Lactobacillus* sp.	*Lactobacillus* sp.	*Pseudomonas* sp. *Streptococcus* sp.	*Pseudomonas* sp. *Streptococcus* sp.	*Micrococcus* sp. *Staphylococcus* sp. *Streptococcus* sp.
BTS2	*Bacillus* sp. *Staphylococcus* sp	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Staphylococcus* sp. *Streptococcus* sp.
BT	*Bacillus* sp. *Lactobacillus* sp.	*Bacillus* sp.	*Pseudomonas* sp. *Serratia* sp.	*Micrococcus* sp.	*Serratia* sp. *Staphylococcus* sp.	*Staphylococcus* sp. *Micrococcus* sp.
CTA1	*Enterococcus* sp.	*Enterococcus* sp.	*Staphylococcus* sp. *Lactobacillus* sp.	*Shigella* sp.	*Shigella* sp.	*Streptococcus* sp.
CTA2	*Proteus* sp.	*Proteus* sp.	*Staphylococcus* sp. *Enterococcus* sp.	*Micrococcus* sp.	*Streptococcus* sp.	*Shigella* sp. *Micrococcus* sp. *Proteus* sp.
CTS1	*Enterococcus* sp.	*Staphylococcus* sp. *Enterococcus* sp.	*Enterococcus* sp.	*Staphylococcus* sp.	*Shigella* sp. *Lactobacillus* sp.	*Shigella* sp.
CTS2	*Micrococcus* sp.	*Enterococcus* sp.	*Lactobacillus* sp. *Pseudomonas* sp	*Micrococcus* sp. *Bacillus* sp.	*Pseudomonas* sp. *Streptococcus* sp.	*Streptococcus* sp.
CT	*Lactobacillus* sp. *Enterococcus* sp.	*Shigella* sp.	*Staphylococcus* sp. *Micrococcus* sp.	*Staphylococcus* sp. *Lactobacillus* sp.	*Shigella* sp. *Lactobacillus* sp.	*Bacillus* sp.

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

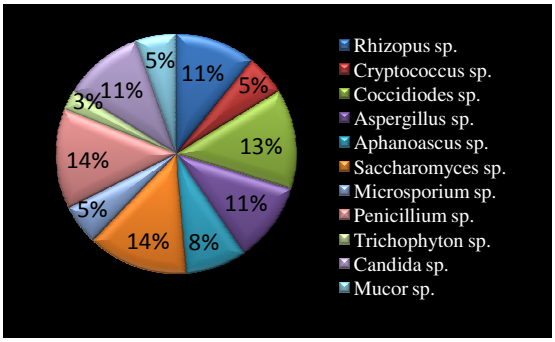

Figure 10. Frequency of occurrence of fungi isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation ATA1, ATA2, ATS1, ATS2 and AT

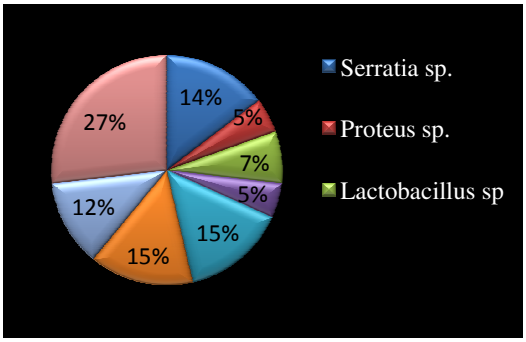

Figure 13. Frequency of occurrence of bacteria isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation ATA1, ATA2, ATS1, ATS2 and AT

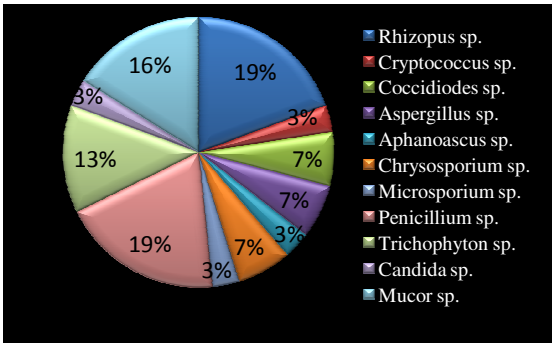

Figure 11. Frequency of occurrence of fungi isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation BTA1, BTA2, BTS1, BTS2 and BT

