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RESEARCH ARTICLE

ASSESSMENT OF 'TYBO' DRINKS PRESERVED WITH ACETIC ACID AND SODIUM BENZOATE BASED ON THEIR SENSORY PROPERTIES AND VITAMINS CONTENT

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ABSTRACT

This study seeks to evaluate sensory properties and vitamins content of tybo drink (blend of zobo drink and tigernut milk) treated with chemical preservative. Zobo (Roselle extract) and tigernut milk in ratios 1:1, 3:1, and 1:3 were prepared. 0.1 % and 0.3 % acetic acid; 0.1 % and 0.3 % sodium benzoate were added to tybo drinks as preservative. Tybo drinks without preservative were the control samples. All the formulations were stored at room temperature (28±2 °C) for 15 Days. Vitamin C, B_2 and B_6 content of the drinks were determined using AOAC methods and sensory evaluation using 9-point Hedonic scale. Vitamin content of each control sample were higher than other tybo drinks of the same tigernut-zobo drink ratio with preservatives. At Day 0, vit. B_2, B_6 and C content of tybo drinks treated with preservatives were in the range of 1.98 - 2.76, 2.06 - 2.79, and 1.94 - 2.77 mg/l while at Day 15, it was 0.96-1.59, 0.68-1.58 and 0.22-0.96 mg/l, respectively. There were significant differences in vitamin content among different formulations of tybo drinks. Average score for each sensory parameter of tybo drinks with preservative were higher than their respective control samples with exception of tybo drink (25 ml zobo + 75 ml tigernut milk + 0.3 % sodium benzoate). Tybo drinks preserved with acetic acid were preferable than other drinks based on sensory results. Since tybo drinks treated with preservatives have acceptable sensory appeal and longer shelf life than zobo drink and tigernut milk, it is recommended to everyone.

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INTRODUCTION

Tybo drink is a locally produced nutritious drink made from a blend of tigernut milk and 'zobo drink' (Ezeh, 2017). Zobo drink (sorrel, zoborodo) is also a local beverage that is non alcoholic prepared by boiling different varieties of dried petals and acid-succulent calyces of *Hibiscus sabdariffa* (flower) (Adelekan *et al*.,2014) whereas tigernut milk is a popular nutritious non-dairy drink prepared using tigernut tubers (*Cyperus esculentus*) (Maduka and Ire, 2018). Zobo drink mainly contains moisture (88.88 %) and carbohydrate (10.64 %) whereas its protein, fat, ash and crude fiber content are less than 1 % each (Samuel and Frederick, 2018). It is rich in vitamin C (Adeniji, 2017). According to Olayemi *et al.,* (2011) vitamin C content of zobo drink is 7.5 mg/g. Available information on other vitamins which could be present in zobo rink is scarce. According to Suleiman *et al*. (2018), 88 -100 % daily vitamin C needs for children aged 4 - 9 years, 77 % for adolescents, 69 % for adults and 52 % for pregnant mothers could be met by consuming 100 g of tigernuts daily. Nutritional composition of tigernut milk has been well researched (Roselló-Soto *et al.* 2019). Therefore, the product is generally referred as a nutritious drink (Musa and Hamza, 2013; Ogbonna *et al.,* 2013). Suleiman *et al.,* (2018) reported that vitamin A and D content in tigernut tubers per 100 g sample was 0.87 and 30.70 g, respectively. Yet, published works which reported quantity of other vitamins which could also be present in tigernut milk is limited. According to Ezeh *et al*. (2017), tybo drink contains 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. Zobo drink has a sour (vinegar) taste which discourages some people from consuming the product regularly. To an extent it has reduced acceptability of the product. Adesokan *et al*., (2013) reported that zobo drink is highly acidic and therefore advised people not to consume it without combining the drink with snacks. Also to be avoided is drinking zobo in an empty stomach. The shelf life of zobo drink kept at room temperature (28±2 ^{0}C) is very short (24 h) unless it is refrigerated.

