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REVIEW ARTICLE

Advances in Preservatives and condiments used in Zobo (a food-drink) production

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ABSTRACT
Zobo is a nutritional drink widely accepted by different socio-economic class of people, age and sex in several parts of Nigeria especially in the Northern region and some neighboring West African countries. Zobo is produced from dried calyces of *Hibiscus sabdariffa,* which is a tropical and semi-tropical annual herb. The short shelf-life span of Zobo drink reduced its health sustainability. This is due to nutritional deterioration caused by spoilage microorganisms in the drink which could cause disease condition when ingested. Several natural preservatives/ spices such as garlic, ginger, mixture of garlic and ginger have been widely used for the production of Zobo drink. This paper reviews advances in the past decade in the preservation of zobo. The preparation of Zobo with long shelf life is a challenging exercise. The review indicated that the use of natural spices such as nutmeg, clove, cinnamon, kola nut, lime and pepper fruit etc in addition to the already existing garlic and ginger as preservatives at moderate concentration could reduce the density and diversity of spoilage microbes of local drink. We conclude by suggesting research on the carbonation of the drink after preparation.

KEY WORDS: *Hibiscus sabdariffa, Nutritional drink, Preservatives, Spoilage microorganisms, Zobo drink*

Introduction

Zobo is a nutritional drink consumed by different class of people irrespective socio economic status (Izah *et al*., 2015; Nwafor, 2012), sex and age in Nigeria especially in the Northern region and other neighboring African countries (Izah *et al*., 2015). The most active ingredient used in the production of zobo drink is *Hibiscus sabdariffa,* which belongs to the Malvaceae family. *H. sabdariffa* are mainly cultivated as vegetables for soup preparations (Fasoyiro *et al*., 2005), folk medicine and tea preparations (Adesokan *et al*., 2013). *H. sabdariffa* is a tropical annual herb. *H. sabdariffa* is native to Asia (India and Malaysia) (Bola and Aboaba, 2004 cited Egbere *et al*., 2007; Ezearigo *et al*., 2014). Specifically, *H. sabdariffa* is native to India from where it was introduced to other part of the World including Central America, West India and Africa (Fasoyiro *et al*., 2005). *H. sabdariffa* was introduced to Central America and West India when the plant became popular in Jamaica in early 18th century (Alo *et al*., 2012; Ilondu and Iloh, 2007). As at today, *H. sabdariffa* is found in several tropical and subtropical countries of the World (Fasoyiro *et al*., 2005) especially in India and Africa (Nwachukwu *et al*., 2007). In Nigeria, *H. sabdariffa* is mainly found in the Middle belt North eastern region (Joseph and Adogbo, 2015; Obadina and Oyewole, 2007 cited Bolade *et al*., 2009). However, two varieties of *H. sabdariffa* are found in Nigeria including red/brown and green (Ilondu and Iloh, 2007; Adanlawo and Ajibade, 2006). The green type is found in Southern guinea savanna while the brown type is prevalent in the Northern calyces of the red variety are used for the production of Zobo drink and soup, while the calyces of the green variety are used to cook soup, stew and sauces (Adanlawo and Ajibade, 2006).

This popular drink is called Zobo or Yakwua (Egbere *et al*., 2007) or Zoborodo (in Hausa), Iseipa (in Yoruba) and Sorrel in English (Adebayo-Tayo and Samuel, 2009), Jamaica flower, and karkade (Cid-Ortega and Guerrero-Beltrán, 2014). Some times Zobo could be spelled as Sobo. However, based on the name of this useful drink in different languages, the popular name it's known in Nigeria is derived from its Hausa name (Ezeigbo *et al*., 2015a, b). Zobo has gained prominence in several parts of the country and are sold in public places. Zobo is one of the nutritional drinks that are served during festivals and in a number of other ceremonies (Umaru *et al*., 2014) in different parts of Nigeria. The increased consumption of zobo is due to the nutritional, medicinal properties and low cost (Oboh and Elusiyan, 2004). At present, zobo drink is consumed by several millions of people cutting across different socio-economic classes in West African (Ogiehor and Nwafor, 2004).

Zobo drink is characterized by short shelf-life span of about 24 hours after production without refrigeration (Omemu *et al*., 2006; Onuoha and Fatokun, 2014; Nwachukwu *et al*., 2007). Most producers depend heavily on the use of preservatives. Electricity supply in which Zobo processors depend mainly on for refrigeration is grossly inadequate and often characterized by frequent power outage. On the overall, when electricity supply is low Zobo could easily be invaded by spoilage microorganisms. Food safety is an essential concern of both the consumers and the producers (Witkowska *et al*., 2013). Microorganism in food is not always detrimental, because their growth may result in pleasant taste and texture (Bukar *et al*., 2010). But microorganisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Vibro cholera, Salmonella, Bacillus, Clostridium* species etc. could contaminate food and transfer a wide range of disease conditions in food (Bukar *et al*., 2010). Also listed are *Pseudomonas, Saccharomyces, Rhizopus, Streptococcus, Bacillus, Erwinia, Aspergillus, Chromobacteria, Penicillium,* Guinea and Sudan savanna (Ilondu and Iloh, 2007). The *Fusarium, Flavobacterium, Xanthomonas, Enterobacter* species as microbes that could biodegrade food materials such as fruits and vegetables (Seiyaboh *et al*., 2013). Specifically, *Bacillus, Streptococcus, Staphylococcus, Leuconostoc, Lactobacillus, Aspergillus, Penicillium, Geotrichum, Fusarium* and *Alternaria* species are potential microbes that could deteriorate zobo drink (Bankole *et al*., 2013). At laboratory, studies have shown that *Bacillus, Aeromonas, Corynebacterium, Veilonella, Micrococcus, Pseudomonas, Streptococcus, Staphylococcus, Lactobacillus, Enterococccus, Escherichia, Proteus* (bacteria), *Aspergillus, Penicillium, Saccharomyces* (Fungi/yeasts) are the genera of microbes that cause spoilage of Zobo drink (Table 1) (Egbere *et al*., 2007; Ezearigo *et al*., 2014; Ezeigbo *et al*., 2015b; Omemu *et al*., 2006; Seiyaboh *et al*., 2013; Braide *et al*., 2012). Generally, contaminated ready–to-eat foods and drinks are the potential source of various food borne disease conditions especially the ones associated with gastroenteritis in human (Bello *et al*., 2014). This paper therefore focused on the preservatives used in enhancing the shelf-life of the Zobo drink.

