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Comparative Study on the Effect of Spices Bioactive Extract on the Stability of Physico-chemical and Sensory Attributes of Zobo Drink under Storage

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ABSTRACT---- *Effect of local spice extracts on the stability of physico-chemical properties and sensory attributes of Zobo-drink during shelf-life was studied. Varied proportions (4, 8, and 12%) of Aframomum danielli and black pepper extracts were incorporated into Zobo-drink (v/v) during preparation. Physico-chemical properties of samples were determined. Samples obtained from both spice treatments were stored under different conditions (ambient, refrigeration and freezing) for fifteen days. The pH, titrable acidity, brix content and microbial counts were examined at every 72h interval during the storage period. Significant difference (P<0.05) was discovered on the properties of Zobo-drink samples. The mean sensory score differ on the samples attributes in both treatments. The mean scores 8.41 and 8.28 for samples contained 12% and 4% Aframomum and black pepper extracts respectively were the most preferred. The values (%) of 86.49, 1.16, 0.26 and 13.56 highest for moisture, protein, ash and carbohydrates were discovered on samples in both spice treatments. The chemical and microbial properties of stored samples varied significantly. The pH 3.25 and 3.17 (control) for Aframomum danielli and black pepper treated samples changed to pH 1.52 and 1.98 under ambient temperature; pH 3.10 and 3.25 for samples under refrigeration and freezing conditions. The brix content of 3.411 and 3.011 control samples changed to 0.980 and 1.281 at fifteenth day of storage in both spice treatments. Aerobic plate counts (cfumL^{-1}) increased from 1.38 x 10^{3} and 2.10 x10^{3} control samples to 6.61 x10^{3} and 6.28 x10^{3} samples stored at ambient temperature during storage. Coliform bacteria were not detected from the samples during the period of storage.*

Keywords--- Zobo-drink, spice extract, *Aframomum danielli*, black pepper

1. INTRODUCTION

Zobo is used to describe non-alcoholic drink obtained from the liquid extraction from *Hibiscus sabdarriffa* flowers with the addition of other ingredient such as sugar and dried clove. It is a refreshing drink produced in a home by local processors, the drink is now becoming very popular among people of various socio-economic classes in West Africa; Nigeria in particular. Zobo drink is an alternative source of cheap and relaxing non-alcoholic drink to most conventional soft drink and non-alcoholic beverage in social gathering (Ogiehor and Nwafor, 2004). Zobo drink beverage appears to be a promising means of economic empowerment among the local producers and socially accepted drink especially amongst the youth in developing nations of tropics.

Nutritionally, the beverage has been shown to be a good source of energy, mineral elements and vitamin C among others which constitute the major reasons for soft drink and fruit juice consumption (Ogiehor and Nwafor, 2004). These components and other chemical characteristics such as pH, total titrable acidity, sugar level and as well as organoleptic attributes of Zobo drink tend to change during distribution, sales and storage period within 24-48h of production, this is due to the activities of associated microorganisms (Akinyosoye and Akinyele, 2007). This change caused short shelf-life of the drink and a major problem faced by the local processor in developing countries. Several microorganisms (*Bacillus, Streptococcus, Staphylococcus, Lactobacillus,* etc) have been reported to be associated with Zobo beverages during storage (Ogiehor and Nwafor, 2004). The proliferation of the associated micro-organisms and other factors such as poor packaging system and handling cause spoilage of zobo drink within few days and cannot competing favorably with most conventional beverages and soft drinks.