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All these factors pose a challenge towards large scale production of zobo drink (Adesokan *et al*., 2013; Adeoye *et al*., 2014; Obi, 2015). Tigernut milk drink has a short shelf life like zobo drink which is less than 24 h (Ibrahim *et al*., 2016). Chemical preservatives such as acetic acid and sodium benzoate have successfully been used to extend the shelf life of zobo (Adesokan *et al*., 2013; Izah *et al.,* 2016). However, limited studies have been done in relation to prolonging the shelf life of tigernut milk using chemical preservatives. Data reported by Adeoye *et al.* (2014) from a survey revealed that 42.25, 30.96, 21.13 and 5.63 % of the study population consumed zobo because of nutritive value, price, availability and organoleptic property, respectively. Meanwhile, 39.28, 28.57, 17.85, 7.14 and 7.14 % of the study population do not consume zobo drink because they disliked the taste, were unaware of its nutritional content, unavailability of the product, dislike the colour and preparatory method for zobo drink, respectively.

Several researchers have successfully enhanced the taste of zobo drink by incorporating spices such as garlic, ginger etc., fruits or artificial sweeteners into the drink (Obi, 2015; Izah *et al.,* 2016). Currently, limited studies focused on improving the taste of zobo by blending the drink with a locally produced non alcoholic beverage that has sugary taste have been reported. In order to improve the sour taste of zobo drink and possibly its vitamin content, this study was aimed at evaluating sensory properties, vitamin C, B_2 and B_6 content of different formulations of tybo drink which is a product of tigernut milk and zobo drink mix together in different ratios and the resulting product were treated with chemical preservative.

MATERIALS AND METHODS

Hibiscus sabdariffa (flower), fresh tigernut tubers, ripe pineapple and fresh ginger used to prepare tybo drink were purchased from Mile 3 market, Port Harcourt and transported to the Food Processing Laboratory, Department of Microbiology, University of Port Harcourt for analysis. Tigernut milk and zobo drink was prepared using the method described by Ogbonna *et al.* (2013) and Obi (2015), respectively with slight modifications.

Different formulations of tybo drink: Three formulations of tybo drink which comprises of 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.

Preparation of preservatives: Two concentrations (0.1 % and 0.3 %) of acetic acid and sodium benzoate which are chemical preservatives were separately prepared. To prepare 0.1 % concentration of the preservatives, 0.1 ml acetic acid was dissolved in 100 ml distilled water; 0.1 ml sodium benzoate was dissolved in 100 ml distilled water. As per 0.3 % concentration of the preservatives, 0.3 ml acetic acid was dissolved in 100 ml distilled water; 0.3 ml sodium benzoate was dissolved in 100 ml distilled water. Three (3) different formulations of tybo drink without preservative added was the control samples. A total of fifteen (15) formulations of tybo drink was prepared and subjected to analysis.

Determination of vitamin C (Ascorbic acid): AOAC (1993) method was adopted. Two milliliter (2 ml) of the standard, sample and blank were separately poured inside 50 ml volumetric flask. Two milliliter (2 ml) sulphuric acid (10 % v/v) and 5 ml ammonium molybdate (10 % w/v) was added to each of the volumetric flask, vigorously mixed and allowed to stand for 50 min at room temperature (28±2 ^{o}C).The content of each volumetric flask was diluted with 25 ml distilled water. Absorbance of the solutions was recorded at 50 nm against blank.

Determination of vitamin B_6 (Pyridoxine hydrochloride): The procedure described by AOAC (1993) was adopted. Two milliliter (2 ml) of standard solution and 2 ml of the sample were separately poured in graduated test tubes. In each test tube, 1 ml ammonium buffer (in water), 1 ml of sodium acetate (in water), 1 ml of 5 % boric acid (in water) and 1 ml of dye (2, 6-di-chloroquinine chromide) solution was added and well mixed. The absorbance of the solution was determined at 650 nm against blank.

Determination of vitamin B_2 (Riboflavin): Five milliliter (5 ml) of standard and sample solution was separately poured inside graduated test tubes. In each test tube, 2 ml hydrochloric acid (1 M), 2 ml glacial acetic acid, 2 ml hydrogen peroxide, 2 ml potassium permanganate (15 % w/v) and 2 ml phosphate buffer (pH 6.8) was added and then well mixed. The absorbance of the solutions was determined at 444 nm against blank.

Sensory evaluation: A total of twelve (12) formulations of tybo drink containing chemical preservatives and three (3) formulations of tybo drinks (control samples) without chemical preservatives stored for 15 Days were subjected to sensory evaluation by ten panelists. Each member of the sensory panelist recorded degree of likeness or dislike of the appearance, colour, taste, consistency/mouth feel and overall acceptability of each formulation of tybo drink using 9-point Hedonic scale.