Zobo drink preparations

Zobo drink is a non-alcoholic nutritive drink prepared from the dried calyces of Roselle (*H. sabdariffa*) (Umaru *et al.,* 2014; Seiyaboh *et al*., 2013; Adebayo-tayo and Samuel, 2009; Fasoyin *et al*., 2005; Adesokan *et al*., 2013; Ezearigo *et al*., 2014; Egbere *et al*., 2007; Braide *et al*. 2012; Amusa *et al*., 2005; Risiquat, 2013; Ayo *et al*., 2004), thus the flowers of *H. sabdariffa* (Nwafor and Ikenebomeh, 2009; Olawale, 2011). Zobo is a red liquid drink with fruit punch taste (Ezeigbo *et al*., 2015a). During zobo preparation, the dried calyces are hurled in water for 1- 2 hours and then allowed to cool prior to sieving (Umaru *et al*., 2014). A stricter soaking period of 10 – 15 minutes has been severally reported by authors (Nwachukwu *et al*., 2007; Ezeigbo *et al*., 2015a; Onuoha and Fatokun, 2014). The resultant filtrate is then consumed as hot tea or taken as a refreshing drink when chilled (Ezeigbo *et al*., 2015b). Thereafter preservatives/spices such as ginger (*Zingiber officinalis* and sugar are added before cooling in the refrigerator (Nwachukwu *et al*., 2007; Umaru *et al*., 2014).

Table 1: Microbial isolates found in Laboratory studies of Zobo drink

Reference	**(Ezearigo *et al*., 2014)**	**(Omemu *et al*., 2006)**		**(Braide *et al*., 2012)**	**(Seiyaboh *et al*., 2013)**	**(Egbere *et al*., 2007)**	**(Ezeigbo *et al*., 2015b)**
Microbes	-	Fresh	2 weeks	-	-	-	-
Bacteria	*B. megaterium, B. pumilus, B. marinus, B. sphaericus*	-	-	*B. subtilis*	*Bacillus sp*	*Bacillus sp*	*Bacillus sp*
	Pseudomonas sp	-	-	-	*Escherichia*	*E. coli*	-
	Aeromonas veronii	*S. fecalis*	-	*Enterococcus fecalis*	-	-	-
	Corynebacterium xerosis, C. kutscheri	-	-	*S. aureus*	-	*S. aureus*	*Staphylococcus sp*
	Veilonella sp.	*L. brevis*	-	-	*Proteus*	*Lactobacillus sp*	*Lactobacillus sp*
	Micrococcus luteus	*L. fermentum*	-	*M. luteus, M. roseus*	*Micrococcus sp*	-	-
Fungi	*A. flavus, A. niger, A. fumigatus*	*A. flavus*	*A. flavus*	*A. flavus*	-	*A. niger*	-
	-	*S. cerevisiae*	*S. cerevisiae*	*S. cerevisiae, S. elliposoideus*	-	*S. cerevisiae*	-
	-	-	-	*R. stolonifer*	-	-	-
	Geotrichum sp	-	-	*F. poae*	-	-	*Penicillum sp*
	-	-	-	*Mucor sp*	-	*Mucor sp*	
	-	-	*Candida krusei*	*P. caeseicolum*	-	-	-

A stricter soaking period of 10 – 15 minutes has been severally reported by authors (Nwachukwu *et al*., 2007; Ezeigbo *et al*., 2015a; Onuoha and Fatokun, 2014). The resultant filtrate is then consumed as hot tea or taken as a refreshing drink when chilled (Ezeigbo *et al*., 2015b). Thereafter preservatives/spices such as ginger (*Zingiber officinalis* and sugar are added before cooling in the refrigerator (Nwachukwu *et al*., 2007; Umaru *et al*., 2014). The choice of preservatives and flavor depend on the processors. After refrigerating, the nutritive drink is ready for consumption. Figure 1 presents the different preparation processes of zobo drink in Nigeria.

More recently, other plant materials are added as a blend in the preparation of zobo drink so as to improve the flavor and their shelf life. The sharp sour taste derived from the raw extract of *H. sabdariffa* is usually sweetened with sugar cane or granulated sugar, pineapple, orange or other fruits depending on choice (Ezearigo *et al*., 2014; Nwachukwu *et al*., 2007; Ezeigbo *et al*., 2015a; Onuoha and Fatokun, 2014; Osueke and Ehirim, 2004). Straw berry is another additives/ flavor used in zobo drink preparation (Egbere *et al*., 2007). The sweetness of zobo does not last probably due to the activities of spoilage microorganism (Nwachukwu *et al*., 2007). Some of these preservatives include garlic and ginger (Adesokan *et al*., 2013; Braide *et al*., 2012), lime (Nwachukwu *et al*., 2007; Ezeigbo *et al*., 2015b; Braide *et al*., 2012), clove, cinnamon and nutmeg (Ezearigo *et al*., 2014), pepper fruit (Ihemeje *et al*., 2013).

Preservatives used in the preparation of Zobo drink

Preservatives are chemical additives that are utilized to improve the shelf-life of food products so as to prevent or retard the growth of microorganisms such as bacteria and mould and prevent changes in the chemicals quality of the food such as color, taste, aroma and texture (Bankole *et al*., 2013). Spices are flavored or aromatic substances obtained from plants and are mostly used as food condiments (Singh *et al*., 2012). However, most spices are also used as preservatives and vice versa. Typically, preservatives are products or substances added to food products to sustain the physicochemical and microbial quality of the food. Several studies have been carried out on the suitability and sustainability of potential preservatives for used in zobo drink. These include physical treatment (freezing, refrigeration and pasteurization), chemical treatment (e.g. acetic acid and sodium benzoate) and biological treatment (several plant extracts).