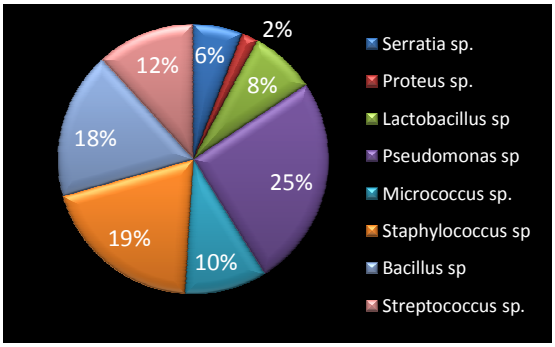

Figure 14. Frequency of occurrence of bacteria isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation BTA1, BTA2, BTS1, BTS2 and BT

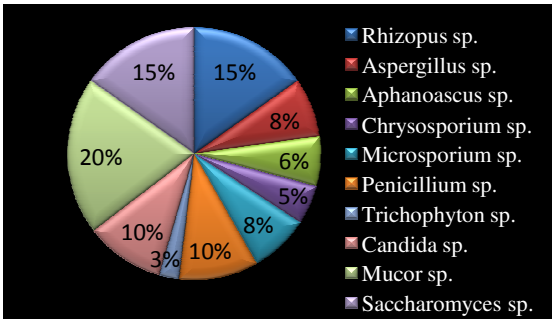

Figure 12. Frequency of occurrence of fungi isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation CTA1, CTA2, CTS1, CTS2 and CT

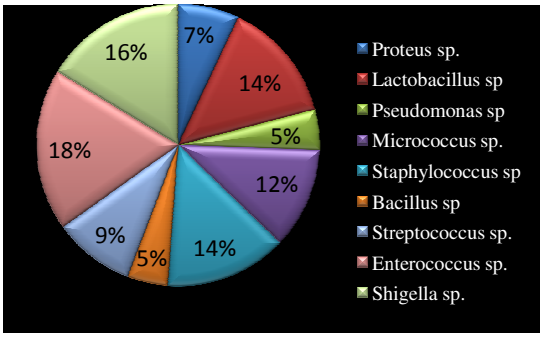

Figure 15. Frequency of occurrence of bacteria isolated at Day 0, 3, 6, 9, 12 and 15 from stored tybo drink formulation CTA1, CTA2, CTS1, CTS2 and CT

T = tigernut milk drink; Z = zobo drink; ATA1 = 50 ml Z + 50 ml T + 0.1 % acetic acid; ATA2 = 50 ml Z + 50 ml T + 0.3 % acetic acid; ATS1 = 50 ml Z + 50 ml T + 0.1 % sodium benzoate; ATS2 = 50 ml Z + 50 ml T + 0.3 % sodium benzoate; AT = 50 ml Z + 50 ml T; BTA1 = 75 ml Z + 25 ml T + 0.1 % acetic acid; BTA2 =75 ml Z + 25 ml T + 0.3 % acetic acid; BTS1 = 75 ml Z + 25 ml T + 0.1 % sodium benzoate; BTS2 = 75 ml Z + 25 ml T + 0.3 % sodium benzoate; BT=75 ml Z + 25 ml T; CTA1 = 25 ml Z + 75 ml T + 0.1 % acetic acid; CTA2 = 25 ml Z + 75 ml T + 0.3 % acetic acid; CTS1 = 25 ml Z + 75 ml T + 0.1 % sodium benzoate; CTS2 = 25 ml Z + 75 ml T + 0.3 % sodium benzoate; CT = 25 ml Z + 75 ml T.