Data analysis: The various parameters tested were done in triplicates. Mean and standard error was calculated. Triplicate result obtained were subjected to statistical analysis using One-way ANOVA aided by using Statistical Package for the Social Sciences (SPSS) software version 21. The mean (M) and Standard Deviation of the triplicate results were calculated at 95 % confidence interval.

RESULTS

Shown in Table 1 is the vitamin B_2, B_6 and C content of different formulations of tybo drink separately preserved with 0.1 % and 0.3 % acetic acid; 0.1 % and 0.3 % sodium benzoate. Vitamin B_2, B_6 and C content of the control (samples without chemical preservative) is also stated in Table 1. The result obtained from sensory evaluation of different formulations of tybo drink containing chemical preservative and control samples are depicted in Fig. 2 - 6.

DISCUSSION

The result presented in Table 1 revealed that vitamin B_2, B_6 and C content of each of the tybo drinks formulated using the ratios 1:1, 1:3 and 3:1 of tigernut milk and zobo drink and treated with chemical preservative including their respective control samples were significantly different except vitamin B_2

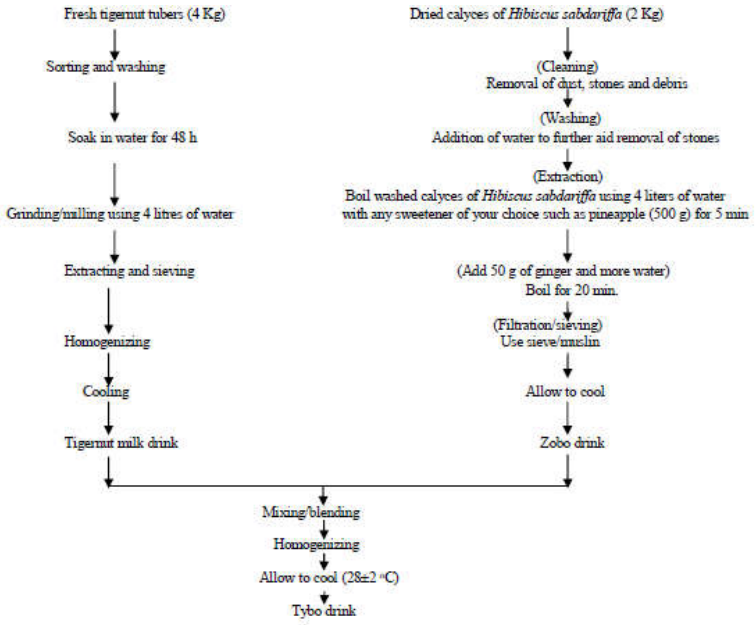

Figure 1. Flow chart for preparation of tybo drink

Table 1. Vitamins B_2, B_6 and C content of different formulations of tybo drink containing chemical preservative and control samples without preservative

Samples	Vit. B_2 (mg/l)		Vit. B_6 (mg/l)		Vit. C (mg/l)	
	Day 0	Day 15	Day 0	Day 15	Day 0	Day 15
ATA1	2.41±0.07^{b}	1.37±0.16^{d}	2.52±0.12^{bc}	1.39±0.14^{bc}	2.32±0.13^{b}	0.82±0.11^{cde}
ATA2	2.38±0.11^{b}	1.59±0.14^{e}	2.68±0.15^{cde}	1.58±0.11^{cd}	2.29±0.19^{b}	0.85±0.07^{cde}
ATS1	2.41±0.08^{b}	1.28±0.10^{cd}	2.60±0.15^{cd}	1.30±0.10^{b}	2.31±0.04^{b}	0.86±0.14^{cde}
ATS2	2.40±0.11^{b}	1.46±0.16^{de}	2.67±0.20^{cde}	1.35±0.13^{bc}	2.31±0.03^{b}	0.89±0.17^{de}
AT	2.51±0.12^{bc}	2.02±0.12^{f}	2.69±0.22^{cde}	1.89±0.24^{e}	2.41±0.18^{bc}	1.73±0.11^{f}
BTA1	1.98±0.08^{a}	1.14±0.09^{abc}	2.16±0.09^{a}	1.27±0.18^{b}	2.04±0.13^{a}	0.57±0.17^{b}
BTA2	1.99±0.15^{a}	1.17±0.10^{bc}	2.29±0.18^{ab}	1.32±0.07^{b}	2.06±0.11^{a}	0.83±0.13^{cde}
BTS1	2.01±0.12^{a}	1.09±0.13^{abc}	2.19±0.09^{a}	1.19±0.16^{b}	2.02±0.09^{a}	0.68±0.10^{bcd}
BTS2	1.93±0.13^{a}	1.12±0.09^{abc}	2.06±0.17^{a}	1.27±0.10^{b}	1.94±0.11^{a}	0.75±0.14^{bcde}
BT	2.37±0.11^{b}	1.96±0.07^{f}	2.52±0.09^{bc}	1.84±0.14^{e}	2.39±0.18^{bc}	1.52±0.18^{f}
CTA1	2.76±0.17^{d}	1.06±0.08^{ab}	2.79±0.13^{de}	1.13±0.12^{b}	2.77±0.19^{d}	0.79±0.10^{bcde}
CTA2	2.69±0.18^{cd}	1.15±0.12^{abc}	2.78±0.08^{cde}	1.36±0.05^{bc}	2.71±0.21^{d}	0.96±0.14^{e}
CTS1	2.68±0.18^{cd}	0.96±0.08^{a}	2.76±0.10^{cde}	0.68±0.16^{a}	2.77±0.09^{d}	0.22±0.12^{a}
CTS2	2.53±0.13^{bc}	1.13±0.09^{abc}	2.69±0.11^{cde}	1.13±0.11^{b}	2.61±0.09^{cd}	0.62±0.10^{bc}
CT	2.84±0.12^{d}	1.93±0.10^{f}	2.87±0.09^{e}	1.72± 0.11^{de}	2.85±0.10^{d}	1.68±0.12^{f}