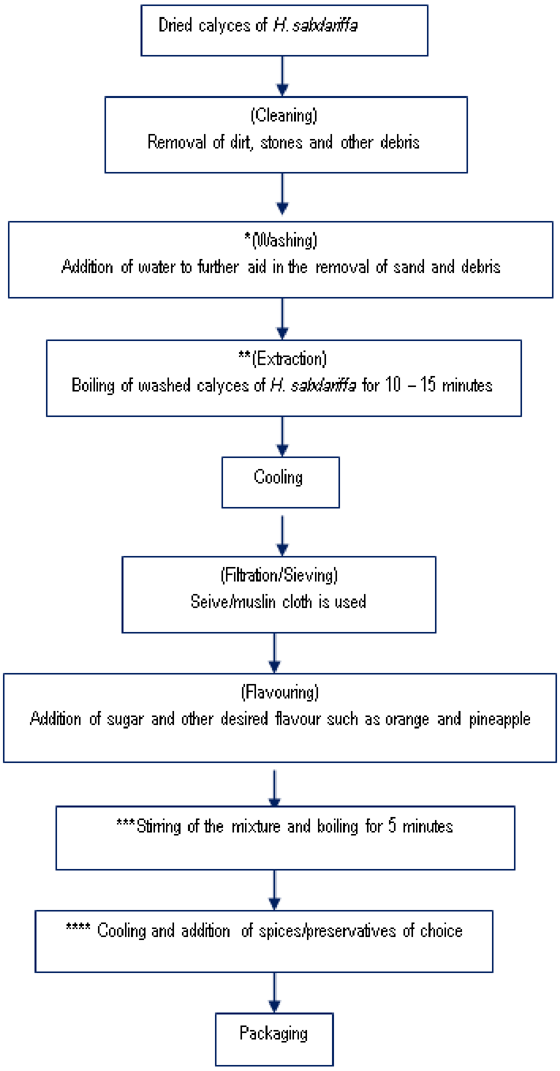

Figure 1: Zobo drink production pathway

(*some processors skip this route; ** some processors add natural spices/preservatives during initial boiling at stage 4 and skip step 8*** and 9****)

Physical and chemical methods

Zobo drink is usually treated with chemical preservatives such as sodium benzoates at varying concentrations. These are basically aimed at reducing the spoilage microorganisms and to enhance the shelf life of the products. Pasteurization is one of the physical methods employed in the preservation of nutritive drink such as zobo. Pasteurization of zobo drink by small holder processors is by immersion of the bottled zobo drink in sterile water and heated to ≤100ºC for few minutes. Other physical methods include freezing and refrigeration. Electricity supply in Nigeria is of low quality and is characterized by unplanned outage. In addition, less than 50% of the about 170 million Nigeria are connected to the national grid (Ohimain, 2014). As such most people rely heavily on gasoline and diesel to power generators. Hence the use of refrigeration as the sole source of preservation could be challenging since most Zobo processors are small-scale producers and this could result in increased cost of production.

Chemical methods used in the preservation of zobo drink are mainly sodium benzoates and acetic acids. The use of acetic acid from synthetic sources for food preservative has received worldwide restriction. Other potential chemical that could be used include sorbate and propionate. However, the use of chemical in the control of spoilage microorganisms could have negative effects on the nutritional composition of the drink especially in the loss of vitamins. Acetic acid produced from biomass via microbial methods such as oxidative fermentation and its derivatives has been recommended as best preservatives for food.

The role of acetic acid is preventing spoilage of Zobo drink is the elimination of spoilage organisms. Acetic acid have antimicrobial properties against *B. subtilis, B. megaterium, B. sphaericus, B. polymyxa, S. aureus E. coli, P. oxalicum, A. flavus, A. luchuensis, Rhizopus stolonifer, Scopulariopsis* and *Mucor* species (Pundir *et al*., 2010). Studies have shown that pasteurization, acetic acid and sodium benzoates act at different concentrations (Table 2) could reduce the microbial load of zobo drink and enhance the shelf life as reported by an author in zobo drink (Braide *et al*., 2012). Acetic acid appears to have a superior effect on bacteria than sodium benzoates. This is because no growth was observed at 24 hours of preservation of zobo drink using 2w/v of acetic acid (Braide *et al*., 2012). The same authors reported that sodium benzoate appears to have a superior effect on the fungi than acetic acid. This is because at 48 hours of preservation the fungi counts was too numerous to count for acetic acid and is in order of 10^{17} using sodium benzoates. Generally, the microbial load of zobo drink increases at the beginning of storage and when the nutrient has been exhausted, the growth begins to decline. This typically occurs between few hours to 4 days of storage depending on the concentration of preservatives and spices added during processing. A laboratory study carried out has showed that bacteria density of zobo drink without any treatment reduced by 90.83% after 3 days of storage. But the pasteurized sample decreased by 99.18% at 14 days of storage and samples pasteurized followed by the addition of 0.1% sodium benzoates decreased by 99.78% after over >40 days of storage (Egbere *et al*., 2007).