DISCUSSION

Physicochemical Properties of Tybo Drink Formulations

The result obtained from this study shows that pH of the tybo drinks at Day 0 was within the range 3.16 – 4.92. Within the period of storage at room temperature (28±2 ^{o}C), pH of the tybo drinks increased to values that ranged between 5.16 - 5.63 at Day 15. In a related study, Babatuyi *et al.* (2019) reported that there was drop in pH of tigernut milk stored at room temperature from 4.7 to 3.6. Similarly, Gbadegesin and Odunlade (2016) reported that pH of zobo drink preserved with chemical preservatives (sodium benzoate and ethylene diamine tetraacetic acid) stored at room temperature dropped from 2.12 – 1.81. Drop in pH reported in both studies which separately involved tigernut milk and zobo drink is not in agreement with our result. Generally, very low pH of food and beverages does not favour growth and multiplication of bacteria. Researchers have observed that few microorganisms grow at pH < 4.0 while most microorganisms grow best between pH 6.6 – 7.5 (Ibrahim *et al.,* 2016). Foods and beverages that have a pH below 5.6 are prone to deterioration resulting from fungal and acidophilic bacteria (Musa and Hamza, 2013). Increase in pH of the tybo drinks during storage does not support unfavourable condition (lowering pH of the drinks) which will inhibit growth of most microorganisms implicated in food spoilage (Gbadegesin and Odunlade, 2016). Fresh ginger and ripe pineapple added to zobo drink used in formulating tybo drink could also have contributed in increasing the pH of tybo drink. This condition could favour increase in population of some pathogenic microorganisms in the drink and result in short shelf life of the product unless adequate quantity of chemical preservative is added and appropriate storage condition adopted. Gbadegesin and Gbadamosi (2017) reported that pH of zobo drink increased as the proportion of pineapple juice added to the drink increased. They reported that pH of zobo drink was 1.89, 2.01, 2.08 and 2.12 when the proportion of pineapple juice added to the drink was 0, 15, 20 and 25 %, respectively.

Total titratable acidity (TTA) of tybo drinks at Day 0 was in the range 0.12 – 0.25 %. At Day 15, the TTA of the drinks reduced to values that range between 0.08 – 0.16 %. In a related study that involved storage of tigernut milk separately obtained from three varieties of tigernut tubers at room temperature (27±2 ^{o}C), Babatuyi *et al.* (2019) reported an increase in TTA from 0.36 - 1.06 % which contradicts trend of TTA result from this study. Eke-Ejiofor and Awaji (2018) also reported a similar trend for TTA of tigernut milk produced using different rations of fresh and roasted tigernut which were stored at room temperature. Egbere *et al.* (2007) reported steady increase in TTA of pasteurized zobo drink preserved with sodium benzoate, pasteurized zobo drink without preservative and control sample stored at room temperature. The TTA values range between 0.038 – 0.053 %. The trend in pH and TTA values of tybo drinks stored at room temperature (28±2 ^{o}C) is in agreement with the postulation that both parameters are inversely related. The implication of both results is increase in microbial population of the tybo drinks during storage (Gbadegesin and Odunlade, 2016).

Bacterial and Fungal Count

Bacterial and fungal population of the tybo drinks at Day 0 was 4.49 and 4.87 $\log_{10}$CFU/ml, respectively. As reported by Musa and Hamza (2013) in a related study, higher fungal population of tybo drinks at Day 0 compared with bacterial population of the same drinks could be as a result of low pH. However, during the period of storage, our result showed that pH of the tybo drinks was on the increase. At Day 15, the bacterial and fungal population of the tybo drinks was within the range 7.26 – 7.6 and 4.30 – 5.55 $\log_{10}$CFU/ml, respectively.