Values are triplicates of samples. Mean scores with same superscripts down the column are not significant at $p<0.05$, while others with different superscripts are significant. T = Tigernut milk; 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 = 50 ml Z + 50 ml T.

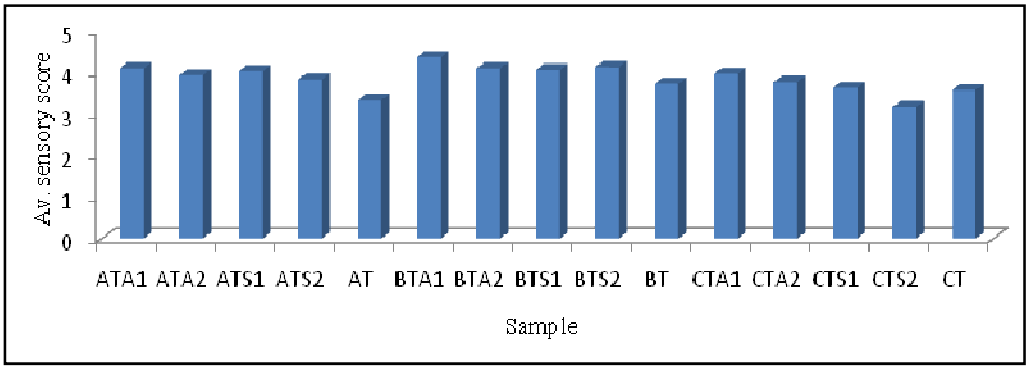

Figure 2. Sensory score for overall acceptability of different formulations of tybo drink

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 = 50 ml Z + 50 ml T.