Typically, storage period influences the microbial load of the zobo drink. Also preservation of zobo drink reduces the nutritional content such as vitamin C. When zobo drink is pasteurized for 0 days the content decreased by 53.41% and by 14 days it further reduced by 60.37% (Egbere *et al*., 2007). Also when zobo is pasteurized and 0.1% sodium benzoates are added at 0 day the vitamin C content reduced by 60.46% and after 40 days it further declined by 70.34% (Egbere *et al*., 2007). On the microbial diversity, the type of preservation used is selective on the microbes. For instance, preserved zobo drink treated with different preservatives including sodium benzoates and pasteurization contain *M. lutens, M. roseus, B. subtilis, S. aureus, E. faecalis* (found in pasteurized samples), while *M. lutens* and *B. subtilis* were only found in samples treated with acetic acid, and *M. lutens, B. subtilis* and *S. aureus* were found in samples treated with sodium benzoates (Braide *et al*., 2012). Furthermore, fungi including *R. stoloifer, A. flavus, F. poae, Mucor* sp, *Saccharomyces cerevisiae, S. ellipsoideus, P. caseicolum* were found in pasteurized samples, and acetic acid treated samples lack only *F. poae* among the fungi diversity and *Mucor* sp, *Saccharomyces cerevisiae, S. ellipsoideus* were found in samples treated with sodium benzoates while the pasteurized samples contained all the type of fungi isolates found in the samples (Braide *et al*., 2012). However, it appears that sodium benzoates is the best chemical method for preserving zobo drink against fungi and yeasts while acetic acid is best against bacteria. Pasteurization only reduces the microbial load slightly. The efficacy of the pasteurization method could be due to short period of denaturation and low temperature i.e. 60 - 70ºC. It was noted that bacteria counts of sodium benzoates treated zobo drink preserved at ambient temperature and refrigerated at 4ºC fluctuates between the storage periods (Ezearigo *et al*., 2014). This reason for this fluctuation remains unknown.

Biological methods

Biological preservation of food is the best method of avoiding microbial food spoilage and contamination. Studies have shown that the use of synthetic chemical for food preservation could have adverse human health. Moreover they are expensive when compared to the financial/economic status of the average local processors (Nwachukwu *et al*., 2007). Some synthetic chemicals contain toxic, mutagenic, clastogenic and genotoxic compounds (Farag *et al*., 1989 cited in Omoruyi and Emefo, 2012). Due to these problems, research has focused on the use of natural products as potential preservatives to mitigate food borne pathogens. To these effects, natural spices have been widely used as food additives or flavor enhancer (Omoruyi and Emefo, 2012). Some of these plant extracts that are used as preservatives are also spices include kola nut or *Cola* species, lime, nutmeg, clove, ginger, garlic, cinnamon, pepper fruit etc.

Lime

Lime (*Citrus aurantifolia*) which belongs to the *Rutaceae* family is widely distributed in Nigeria and several other tropical countries. Typically, the sub-genus Citrus (Swingle) comprises of *C. aurantium, C. sinensis, C. reticulata* (*Tangerine*), *C. limon, C. aurantifolia, C. grandis, C. paradisi, C. indica* and *C. tachibans* etc (Nwauzoma *et al*., 2013; Piccinelli *et al*., 2008). *C. aurantifolia* have several medicinal properties. For instance, in traditional setting especially in rural area in Nigeria, *C. aurantifolia* is used for the treatment of several types of skin diseases. Other uses include antioxidants, throat and mouth wash. Typically, *C. aurantifolia* are rich in phytonutrients with health benefits. *C. aurantifolia* contain bioactive metabolites including alkaloids, flavonoids, phenols, saponins and tannins; vitamins including ascorbic acid, thiamine, riboflavin; and nutrients including

Table 2: Effects of physical and Chemical preservation methods on Zobo drink

Hours / Preservatives	Unspiced	Pasteurization	Acetic acid	Pasturization + 0.1% Sodium benzoate	Sodium benzoate	Sodium benzoate			References
	-	-	0.02% (2ml:98ml)	-	0.02% (2ml:98ml)	1%	0.1%	0.01%	
24	0.00	-	-	-	-	8.00 x10^{9}	0.00	5.00 x10^{8}	*(Ezearigo *et al*., 2014)
	TNTC	-	-	-	-	2.00 x10^{9}	7.00 x10^{9}	3.50 x10^{9}	**(Ezearigo *et al*., 2014)
	2.01 x10^{10}	0.00	0.00	-	5.1 x10^{10}	-	-	-	(Braide *et al*., 2012)
48	2.76 x10^{10}	2.31 x10^{10}	1.60 x10^{10}	-	TNTC	-	-	-	
72	4.64 x10^{10}	1.76 x10^{10}	TNTC	-	2.26	-	-	-	(Braide *et al*., 2012)
	5.0 x10^{9}	-	-	-	-	0.00	3.00 x10^{9}	3.00 x10^{9}	*(Ezearigo *et al*., 2014)
	1.1 x10^{9}	-	-	-	-	5.00 x10^{8}	0.00	0.00	**(Ezearigo *et al*., 2014)
96	5.2 x10^{9}	TNTC	3.0 x10^{8}	-	1.1 x10^{9}	-	-	-	(Braide *et al*., 2012)
120	9.4 x10^{9}	TNTC	1.8 x10^{8}	-	1.7 x10^{10}	-	-	-	(Braide *et al*., 2012)
144	5.2 x10^{9}	1.62 x10^{10}	6.6 x10^{9}	-	1.9 x10^{9}	-	-	-	(Braide *et al*., 2012)
168	8.6 x10^{9}	3.55 x10^{10}	4.1 x10^{9}	-	0.00	-	-	-	(Braide *et al*., 2012)
288	2.00 x10^{9}	-	-	-	-	2.00 x10^{9}	2.00 x10^{9}	4.00 x10^{9}	*(Ezearigo *et al*., 2014)
	0.00	-	-	-	-	0.00	2.00 x10^{9}	0.00	**(Ezearigo *et al*., 2014)
336	2.14 x10^{9}	3.0 x10^{9}	1.0 x10^{8}	-	0.00	-	-	-	(Braide *et al*., 2012)
	5.00 x10^{3}	9.80 x10^{2}	-	-	-	-	-	-	(Egbere *et al*., 2007)
432	2.00 x10^{9}	-	-	-	-	0.00	0.00	4.00 x10^{9}	*(Ezearigo *et al*., 2014)
	0.00 x10^{9}	-	-	-	-	1.00 x10^{9}	1.00 x10^{9}	1.00 x10^{9}	**(Ezearigo *et al*., 2014)
600	6.00 x10^{9}	-	-	-	-	2.00 x10^{9}	3.00 x10^{9}	TNTC	*(Ezearigo *et al*., 2014)
	3.00 x10^{9}	-	-	-	-	1.00 x10^{9}	3.00 x10^{9}	2.00 x10^{9}	**(Ezearigo *et al*., 2014)
	1.60 x10^{3}	-	-	2.80 x10^{2}	-	-	-	-	(Egbere *et al*., 2007)

*= samples are preserved in ambient temperature

** = samples are refrigerated at 4°C

TNTC= Too numerous to count

potassium, phosphorus, magnesium, sodium and calcium (Okwu, 2008).