According to Nwobosi *et al*. (2013), milk sample that contains bacterial population above 7.30 $\log_{10}$CFU/ml is bad for human consumption. Meanwhile, 6.30 $\log_{10}$CFU/ml is passable, 4.00 $\log_{10}$CFU/ml is fairly good while 3.70 $\log_{10}$CFU/ml is good for consumption. Recommended limit for total aerobic plate counts by International Commission on the Microbiological Specifications for food (ICMSF) is 7.00 $\log_{10}$CFU/ml (Mohammed *et al.,* 2017). Adesokan *et al*. (2013) reported that unspiced zobo drink stored at room temperature for 7 Days experienced increase in total viable counts from 4.41 – 7.57 $\log_{10}$CFU/ml whereas that of spiced zobo drink though lower than that of unspiced zobo drink also increased from 3.08 – 6.74 $\log_{10}$CFU/ml during storage. In another related study, total bacterial and fungal count reported by Braide *et al*. (2012) ranged from 8.00 – 10.67 and 7.0 – 10.20 $\log_{10}$CFU/ml, respectively.

The result obtained from this study revealed that average bacterial population of each tybo drink stored at room temperature (28±2 ^{o}C) was higher than average fungal population of similar tybo drink subjected to the same treatment. This could be attributed to antifungal properties of some bacteria species in the tybo drinks. According to Salas *et al*. (2017), some strains of *Lactobacillus casei* could inhibit wide spectrum of fungi. Similarly, some strains of *Bacillus* sp. have demonstrated large activity spectrum against some fruit molds which are pathogenic species grouped under *Aspergillus*, *Penicillium*, *Fusarium*, *Phoma* and *Rhizopus* genera. Although bacterial and fungal population in the tybo drinks increased during the period of storage, increase in pH of the drinks might have favoured increase in bacteria population above that of fungi. Tigernut milk and zobo drink are acidic in nature. Musa and Hamza (2013) attributed acidic nature of both drinks to be as a result of activities of lactic acid bacteria (LAB) such as *Lactobacillus* sp. The LAB is usually .present in tigernut milk and zobo drink left at room temperature for several hours. Based on average fungal count of the tybo drinks stored for 15 Days, our results revealed that each formulation of tybo drink preserved with 0.1 % and 0.3 % concentration of acetic acid recorded higher fungal count than the drinks of the same formulation preserved with the same concentration (0.1 % or 0.3 %) of sodium benzoate with few exceptions. This is an indication that sodium benzoate had greater inhibitory effect against fungi than acetic acid.

According to Ibrahim *et al*. (2016), at pH < 5, sodium benzoate is effective in slowing down growth and survival of several microorganisms associated with food spoilage. This condition is favourable for fungal growth. Similarly, acetic acid carries out its antibacterial activity by reducing pH of the substrate, altering cell membrane permeability by disrupting substrate transport and ionization of un-dissociated acid molecule leading to depression of intracellular pH (Ikawa, 1995). The pH of food product largely influences the activity of sodium benzoate used as a food preservative. Optimum inhibitory effect resulting from the ability of undissociated form of sodium benzoate to move freely across plasma membrane into the cytoplasm of microorganisms associated with spoilage is favourable at low pH. Apart from benzoate, other chemical preservatives which can be used to prevent fungal spoilage are sorbate, nitrate, nitrite and sulfites (Salas *et al.,* 2017). Based on average bacterial count of the tybo drinks stored for 15 Days, this study revealed that average bacterial count of each formulation of tybo drink preserved with 0.1 % and 0.3 % concentration of acetic acid was lower than that of tybo drinks of the same formulation preserved with the same concentration (0.1 % or 0.3 %) of sodium benzoate with one exception. This suggests that acetic acid was more effective than sodium benzoate in reducing the bacterial population in the drink.

In a related study, Braide *et al.* (2012) also reported that acetic acid treated zobo drinks had higher inhibitory effect on bacteria population compared with sodium benzoate treated zobo drink. According to Braide *et al.* (2012), zobo drink treated with sodium benzoate was more effective in reducing fungal population in the drink than that of acetic acid treated zobo drink.