In Nigeria and like other developing countries of the world, recent government policy emphasized the need to exploit local material in manufacturing process and less dependent on foreign food items through stringent policy on importation of goods and material use in production. However, the uses of local material in food formulation and processing become inevitable. The use of local spices to control the activities of micro-organisms in food has been reported to be effective (Akpomedaye and Ejechi, 1998; Adegoke *et al.*, 2000) in addition to different preservation or storage methods to retard microbial growth and stabilize the chemical component in food has been documented (Ogiehor *et al*., 1998). Apart from the antimicrobial properties, spices in powdery or aqueous extract forms are believed to have desirable influence on the overall organoleptic quality when added to food. *Aframomum danielli* belongs to the genus *Aframomum*, of the family *zingiberace*. It is commonly known as 'Atare-oburo', 'Upoe' and 'Urima' in Yoruba, Igbo and Edo languages respectively in Nigeria where they are used for the preparation of traditional cuisine (Adegoke and Skura, 1994). Black pepper (*Piper nigrum*) the king of spices, is one of the oldest and the most popular spices in the world. The hot-pungent spice made from its berries is one of the earliest spices. It is used as an important component of many recipes and to flavor foods (FAO 2000). *Aframomum danielli* and black pepper have been reported to be a good antioxidant and have good inhibitory properties on many pathogenic microorganisms (*Salmonella enteriditis*, *Pseudomonas fragi*, *Pseudomonas fluorescence*, *Staphylococcus aureus*, *Staphylococcus epidermidis Streptococcus faecalis*, *Gram-negative Escherichia coli*, *Aspergillus flavors*, *Aspergillus parasiticu*) Adegoke *et al.* (1998); Fasoyiro *et al.* (2001);Martins *et al.* (2001) and on food spoilage yeasts like *Torulopsis candida*, *Candida tropicalis* and *Aflatoxigenic* moulds like *Aspergillus flavus*, *Aspergillus parasiticus* (Adegoke *et al.* 2000). *Aframomum danielli* has been used in the preservation of a variety of food commodities, including maize and soybean against mould growth and insect infestation (Adegoke *et al.* 2000). This objective of this work was to examine the potential of spice aqueous extract on the physico-chemical and sensory stability of Zobo drink during storage.

2. MATERIALS AND METHODS

Materials

Dried *Hibiscus sabdarriffa* flower, sugar and clove were obtained from Ama-Hausa and Owerri main markets respectively in Owerri, Imo State. The Matured *Aframomum danielli* pods were harvested from a local farm at Warri, Delta State and black pepper was purchased from Owerri main market, Imo State both in Nigeria.

Aqueous Spice Extraction, Preparation and Storage of Zobo drink

The dried seeds of each spice was made into powdery form by pulverization using Warring blender (model: HGB2WTG4) and sieved (200 µm aperture) into fine particle size respectively. The each powder was subjected to cold extraction by dispersing in water at ratio 1:1 (w/v) at ambient condition for 48h. Filtrate was obtained after passing the suspension through Whatman filter paper No. 2. The dried cleaned petals of *Hibiscus sabdarriffa* (500g in 4L) was boiled in water for 5min with addition of 1% clove. The different proportions 4; 8; 12% aqueous extract of the spice per volume of Zobo-drink (1L) respectively was added along with 4% syrup to Zobo-drink at 40^{o}C and then sieved using sieve of 200µm and bottled prior to storage the following conditions; under ambient (29-32^{o}C), refrigeration (4^{o}C) and freezing (-18^{o}C) conditions. The following chemical properties; pH, Total Soluble Solid (as ^{o}Brix), acidity were determined and microbial load of Zobo-drink were monitored at every four (4) interval for fifteen days. Figure 1 showed the steps used for the preparation of samples.

Chemical Analysis

pH

Five millitre (5mL) of liquid samples was thoroughly mixed for 5min and the pH was measured using a digital pH meter (Orion Research Inc. USA model 720A).

Total Titrable Acidity

The sample was thoroughly mixed for 5mins; l0mL of the solution was titrated with 0.IN NaOH using phenolphthalein indicator solution. The total titrable acidity was expressed as % (w/v), 1ml 0.IN 0.009g lactic acid as described by A.O.A.C (2000).