The effects of lime on the preservation of zobo drink have been demonstrated (Table 3 and Table 4). For instance, lime as low as 0.1 concentrations could reduce the population of coliforms and total viable counts in Zobo drinks (Nwachukwu *et al*., 2007; Onuoha and Fatokun, 2014).

The ability of the lime to reduce the microbial load of the zobo drink could be due to the acidic nature of the lime. Most microorganisms cannot survive an acidic condition as such their survival in the zobo could be inhibited (Nwachukwu *et al*., 2007; Onuoha and Fatokun, 2014). Lime has lesser microbial load among other organic spices/preservatives such as garlic, ginger and clove (Braide *et al*., 2012). The authors also reported a lesser microbial population compared to sodium benzoate at the same concentration of preservatives added at 24 hours of preservation. But thereafter, the bacterial density was similar to other preservatives from 24 hours to 336 hours. On effect of lime on fungal spoilage organisms on zobo drink, higher fungi density have been reported among other organic preservatives from 24 hours to 48 hours and from 12 hours to 336 hours the growth was too numerous to count (Braide *et al*., 2012). The reduction in the population of microbial counts in zobo drink that lime was added to and completely control *S. aureus* and *F. poae* from the Zobo samples but other microbes including *M. lutens, M. roseus, B. subtilis, Enterobacter fecalis* (bacteria) *R. stoloifer, A. flavus, Mucor sp, Sacchraomyces cerevisiae, S. ellipsoideus, P. caseicolum* (fungi) still persist in the zobo drink (Braide *et al*., 2012**)**, though the population is lower than the sample without lime.

Nutmeg (*Myristica fragrans* Houtt.)

Nutmeg (*Myristica fragrans* Houtt) belongs to the *Myristicaceae* family. *M. fragrans* is the most common commercial evergreen tree native to Banda Islands in eastern Indonesia (Moluccas) and is found in Banda Island (Saxena and Patil, 2012; Pooja *et al*., 2012). Typically, nutmeg tree which grows slowly and could reach about 20 m in height and are mainly cultivated in India, Ceylon, Malaysia and Granada (Pooja *et al*., 2012; Sanghai-Vaijwade *et al*., 2011), Caribbean, South India, Sri Lanka, Sumatra, and Brazil (Pooja *et al*., 2012). When the fruit of nutmeg tree matures, it splits open and the stony endocarp or seed surrounded by a red, slightly fleshy aril are laid bare (Sanghai-Vaijwade *et al*., 2011). The resultant nut is then dried and called nutmeg. The dried kernel seed of nutmeg is broadly utilized as spice and active ingredient by the alternative medicine practitioners. The *M. fragrans* produced essential oil which is useful in the pharmaceutical sector and some perfume production industry (Saxena and Patil, 2012). The oil of *M. fragrans* is colourless or light yellow and is used in the oleochemical industry and natural food flavorings including baked foods, syrups, beverages and sweets (Saxena and Patil, 2012). Nutmeg has antioxidant and antimicrobial properties and contains active phytochemicals such as vitamins, carotenoids, terpenoids, alkaloids, saponins, flavonoids, lignans, terpernoids, phlobatanins, tannins, quionines and phenolics etc (Gupta *et al*., 2013; Kumari *et al*., 2014). Also *M. fragrans* essential oil contains secondary metabolites including steroids, tannins, alkaloids, flavonoids, phenolics and glycosides and lacks saponins (Saxena and Patil, 2012). Nutmeg has strong antioxidant potentials and a good preservative agent (Tan *et al*., 2013). Nutmeg contain active molecules including myristicin (main psychoactive constituent), macelignan (Lignans are a class of phytoestrogens having several potential pharmacological activities such as anticancer, anti-inflammatory, antimicrobial, antioxidative, and immunosuppressive activities), eugenol (4-allyl-2-methoxyphenol) which is mainly used as antiseptic, analgesic and antibacterial agent (Demeco, 2013).

Nutmeg have antimicrobial properties against *B. subtilis, S. aureus, P. putida, P. aeruginosa, A. fumigates, A. niger* and *A. flavus* (Gupta *et al*., 2013), *P. fluorescens* (Witkowska *et al*., 2013), *S. aureus* ATCC 26923, *E. fecalis* ATCC 2912 (Lawal *et al*., 2014), *Candida albicans* and *A. niger* (Pooja *et al*., 2012). Also, Omoruyi and Emefo (2012) reported that *M. fragrans* have antibacterial activity to both gram positive and gram negative microbes including *S. aureus*, *S. epidermidis*, *Klebsiella pneumoniae*, *B. cereus, E. coli, Salmonella typhi* and *P. aeruginosa.* Nutmeg have been tried as a possible preservatives of zobo drink but the bacteria growth at 0 days of preparation at ambient storage temperature were higher than that of other organic preservative such as ginger, garlic, cinnamon and even samples without preservatives (Table 4) (Ezearigo *et al*., 2014).

Garlic (*Allium sativum* L.)

Garlic (*Allium sativum*) which belongs to the family *Alliaceae*, is a common spice used to add flavor in food.