Bacteria and Fungi Isolated from Tybo Drink Formulations

A total of twenty two (22) genera of microorganisms which comprises ten (10) bacteria and twelve (12) fungi were isolated from different formulations of tybo drinks stored at room temperature (28±2 ^{o}C) for fifteen (15) Days. The bacterial isolates were *Bacillus* sp. (from sample BTA1, BTA2, BTS2, BT, CTS2 and CT); *Micrococcus* sp. (from sample ATA2, ATS1, AT, BTA2, BTS1, BT, CTA2, CTS2 and CT); *Shigella* sp. (from sample ATA2, ATS1, ATS2, AT, CTA1, CTA2, CTS1 and CT); *Enterococcus* sp. (from sample ATA2, AT, CTA1, CTA2, CTS1, CTS2 and CT); *Proteus* sp. (from sample AT, BTA1 and CTA2); *Lactobacillus* sp. (from sample ATA1, ATA2, ATS1, BTS1, BT, CTA1, CTS1, CTS2 and CT); *Staphylococcus* sp. (from sample ATA1, ATA2, ATS1, ATS2, AT, BTA1, BTA2, BTS1, BTS2, BT, CTA1, CTA2, CTS1 and CT); *Streptococcus* sp. (from sample BTA2, BTS1, BTS2, CTA1, CTA2 and CTS2); *Serratia* sp. (ATA1, AT, BTA1 and BT) and *Pseudomonas* sp. (from sample ATA2, ATS2, BTA1, BTA2, BTS1, BTS2, BT and CTS2), whereas the fungal isolates were *Coccidioides* sp. (ATA1, ATA2, AT and BTA1); *Aspergillus* sp. (from sample ATS1, ATS2, AT, BTS1 and CTS1); *Penicillium* sp. (from sample ATA2, AT, BTA1, BTA2, BTS1, BT, CTA1, CTA2 and CT); *Microsporium* sp. (from sample ATA2, BTS2, CTS1 and CTS2); *Trichophyton* sp. (from sample AT, BTA1, BTA2 and CT); *Cryptococcus* sp.(from sample AT and BTA2); *Saccharomyces* sp. (from sample ATA1, ATS1, CTA1, CTA2, CTS2 and CT); *Aphanoascus* sp. (from sample ATS2, AT, BTA2 and CT); *Candida* sp. (from sample ATA2, ATS1, ATS2, BT, CTA1, CTS2 and CT); *Chrysosporium* sp. (from sample BTS2 and CTS1); *Mucor* sp. (from sample ATS2, BTA2, BTS1, BTS2, BT, CTA2, CTS1, CTS2 and CT); and *Rhizopus* sp. (from sample ATA1, AT, BTS1, BTS2, BT, CTA1, CTS1, CTS2 and CT).

Currently, there is dearth of information about bacteria and fungi that are present in tybo drinks stored at room temperature (28±2 ^{o}C). However, some of the bacteria genera identified in tybo drinks in this study is in agreement with the result reported by Babatuyi *et al*. (2019) from a related study that involved microbial analysis of tigernut milk stored at room temperature. In another related study, Braide *et al.* (2012) reported the presence of bacteria and fungi genera in pasteurized zobo drink preserved with acetic acid and sodium benzoate stored at room temperature which is similar with our research findings. Possible sources of bacteria and fungi isolated from the tybo drinks could be from the materials used in preparing the drink namely *Hibiscus sabdariffa* Calyx, tigernut tubers, pineapple and ginger which are usually exposed in the markets. Tigernut tubers are usually contaminated with soil while growing in the field and during harvesting of the tubers (Ibrahim *et al*., 2016). *Staphylococcus aureus* is usually present in the nose, skin, throat, hairs and palms as part of normal flora. The *Steptococci* sp. is also part of flora of the throat and the buccal cavity. *Micrococcus* sp. is present on the skin of animals and humans. They are harmless saprophytic bacteria (Seiyaboh *et al*. 2013). *Pseudomonas* sp. colonizes the skin surface which is part of its flora (Oku *et al*., 2018). Passage of *Serratia* sp. through gastrointestinal tract has not been established to cause gastrointestinal infection but it could be pathogenic if it enters the body through other portals (Timothy, 2013). The genera *Enterococcus* is among lactic acid bacteria (LAB) found in fermented foods (Salas *et al.,* 2017).