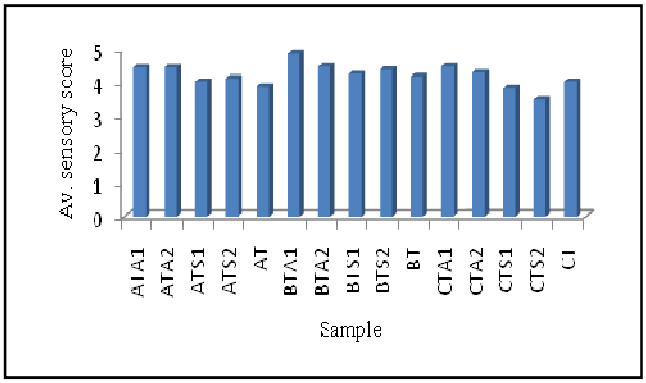

Figure 3. Sensory score for appearance of different formulations of tybo drink

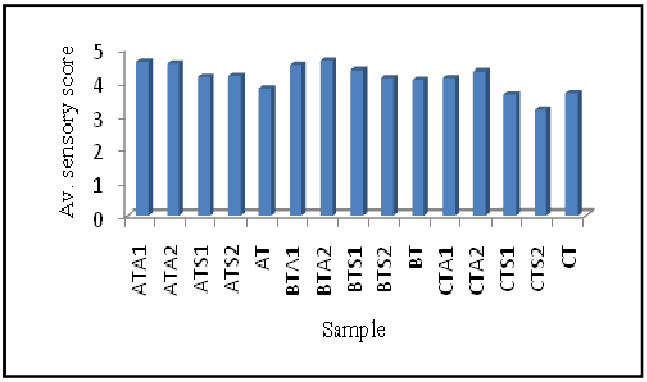

Figure 4. Sensory score for colour of different formulations tybo drink

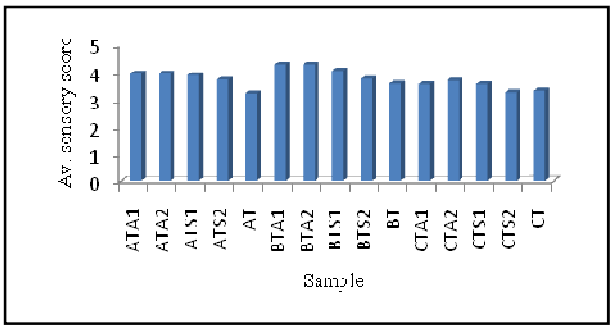

Figure 5. Sensory score for taste of different formulations of tybo drink

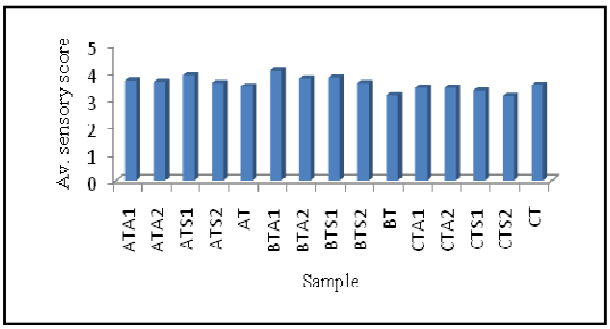

Figure 6. Sensory score for consistency/mouth feel of different formulations of tybo drink

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 l 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 = 50 ml Z + 50 ml T.

content of tybo drink formulated using 75 ml zobo drink + 25 ml tigernut milk + 0.1 % sodium benzoate, 75 ml zobo drink + 25 ml tigernut milk + 0.3 % sodium benzoate, 75 ml zobo drink + 25 ml tigernut milk + 0.1 % acetic acid and 75 ml zobo drink + 25 ml tigernut milk + 0.3 % acetic acid at Day 0; 50 ml zobo drink + 50 ml tigernut milk + 0.1 % sodium benzoate, 50 ml zobo drink + 50 ml tigernut milk + 0.3 % sodium benzoate, 50 ml zobo drink + 50 ml tigernut milk + 0.1 % acetic acid and 50 ml zobo drink + 50 ml tigernut milk + 0.3 % acetic acid at Day 0. According to Achoribo and Ong (2017), tigernut contains vitamin B. This vitamin could help in balancing the central nervous system and also assist the body adapt to stress. Tigernut milk is also a good source of vitamin C (Sánchez-Zapata *et al*., 2012). Zobo drink contains reasonable quantity of vitamin C (Olayemi *et al.,* 2011). This vitamin is an antioxidant (Suleiman *et al.,* 2018). Treatment of disorders such as diabetes, glaucoma, cataracts, stroke, heart diseases and cancer could be done using vitamin C. However, vitamin C deficiency in human body could lead to scurvy, bleeding gums, poor wound healing and anemia (Dave and Patil, 2017). Although vitamins are required in small amounts by humans, they help the body maintain good health and vitality (Adelekan *et al*., 2014). At Day 0 and 15, our result shows that vitamin C, B_6 and B_2 content of all the tybo drinks preserved with acetic acid or sodium benzoate with the exception of sample CTS1 (25 ml zobo drink + 75 ml tigernut milk drink + 0.1 % sodium benzoate) were lower than their respective control samples.