Table 3: Effects of different concentration of Lime on the microbial quality of zobo drink

Concentrations	0.0%	0.1%	0.5%	1.0%	1.5%	2.0%	References
Total coliform, cfu/ml x10^2	4.2	1.8	0.5	0.1	0.0	0.0	(Nwachukwu *et al*., 2007)
Total viable counts, cfu/ml x10^2	6.2	3.1	1.6	1.6	0.6	0.1	
Total coliform, cfu/ml x10^2	4.5	-	2.0	0.8	0.2	0.0	(Onuoha and Fatokun, 2014)
Total viable counts, cfu/ml x10^2	6.0	-	3.5	2.2	1.4	0.1	
Concentrations	6.25%	12.5%	25%	50%	75%	100%	
Total viable counts, cfu/ml x10^3	(1.0) [8.0] {14.0} <19.0>	(1.0) [4.0] {9.0} <12.0>	**[1.0] {4.0} <10.0>	{1.0} <4.0>	-	-	*(Ezeigbo *et al*., 2015b)
Total coliform, cfu/ml x10^3	[3.0] {9.0} <12.0>	[3.0] {6.0} <10.0>	{1.0} <8.0>	<1.0>	-	-	
Total fungi, cfu/ml x10^3	(3.0) [7.0] {1.0} <15.0>	(2.0) [4.0] {8.0} <13.0>	**[1.0] {4.0} <1.0>	<4.0>	-	-	

( )= day 1; [ ] = day 2 and 3; { } = day 4; < > = day 5

** = occurred only in day 3; *In Ezeigbo *et al*. (2015b), no total viable aerobic counts, coliform and fungi was observed for zobo drink with 75% -100% concentration of lime for the five days of storage. Also no microbial growth was observed when 50% and 25% of lime was added for first 3 days and 2days of storage respectively. No coliform growth was observed at 6.25 – 12.5% concentration of lime in day 1, 25% in day 2 and no coliform and fungi in 50% in day 4.

Garlic is an erect bulbous herb that reaches 30–60 cm tall, and possess strong smell when crushed (Enyi-Idoh *et al*., 2011). *A. sativum* is aromatic sulphur based compounds, which contribute to its odour and taste properties (Pundir *et al*., 2010). *A. sativum* contain bioactive constituents including terpernoids, glycosides (Youssef *et al*., 2013), flavonoids (Enyi-Idoh *et al*., 2011; Youssef *et al*., 2013), saponin, tannins and hydrocynaides (Enyi-Idoh *et al*., 2011). As such, *A. sativum* has antimycotic properties against *A. flavus*, *A. niger*, *A. ostianus*, *Alternaria alternate, Fusarium solani* and *C. albicans* (Youssef *et al*., 2013), *B. subtilis, B. megaterium, B. sphaericus, B. polymyxa, S. aureus, E. coli, P. oxalicum, A. flavus, A. luchuensis, Rhizopus stolonifer, Scopulariopsis* and *Mucor* species (Pundir *et al*., 2010). The antimicrobial properties of *A. sativum* to its key component allicin, which is a volatile molecule that gives garlic its odour (Pundir *et al*., 2010). Garlic is effective against heart disease, stroke and hypertension (Padhye *et al*., 2014).

Garlic has been severally used as a spice for the preparation of Zobo drink (Table 4). A lower microbial counts for ginger treated zobo drink as compared to the sample without any treatment (Braide *et al*., 2012; Adesokan *et al*., 2013). Specifically, garlic reduce the population of *M. lutens, M. roseus, S. aureus, B. subtilis, Enterobacter faecalis* and completely eliminate *R. stoloifer, A. flavus, P. caseicolum*, *F. poae* and reduces the population of *Mucor* species and do not have effect on the population of *S. cerevisiae, S. ellipsoideus* (Braide *et al*., 2012).

Ginger (*Zingiber officinale* Rosc.)

Ginger (*Zingiber officinale* Rosc), is a tropical and subtropical plant native to South East Asia from where it was introduced to other parts of the world (Lawal *et al*., 2014). Presently, it is extensively cultivated in Jamaica, Nigeria, China, India, Fiji, Sierra Leone and Australia (Bhargava *et al*., 2012). Ginger which belongs to the *Zingiberaceae* family, is one of the most widely utilized herbs used for food flavouring and it possesses medicinal properties (Lawal *et al*., 2014; Gaurav et al., 2013).

Ginger aids in stimulation of the heart and circulatory system its ability to reduce inflammation (Padhye *et al*., 2014). In addition garlic have hepatoprotective, nephroprotective, antioxidant, larvicidal, anti-diabetic, anti-diarrhea, anti-inflammatory, antifungal, antibacterial, anti-helminthes, cytotoxic and analgesic effects (Gaurav *et al*., 2013). Ginger has been widely employed for broad range of ailment including arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis (Ali *et al*., 2008). *Z. officinale* have variously reported to have antimicrobial properties including *S. aureus* 25923 MRSA (Lawal *et al*., 2014), *P. aeruginoa, E. coli, S. aureus* (Bhargava *et al*., 2012; Bello and Adeleke, 2012), *E. faecalis* (Bhargava *et al*., 2012), *K. pneumoniae, B. cereus, E. aerogenes and P. mirabilis* (Bello and Adeleke, 2012).