Presence of *Lactobacillus* sp. from samples of zobo drink was reported by Nwachukwu *et al.* (2007). Survival of *Bacillus* sp. irrespective of the concentration of acetic acid or sodium benzoate added to tybo drinks as preservatives could be as a result spore formation. The chance of survival of *Bacillus* spores during food processing is high (Oku *et al*., 2018). The spores of *Bacillus* sp. are prevalent in the environment. Since *Proteus* sp. is present in the soil, also isolating this bacterium from the tybo drinks could be as a result of using contaminated tigernut tubers to prepare the drink (Seiyaboh *et al*., 2013). The presence of *Shigella* sp. in tybo drinks raises some health concern. *Shigella* sp. usually inhabit the gastrointestinal tract and causes bacilliary dysentery. In a related study, Bristone *et al.* (2018b) reported the presence of *Shigella* sp. in retailed samples of zobo drink.

Penicillium sp., *Rhizopus* sp., *Aspergillus* and *Mucor* cause postharvest diseases of concern (Salas *et al.,* 2017). Most of these fungal genera are cosmopolitan and ubiquitous organisms (Oku *et al*., 2018). The ability of fungal genera *Aspergillus* and *Penicillium* to demonstrate antifungal and antibacterial activities have been reported. The presence of yeast belonging to the genera *Candida* and *Cryptococcus* in food could cause off-flavours. *Chrysosporium* sp. is a fungi commonly isolated from dusty floor or soil. *Trichophyton* sp. has also been identified from this source. Other fungal genera that inhabit floor dust particles include *Rhizopus* and *Aspergillus* while *Aphanoascus* is present in the soil (Maghraby *et al*., 2008). *Coccidioides* spp. inhabit the soil and the spores spread through air (Reyes-Montes *et al*., 2016). *Saccharomyces* sp. is ubiquitous and has the ability to survive low nutrient availability as well as other extreme conditions (Braide *et al.,* 2012). Some of the fungi genera isolated from the tybo drinks were also reported by Samuel and Frederick (2018) as fungi that were present in zobo drinks.

A striking result from this study revealed that chemical preservative treatment of tybo drinks could be considered not to be very effective because increasing population of diverse bacterial and fungal genera in the drink during storage at room temperature was reported. According to Izah *et al.* (2016), zobo drink could be subjected to different preservative treatment which is usually selective on microbes. Pundir and Jain (2011) tested the efficacy of five chemical food preservatives which includes acetic acid and sodium benzoate against *Bacillus subtilis*, *B. megaterium*, *B. sphaericus*, *B. polymyxa*, *Escherichia coli* and *Staphylococcus aureus*. Their finding shows that acetic acid and sodium acetate was the most and least effective antibacterial agent, respectively. According to Samuel and Frederick (2018), a wide range of microorganisms have the ability to grow and proliferate in zobo drink treated with preservatives and the ones that were not treated with preservatives. Microbial growth can also occur in tigernut milk stored at room temperature whether preservative was added to the milk or not (Nwobosi *et al.*, 2013). Their research findings corroborate our result which involves storage of tybo drinks preserved with acetic acid and sodium benzoate at room temperature.

CONCLUSION

This study reported the presence of ten bacterial and twelve fungal genera from tybo drinks treated with acetic and sodium benzoic acid which were stored for fifteen (15) days at room temperature (28±2 ^{o}C). During the period of storage, there was increase in pH, total bacterial and fungal count but reduction in total titratable acidity. Based on total aerobic plate counts of foods recommended by International Commission on the Microbiological Specifications for Food (ICMSF), the tybo drinks is considered safe for human consumption within nine (9) days from date of production.

However, the population of diverse bacterial and fungal genera identified in the tybo drinks during storage at room temperature raises some health concern. Therefore, storage conditions such as refrigeration as well as application of Hazard Analysis and Critical Control Points (HACCP) and hurdles technology during preparation of the tybo drinks is recommended.

COMPETING INTEREST

The authors declare that no competing interests exist.

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