Total Soluble Solids (^{o}Brix)

Total soluble solids content was determined at 29 ± 2°C using Abbey Hand Refractometer (Atago Co. Ltd. Japan). Percentage total soluble solids content was calculated as sucrose, using sucrose conversion table corrected to 20^{o}C as described by Pearson (1976).

Proximate Composition analysis

The moisture content, fat, protein, crude fiber, ash content were determined by methods described by A.O.A.C (2000) and carbohydrate was determined by total difference.

Dried petals of *Hibiscus sabdarrifa*

↓

Washed in Sterile Water

↓

Boiled in Water for 5min (500g in 4litre of water)

↓

Addition of Clove

↓ ← Addition of 4; 8; 12 % of Spice Bioactive extract

Cooled and Filtered

↓

Addition of 4% Syrup

↓

Bottling

↓

Cooling

↓

Zobo Drink

Figure 1: Flow chart used for the preparation of Zobo drink

Determination of Microbial Population

Microbial count was done according to ICMSE (1997) on each sample under investigation. A 10mL of each sample was dispersed in 90mL of sterile distilled water, homogenized by shaking vigorously and further diluted up to 10^6. An aliquot portion (0.1ml) of the 6th dilution was inoculated in duplicate onto surface nutrient and MacConkey agar respectively. Also 0.1mL of the 4th dilution was transferred in duplicate on Sabouraud dextrose agar. The plates was spread evenly with a sterile spreader and incubated for 48hrs at 28°C for heterotrophic fungi and at 37°C for 24h for bacteria and coliform (Pelezar *et al*.1993; Cheesbrough, 2000).

Sensory Evaluation

The scoring method was used to determine the preference test on the samples of the Zobo drink produced with added spice bioactive extract. Twenty (20) trained panelists were draw from the staff and students of Department for the evaluation exercise. The samples were coded with arbitrary codes which were presented to the panelist and presentation was randomized. Nine (9) points hedonic scale was used in the rating. The evaluation was conducted on four sensory attributes namely color, aroma, taste, and overall acceptability.

Statistical Analysis

The data obtained were subjected to various statistical analyses which include simple descriptive mean, standard deviation and analysis of variance (ANOVA), while Duncan's test was used to separate the means using SPSS 20.0 Software Inc. USA.

3. RESULTS AND DISCUSSION

Sensory evaluation of Zobo-drink

Table 1 showed the mean values of Zobo-drink treated with bioactive extract from *Aframomum danielli* and black pepper. Apart from colour significant differences ($P<0.05$) were discovered on the sensory properties of Zobo-drink samples treated with varied proportion of bioactive extracts (*A. danielli* and black pepper) respectively. This observation was in agreement with findings of Oluwalana *et al*. (2013) and Bolade *et al*. (2009) in separate reports where significant difference ($p<0.05$) was discovered on the sensory properties of sorghum sheath beverage 'poporo' treated with *Aframomum meleguata* family of *Zingiberace* and optimized production of Roselle beverage with Hot water extraction different sweetness level respectively. In the same view from the result, there was no significant difference ($p<0.05$) on the color perception of the drink samples treated with spice bioactive extract. This observation was similar when compared in both spices used and control. This could be due to the interaction of the pigment during co-pigmentation of bioactive extract from the spices used and extract from Roselle leaves 'Zobo' despite variation in the proportion used. In addition, the color rating slightly decreased in value when the proportion of added bioactive flavor extract increased from 4-12%. Mazza and Brouillard (1990) reported that anthocyanin in Roselle beverage has been observed as one of the factors influencing color intensity in the beverage. Furthermore, mean values of aroma and taste decreased significantly when the incorporation of bioactive flavour extract was increased from 4-12% in both treatments (*Aframomum danielli* and black pepper extracts) respectively. The overall acceptability of zobo-drink treated *Aframomum danielli* and black pepper differs. The zobo-drink contained 12% of *Aframomum danielli* and 4% black pepper in each treatment was most preferred. This may be attributed to the different bioactive content of the spices used. The quantity or proportion of bioactive flavour extract needed in food flavoring could differ from one spice to another.