On nutritional and proximate composition, the moisture, protein, fat, insoluble fiber, soluble fiber, carbohydrate, total carotenoids, ash, iron, calcium, phosphorous, zinc, copper, chromium and manganese and vitamin C of garlic respectively as 15.02, 5.087g, 3.72g, 23.5%, 25.5%,38.35g, 79mg, 3.85g, 8.0 mg, 88.4 mg, 174 mg, 0.92 mg, 0.545 mg, 70 µg, 9.13mg and 9.33mg per 100 g of sample respectively (Shirin and Jamuna, 2010). Two varieties of *Z. officinale* are found in Nigeria i.e. white and yellow types (Ajayi *et al*., 2013). The nutritional composition (proximate, amino acids, and nutrients) of two varieties of garlic (white and yellow types) respectively include crude fiber (21.90 and 8.30%), fat (17.11 and 9.89%), carbohydrate (39.70 and 58.21%), crude protein (12.05 and 11.65%), ash (4.95 and 7.45%) and moisture (3.95 and 4.63%), calcium (0.68 and 0.41 ppm), magnesium (0.04 and 0.11 ppm), sodium (0.26 and 0.39 ppm), potassium (0.98 and 1.38 ppm), phosphorus (0.42 and 0.47 ppm), manganese (0.03 and 0.07 ppm), iron (0.29 and 0.14ppm), zinc (0.04 and 0.03 ppm), copper (0.01 and 0.02 ppm), lysine (2.70 and 15.90 mg/100 g protein), histidine (10.40 and 5.00 mg/100 g protein), arginine (41.40 and 26.80 mg/100 g protein), aspartic acid (29.80 and 31.60 mg/100 g protein), threonine (9.10 and 23.20 mg/100 g protein), serine (23.10 and 10.20 mg/100 g protein), glutamic acid (56.80 and 35.80 mg/100 g protein), proline (15.00 and 8.10 mg/100 g protein), glycine (22.60 and 17.10 mg/100 g protein), alanine (10.60 and 9.90 mg/100 g protein), cystine 4.60 and 4.60 mg/100g protein), valine (22.00 and 23.70 mg/100 g protein), methionine (5.70 and 4.70 mg/100 g protein), isoleucine (10.70 and 10.40 mg/100 g protein), leucine (42.00 and 56.00 mg/100 g protein), tyrosine (11.10 and 14.20 mg/100 g protein) and phenylalanine (10.00 and 27.40 mg/100 g protein). Garlic is also rich in tannins, flavonoids (Bhargava *et al*., 2012; Shirin and Jamuna, 2010), total polyphenol, oxidant activity (Shirin and Jamuna, 2010), alkaloid, phlobotannins, glycosides, saponins, and terpernoids (Bhargava *et al*., 2012). Also both white and yellow varieties of ginger contain saponin, anthraquinones, phlobatannin, glycoside general, and glycosides with steroidal ring (Ajayi *et al*., 2012).

Ginger is a major spice used in the production of Zobo drink. Typically, ginger reduces the microbial density of the zobo drink (Table 4) (Braide *et al*., 2012; Adesokan *et al*., 2013). Like garlic, ginger reduced the population of *M. lutens, M. roseus, S. aureus, B. subtilis, Enterobacter faecalis, R. stoloifer, A. flavus, F. poae* and *P. caseicolum*, but do not have effect on the population of *S. cerevisiae, S. ellipsoideus* (Braide *et al*., 2012). Typically, the ability of ginger to have effects on the microbial quality of zobo could be due to the presence of secondary metabolites found in them. Also blended ginger and garlic has superior effect on the bacterial density of zobo as when compared to separate blends (Adesokan *et al*., 2013).

Clove (*Syzygium aromaticum* L.)

Clove (*Syzigium aromaticum*) belongs to the family of *Myrtaceae*. Cloves are an evergreen aromatic herb that could reach 30 – 40 feet high with leathery textured leaves covered with many depressions (Bhowmik *et al*., 2012). Cloves thrive in tropical climates such as the Islands of Indonesia (Bhowmik *et al*., 2012). *S. aromaticum* is native to India, Indonesia, Zanzibar, Mauritious and Ceylon (Reji and Rajasekaran, 2015). The flower buds of the plant produced oil known as clove oil (Bhowmik *et al*., 2012; Ayoola *et al*., 2008). Clove oil contains ß-caryophyllene, representing 14-21% of its compounds, 10-13% of tannins as well as sesquiterpenes and phenols (Rodríguez *et al*., 2014). Also, phenylpropene is the most important part of clove oil, apart from eugenol, which is responsible for the scent properties of the plant and its main component (Rodríguez *et al*., 2014).

Table 4: Bacterial counts on organic preserved zobo drink

Hours / Spiced	Unspiced	Ginger	Garlic	Ginger and Garlic	Clove	Lime	Cinnamon	Nutmeg	*C. nitda*	*C. acuminata*	References
0	2.0 x10^{4}	-	-	-	-	-	-	-	2.0 x10^{4}	2.0 x10^{4}	***(Bankole *et al.*, 2013)
24	2.6 x10^{4}	3.0 x10^{3}	1.5 x10^{3}	1.2 x10^{3}	-	-	-	-	-	-	(Adesokan *et al.*, 2013)
	0.00	0.00	0.00	-	-	-	0.00	2.5 x10^{9}	-	-	*(Ezearigo *et al.*, 2014)
	TNTC	6.00 x10^{9}	2.50 x10^{9}	-	-	-	2.00 x10^{9}	2.50 x10^{9}	-	-	**(Ezearigo *et al.*, 2014)
	3.2 x10^{4}	-	-	-	-	-	-	-	1.8 x10^{4}	1.9 x10^{4}	***(Bankole *et al.*, 2013)
	2.01 x10^{10}	2.9 x10^{9}	1.7 x10^{10}	-	TNTC	2.3 x10^{8}	-	-	-	-	(Braide *et al.*, 2012)
48	2.76 x10^{10}	2.75 x10^{10}	2.5 x10^{10}	-	2.30 x10^{10}	TNTC	-	-	-	-	
	2.5 x10^{5}	-	-	-	-	-	-	-	1.5 x10^{3}	1.4 x10^{4}	***(Bankole *et al.*, 2013)
72	4.64 x10^{10}	1.92	2.60 x10^{10}	-	1.89 x10^{10}	2.24 x10^{10}	-	-	-	-	(Braide *et al.*, 2012)
	3.2 x10^{5}	-	-	-	-	-	-	-	1.8 x10^{2}	1.2 x10^{4}	***(Bankole *et al.*, 2013)
	5.0 x10^{9}	0.00	0.00	-	-	-	0.00	0.00	-	-	*(Ezearigo *et al.*, 2014)
	1.1 x10^{9}	0.00	1.50 x10^{9}	-	-	-	4.00 x10^{9}	0.00	-	-	**(Ezearigo *et al.*, 2014)
96	3.5 x10^{5}	3.7 x10^{3}	1.9 x10^{3}	1.3 x10^{3}	-	-	-	-	-	-	(Adesokan *et al.*, 2013)
	5.2 x10^{9}	2.03 x10^{10}	1.5 x10^{9}	-	TNTC	4.1 x10^{9}	-	-	-	-	(Braide *et al.*, 2012)
	4.2 x10^{5}	-	-	-	-	-	-	-	1.5 x10^{2}	1.0 x10^{3}	***(Bankole *et al.*, 2013)
120	9.4 x10^{9}	8.0 x10^{8}	2.12x10^{10}	-	1.0 x10^{10}	2.0 x10^{9}	-	-	-	-	(Braide *et al.*, 2012)
144	5.2 x10^{9}	2.90 x10^{10}	1.60 x10^{10}	-	9.9 x10^{9}	2.4 x10^{9}	-	-	-	-	(Braide *et al.*, 2012)
168	3.7 x10^{7}	5.5 x10^{6}	2.5 x10^{4}	2.6 x10^{4}	-	-	-	-	-	-	(Adesokan *et al.*, 2013)
	8.6 x10^{9}	4.6 x10^{9}	7.5 x10^{9}	-	6.6 x10^{9}	2.2 x10^{9}	-	-	-	-	(Braide *et al.*, 2012)
288	2.00 x10^{9}	4.00 x10^{9}	0.00	-	-	-	1.00 x10^{9}	3.00 x10^{9}	-	-	*(Ezearigo *et al.*, 2014)