At Day 15, the vitamin B_2 and B_6 content of sample CTS1 was 0.96 and 0.68 mg/l, respectively whereas that of other tybo drinks including the control samples were above 1.0 mg/l. Meanwhile, vitamin C content of sample CTS1 (0.22 mg/l) at Day 15 was lower than that of other tybo drinks containing chemical preservatives including the control samples. Higher quantity of vitamin C, B_6 and B_2 content of the control samples at Day 0 and 15 compared with other tybo drinks of the same tigernut-zobo drink ratio with preservatives could be as a result of acetic acid and sodium benzoate was not added to the drink. Egbere *et al*. (2007) and Chibueze *et al.* (2019) reported a similar result from a related study. According to their findings, vitamin C content of zobo drink preserved with organic acid was lower than that of zobo drink without organic acid added to it. Although chemical preservatives were not added to the control samples stored at room temperature, our result revealed that its vitamins C, B_6 and B_2 content reduced during the storage period. Similarly, vitamin C, B_6 and B_2 content of other tybo drinks treated with chemical preservative was reducing during the period of storage. At Day 0,vitamin B_2, B_6 and C content of all the tybo drinks was within the range 1.93 - 2.84, 2.06 - 2.87 and 1.94 – 2.85 while at Day 15 it was 0.96 – 2.02, 0.68 - 1.89 and 0.22 – 1.73 mg/l, respectively. According to Gbadegesin and Odunlade (2016), food processing and length of food storage is capable of degrading vitamin C content in food. It is reported that vitamin C content in food products and drinks are easily denatured by slightest stress (Singh, 2004).

In a related study, Babatuyi *et al.* (2019) reported that vitamin C content of tigernut milk stored at room temperature reduced from 30.21 – 20.14 mg/ml. Chibueze *et al.* (2019) reported that combined effect of the preservatives and activities of microorganisms in zobo drink could be responsible for the reduction in vitamin C content during storage.

In terms of average sensory score for overall acceptability, Fig. 2 shows that tybo drink without chemical preservative was least preferred compared with other tybo drinks of the same tigernut-zobo drink ratio treated with acetic acid or sodium benzoate with the exception of tybo drink (25 ml zobo and 75 ml tigernut milk drink preserved with 0.3 % benzoate). This result suggests that addition of chemical preservatives to tybo drink preserved its sensory attributes better than the tybo drinks without chemical preservatives. Fig. 2 shows that average sensory score for overall acceptability, appearance and consistency/mouth feel of sample BTA1 (75 ml zobo drink + 25 ml tigernut milk drink + 0.1 % acetic acid) was the highest compared with other tybo drinks. On the contrary, sample CTS2 (25 ml zobo drink + 75 ml tigernut milk + 0.3 % sodium benzoate) had the lowest average sensory score for all the sensory attributes with exception of taste. In a related study, Braide *et al*. (2012) reported that zobo drink preserved with sodium benzoate maintained the characteristic red colour zobo drink is known for and also had a sweet taste whereas the colour of zobo drink preserved with acetic acid turned dark red, the product became sour and retained strong pungent smell acetic acid is known for. The ratio of tigernut milk mix together with zobo drink as well as concentration of chemical preservatives added to the drink could be responsible for influencing sensory results obtained from this study.

The results presented in Fig. 3, 4, 5 and 6 shows that appearance, colour, taste and consistency/mouthfeel, respectively of different formulations of tybo drink preserved with 0.1 or 0.3 % acetic acid had average sensory scores that were slightly higher than that of tybo drinks of the same tigernut-zobo drink ratio preserved with 0.1 or 0.3 % sodium benzoate. Therefore, acetic acid used as a chemical preservative was preferable than sodium benzoate in terms of sensory properties of different formulations of tybo drink preserved with these chemicals. In a related study, Braide *et al*. (2012) reported that sodium benzoate added to zobo drink maintained the red characteristic colour and sweet taste of the drink preserved at ambient temperature for 14 days. Meanwhile, zobo drink preserved with acetic acid became dark red, sour and the strong pungent smell this preservative is known for was retained within the same period of storage at ambient temperature. In other words, zobo drink preserved with sodium benzoate was preferable than the one preserved with acetic acid. This is not in agreement with our sensory results which showed that different formulations of tybo drinks preserved with acetic acid were preferable than the drinks preserved with sodium benzoate.

Conclusion

Addition of sodium benzoate and acetic acid to different formulations of tybo drink, length of storage and storage condition resulted in reduction of vitamin B_2, B_6 and C content of the drink. Most of the sensory properties of different formulations of tybo drinks treated with chemical preservatives were preferable than tybo drinks without chemical preservatives.

Based on sensory results, different formulations of tybo drinks preserved with acetic acid were preferable than the drinks preserved with sodium benzoate.

Competing interests: The authors declare that no competing interests exist.

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