Table 1: Mean value of sensory Attributes of Zobo-drink

Sample of Zobo-drink	Proportion of spice extract %	Color	Aroma	Taste	Overall acceptability
AF-Zobo-drink B_1	4	7.82^a	8.38^b	8.08^a	8.35^a
AF-Zobo-drink B_2	8	7.80^a	8.44^a	8.11^a	8.40^a
AF-Zobo-drink B_3	12	7.81^a	8.10^d	7.98^b	8.41^a
BPF-Zobo-drink B_4	4	7.80^a	8.40^{ab}	8.16^a	8.28^b
BPF-Zobo-drink B_5	8	7.84^a	8.12^d	8.14^a	8.10^c
BPF-Zobo-drink B_6	12	7.78^a	7.88^e	7.82^b	8.0^d
Control	0	7.81^a	8.20^c	8.10^a	8.22^b

Mean score with different superscript letter along the column are significantly different (P<0.05) AF- *Aframomum danielli* Flavoured Zobo-drink (B_{1-3}); BPF-Black pepper flavoured Zobo-drink (B_{4-6}) = Blend samples.

Proximate and chemical properties of Zobo-drink Flavoured with Spice Extracts

The proximate composition and chemical properties of Zobo-drink treated with varied proportions of bioactive flavour extract from *Aframomum danielli* and black pepper respectively was presented in Table 2. Significant ($p< 0.05$) variation was discovered on the properties of Zobo-drink examined. The moisture content of the Zobo-drink ranged from 85.21% to 86.49%. These values were decreased when the proportion of spice bioactive extract increased from 4% to 12% in both treatments (*Aframomum danielli* and black pepper). This observation was in agreement with findings of Oluwalan *et al.* (2013) when sorghum stem sheath beverage was treated with varied concentration of alligator pepper extract and higher when compared with 78.24 - 84.4% moisture of Roselle drink contained orange and pineapple flavour (Fasoyiro *et al.* 2005). The variation observed may be due to the difference in the methods and materials used in 'Zobo' preparation. The high moisture content found in Zobo-drink is desirable as refreshing and thirst-quenching drink, but an indication of possibility of spoilage may require further preservation during storage.

Table 2: Proximate and chemical properties of Zobo-drink flavoured with *Aframomum danielli* and Black pepper extracts

Sample of Zobo-drink	Proportion of spice extract	★Moisture %	★Crude protein %	★Crude fat %	★Ash %	★Total carbohydrates %	★pH	°Brix content	★Dry matter %	★Titrable acidity %
AF-zobo-drink B_1	4	86.49^a	0.98^{de}	ND	0.16^d	12.37^d	3.15^a	2.721^c	13.51^d	0.210^c
AF-zobo-drink B_3	8	85.98^c	1.07^c	ND	0.18^c	12.77^c	3.21^a	3.101^b	14.02^b	0.230^b
AF-zobo-drink B_3	12	85.21^d	1.16^a	ND	0.21^b	13.42^a	3.25^a	3.410^a	14.79^a	0.241^b
BPF-zobo-drink B_1	4	86.22^b	1.01^d	ND	0.14^e	12.84^b	3.17^a	3.011^b	13.99^b	0.191^c
BPF-zobo-drink B_2	8	85.88^c	1.10^c	ND	0.20^b	12.83^b	3.16^a	3.181^b	14.12^b	0.220^c
BPF-zobo-drink B_3	12	85.04^d	1.14^b	ND	0.26^a	13.56^a	3.28^a	3.501^a	14.96^a	0.280^a
Control	0	86.22^b	0.88^e	ND	0.17^d	12.73^c	3.13^a	2.401^d	13.78^{bc}	0.181^c

★Mean of triplicate values; Mean values with different superscript letter along the column are significantly different ($p<0.05$) AF- *Aframomum danielli* Flavoured zobo-drink; BPF-Black pepper flavoured zobo-drink.

The crude protein increased from 0.98 – 1.16% and 1.01 – 1.14% in samples containing varied proportion of *Aframomum danielli* and black pepper respectively. The values of protein in both treatments were higher than 0.88% control (without spice bioactive extract). This observation was not in agreement with the findings of Oluwalana *et al.* (2013) who reported a progressive significant decrease in the protein content of sorghum stem sheath beverage flavoured with 0.5 – 2.5% Alligator pepper. The variation and increase of protein could be due to difference in chemical composition of raw materials used *Hibiscus sabdariffa* L. and spices or other plant flavoring materials), also, point (after boiling) at which the bioactive extract was introduced during Zobo-drink preparation. However, Oluwaniyi *et al.* (2009) stated that effect of heat processing involved in extraction may destroy some amino acids and consequently reduce total nitrogen content of the resulting beverage. The ash content of Zobo-drink samples was valued at the range of 0.14 – 0.26%. These values represent the mineral elements present in the drink. Total carbohydrates slightly increased significantly in value 12.37 – 13.42% and 12.84 – 13.56% as the proportions of bioactive extracts in both treatments increased (4-12%) respectively. Furthermore, the values were closely related to the report of Amo (2006) who discovered 12.27% total carbohydrates on sorghum stem sheath beverage.

The pH of Zobo-drink did not show significant different ($p > 0.05$) among the samples despite different types and proportion of spice extract used. The pH was found between the range of 3.15 and 3.28. These values were similar to pH 3.10 – 3.62 reported for Roselle drink flavoured with apple, pineapple and orange respectively (Fasoyiro *et al.* 2005). The low pH value is an indication of acidity level in Zobo-drink which is an important property or parameter used in food handling such as packaging material selection and storage condition. The total soluble solid discovered as ^{o}Brix content of treated Zobo-drink ranged between 2.751- 3.410 and 3.011 – 3.501 for both treatments, these were higher than the 2.401 (control). The pH increased with increasing proportion of spice extract in the blends. The ^{o}Brix content found was in agreement with Bolade and Oluwalana (2009) who reported 2.4 – 3.9% total solid for Roselle beverage ('soborodo') prepared from different dried calyces/water ratios and boiling duration. But, the ^{o}Brix content obtained differs from range of 8.20 – 11.21 of Roselle-drink blended with apple, pineapple and orange flavors respectively (Fasoyiro *et al.* 2005). The differences in reports of earlier researches could be due to variations in quantity, nature and chemical properties of raw materials used as flavoring agent of Zobo-drink. The titrable acidity (%) is a measure of acidity level present in a food. The percentage found was between 0.210 – 0.241 and 0.191 – 0.280 on Zobo-drink treated with both spice extract respectively. Amo (2006) reported 0.06 – 0.07% from extract of calyces of Roselle as a source of 'Zobo-drink'. The findings on the properties of Zobo-drink shows that combination of Roselle extract with spice bioactive extract tends to improve the proximate composition and chemical properties of non-alcoholic drink.

Effect of Spice Bioactive Extracts on Chemical and Microbial Properties Zobo-drink under storage conditions

Table 3 showed the chemical and microbial qualities of treated Zobo-drink samples at three different conditions (temperatures) of storage. Significant difference ($p < 0.05$) was discovered among the properties of Zobo-drink monitored under ambient, refrigeration and freezing conditions. The pH of the stored samples observed in both treatments (with *Aframomum danielli* and black pepper extracts) under ambient condition showed a significant and gradual decline to 2.87 and 2.77 at eight day and continued to 1.52 and 1.98 at fifteenth day of storage in both treatments used. The change in values was differed to pH of 3.25 and 3.17 of control samples (1st day of storage). The pH 3.10, 3.25 and 3.16 of samples stored under refrigeration and freezing conditions in both spice extracts treatments were discovered at fifteenth day of storage. However, Dnyaneshwar *et al.* (2013) reported a change in the pH of carbonated pineapple from initial pH 4.2 to 3.63. Iqual *et al.* (2010) discovered change in pH from 3.09 to 2.92 and 3.20 to 2.90 of grape fruit juice stored at 4^{o}C and -18^{o}C respectively within two months. The extent of time (8th day) to which the pH of samples was stable at room temperature may be attributed to the effect of spice bioactive flavour extract which has been reported to have antioxidant property. Natural antioxidant is capable of delaying, retarding or preventing the development in food of rancidity or other flavour deterioration due to oxidation. Antioxidants delay the development of off-flavour thereby extending the induction period (Michael, 2001). The titrable acidity (%) of Zobo-drink samples increased gradually to 0.256 and 0.308 % at fourth day and further increased to 0.386 and 0.357% under room temperature compared with initial value of 0.240 and 0.308% (control samples) in each treatments. Samples under freezing condition differ in titrable acidity, the values of 0.244 and 0.291% in each treatment showed no significant difference during period of storage. This indication could be attributed to combined effect of the spice bioactive extract and freezing temperature. The ^{o}Brix content of the samples showed a decline from 3.411 and 3.011 control samples in each spice treatment to 0.980 and 1.281 of samples at room temperature at the end of storage time. The ^{o}Brix was relatively stable under refrigeration and freezing conditions without any significant ($p>0.05$) difference with final values of 3.390 and 3.0 for *Aframomum danelli* and black pepper treated samples. The decline ^{o}Brix content at ambient storage condition could be attributed to the fermentation process that was influenced by ambient temperature. Furthermore, the percentage dry matter was reduced during storage period at room temperature. The dry matter reduced from 14.79 to 4.22% and 13.99 to 5.10% in both treatments at ambient but stable relatively under refrigeration and freezing conditions when compared.

The aerobic plate counts (cfumL^{-1}) of samples monitored showed significant ($p < 0.05$) increase on population at ambient temperature. The microbial load changed from initial values of 1.38×10^{3} and 2.10×10^{3} control samples to 6.61×10^{3} and 6.28×10^{3} at fifteenth day of storage in both treatments respectively. The values of 2.28×10^{3} and 1.52×10^{3}cfumL^{-1} were obtained on samples at refrigeration condition and 4.21x10^{3} and 2.10x10^{3}cfumL^{-1} at freezing condition. The result agreed with findings of Emmanuel et *al.* (2006) who reported increase in the number of mesophilic aerobic counts of pasteurized soursop juice stored at 4^{o}C. In addition, Ajibola *et al.* (2013) indicated no inhibition of bacteria from Kunun-zaki beverage contained water and ethanol extracts from *Piper guineensis* and *Monodora myristica*. Natural aromatic plants and spices have been widely used in many food products as flavouring, seasoning agent (Elegayyar *et al.*, 2001) and for preservation value (Reddy *et al.* 2005). The coliform bacteria were found absent from the samples studied at different conditions of storage. This could be attributed to the good manufacturing practice adopted during raw materials assembling and samples preparation. The findings from this work on bioactive extract from local spices increased their potential in food processing and preservation.

Table 3: Chemical and microbial properties of Stored Zobo-drink flavoured with *Aframomum danelli* and Black pepper

		Storage time (day) Storage at 4th day			storage at 8th day			storage at 12th day			storage at 15th day		
Properties	**Control**	**Amb**	**Ref**	**Fr**	**Amb**	**Ref**	**Fr**	**Amb**	**Ref**	**Fr**	**Amb**	**Ref**	**Fr**
Aframomum danelli													
pH	3.25^{a}	3.11^{a}	3.22^{a}	3.24^{a}	2.87^{b}	3.21^{a}	3.25^{a}	2.01^{c}	3.19^{a}	3.25^{a}	1.52^{d}	3.10^{a}	3.25^{a}
Titrable acidity %	0.240^{e}	0.256^{d}	0.248^{de}	0.240^{e}	0.291^{b}	0.251^{d}	0.241^{e}	0.311^{b}	0.254^{d}	0.241^{e}	0.386^{a}	0.260^{c}	0.244^{e}
^{o}Brix content	3.411^{a}	3.281^{a}	3.391^{a}	3.410^{a}	2.961^{ab}	3.361^{a}	3.40^{a}	1.841^{c}	3.301^{a}	3.40^{a}	0.980^{d}	3.281^{a}	3.390^{a}
Dry matter %	14.79^{a}	14.01^{a}	14.73^{a}	14.77^{a}	12.21^{b}	14.60^{a}	14.76^{a}	8.66^{c}	14.18^{a}	14.73^{a}	4.22^{d}	14.04^{a}	14.70^{a}
Aerobic plate count cfumL^{-1}	1.38^{f} x10^{3}	1.52^{e} x10^{3}	1.42^{f} x10^{3}	1.40^{f} x10^{3}	3.10^{c} x10^{4}	1.62^{d} x10^{3}	1.41^{f} x10^{3}	5.11^{b} x10^{6}	2.01^{d} x10^{3}	1.51^{e} x10^{3}	6.61^{a} x10^{3}	2.28^{d} x10^{3}	1.52^{e} x10^{3}
Coliform count cfumL^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Black pepper													
pH	3.17^{a}	2.98^{b}	3.16a	3.17^{a}	2.77^{b}	3.14^{a}	3.16^{a}	2.28^{c}	3.13^{a}	3.16^{a}	1.98^{d}	3.10^{a}	3.16^{a}
Titrable acidity %	0.290^{d}	0.308^{b}	0.292^{d}	0.290^{d}	0.311^{b}	0.294^{cd}	0.291^{d}	0.322^{b}	0.292^{d}	0.291^{d}	0.357^{a}	0.298^{bc}	0.291^{d}
^{o}Brix content	3.011^{a}	2.831^{b}	2.990a	2.60^{c}	2.60^{c}	2.961^{a}	3.01^{a}	2.181^{d}	2.951^{a}	3.01^{a}	1.281^{e}	2.922^{a}	3.0^{a}
Dry matter %	13.99^{a}	12.76^{ab}	13.88a	12.01^{b}	12.01^{b}	13.83^{a}	13.98^{a}	9.36^{c}	13.22^{a}	13.96^{a}	5.10^{e}	13.60^{a}	13.97^{a}
Aerobic plate count cfumL^{-1}	2.10^{d} x10^{3}	4.3^{b} x10^{3}	2.96^{c} x10^{3}	2.11^{d} x10^{3}	6.21^{a} x10^{4}	2.0^{d} x10^{3}	2.0^{d} x10^{3}	4.52^{b} x10^{4}	4.20^{b} x10^{3}	2.10^{d} x10^{3}	6.28^{a} x10^{3}	4.21^{c} x10^{3}	2.10^{d} x10^{3}
Coliform count cfumL^{-1}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Mean values with different superscript letter along the column are significantly different ($p<0.05$); Amb- Ambient condition, Ref-refrigeration condition; Fr. - Freezing condition and ND-Not detect.

4. CONCLUSION

The findings on the properties of Zobo-drink showed that combination of Roselle extract 'Zobo-drink' with spice bioactive extracts tend to improve the proximate composition and chemical properties of non-alcoholic Zobo-drink especially during storage time. The keeping quality of "Zobo-drink is largely based on stability of physico-chemical properties and sensory attributes such as flavour, aroma, taste and colour during distribution and sales by local producers. Natural antioxidants and antimicrobials properties of local spices used increased shelf-life of Zobo-drink and absence of synthetic preservative agents made the product safe with no side effects on human health. The composition, structure as well as functional groups in the spice bioactive fraction could responsible for the eight day stability of the drink against changes in chemical properties and increase in microbial load under room temperature and fifteen days shelf-life at refrigeration and freezing conditions as observed in this work.

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