	0.00	3.00 x10^9	5.00 x10^8	-	-	-	1.50 x10^9	2.50 x10^9	-	-	**(Ezearigo *et al*., 2014)
336	2.14 x10^9	2.31 x10^9	2.0 x10^8	-	3.0 x10^9	1.6 x10^9	-	-	-	-	(Braide *et al*., 2012)
432	2.00 x10^9	0.00	1.00 x10^9	-	-	-	0.00	0.00	-	-	*(Ezearigo *et al*., 2014)
	0.00 x10^9	2.00 x10^9	2.00 x10^9	-	-	-	0.00	1.00 x10^9	-	-	**(Ezearigo *et al*., 2014)
600	6.00 x10^9	5.00 x10^9	4.00 x10^9	-	-	-	4.00 x10^9	7.00 x10^9	-	-	*(Ezearigo *et al*., 2014)
	3.00 x10^9	3.00 x10^9	4.00 x10^9	-	-	-	3.50 x10^9	4.00 x10^9	-	-	**(Ezearigo *et al*., 2014)

In Braide *et al*. (2012) study the ratio of 1:50 was used as spice to Zobo drink

In Bankole *et al*. (2013) study the ratio of 1:10 was used as spice to Zobo drink

*= samples are preserved in ambient temperature

** = samples are refrigerated at 4ºC

***= *E. coli* counts only

Different parts of Clove plants (oils, dried flower buds, leaves, and stems) have several medicinal properties including expectorant, enhancing blood circulation and increasing body temperature slightly, the aromatic oil have stimulating and irritant effects, enhancing the flow of saliva and gastric juices, relieving chronic rheumatism, toothache, lumbago stomach pain, muscle cramps and some nerve conditions, nausea and vomiting, mouth and throat inflammation, diarrhea, hernia, and bad breath (Bhowmik *et al*., 2012). *S. aromaticum* also has diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment activity and condiment with carminative and stimulant properties as well as antibacterial and anti-inflammatory properties (Mishra and Sharma, 2014), antimicrobial effects against *E. coli*, *E. cloacae*, *K. pneumoniae*, *S. paratyphi* and *Citrobacter* sp, *S. aureus* ATCC 25923 and *C. albicans* (Ayoola *et al*., 2008), antimycotic properties against *A. flavus*, *A. niger*, *A. ostianus*, *Alternaria alternate, Fusarium solani* and *Candida albicans* (Youssef *et al*., 2013). Pundir *et al*. (2010) also reported that *S. aromaticum* have antimicrobial properties against *B. subtilis, B. megaterium, B. sphaericus, B. polymyxa, S. aureus E. coli, P. oxalicum, A. flavus, A. luchuensis, Rhizopus stolonifer, Scopulariopsis* and *Mucor* spp. Reji and Rajasekaran (2015) also reported that *S. aromaticum* can be used against *E. coli*, *P. mirabilis* and *K. pneumoniae*. *S. aromaticum* contain several metabolites including terpernoids, glycosides (Youssef *et al*., 2013; Mishra and Sharma, 2014), phylobatannin, tannins, saponins, sugars, steroids (Mishra and Sharma, 2014), flavonoids and coumarins (Youssef *et al*., 2013).

Clove has been studied as a preservative for zobo drink (Table 4). Braide *et al.* (2012) showed that at 24 hours of preservation, the bacterial density were higher than the control, but reduced from 48 – 72 hours before increasing again at 96 hours. The authors further reported that no fungal growth was observed at 24 hours and the population was lesser than the control throughout the period 24 – 336 hours (Table 4). It appears that clove has superior effects on the fungal spoilage organisms of zobo as compared to bacteria. Clove reduces the density of *M. lutens, B. subtilis, R. stoloifer, A. flavus, S. cerevisiae* and *S. ellipsoideus* moderately and scanty growth of *M. roseus, S. aureus, Enterobacter faecalis, Mucor sp* and *P. caseicolum*, and completely eliminate the growth of *F. poae* (Braide *et al*., 2012).

Cinnamon (*Cinnamomum zeylanicum* L.)

Cinnamon (*Cinnamomum zeylanicum* L.) which belongs to the *Lauraceae* family is an ever green plant that could reach 10-15 m tall and originated from Sri Lanka and South India (Reji and Rajasekaran, 2015). The flower of *C. zeylanicum* is green in color with distinct odour and arranged in panicles, while the berry fruit contain a single seed (Reji and Rajasekaran, 2015). Like most spices, *C. zeylanicum* can be used to treat diarrhea, and other digestive system upset; the plant also has antioxidant activity and the oil is effective against microbes, which help in some food preservation (Reji and Rajasekaran, 2015). Bioactive agents found in *C. zeylanicum* include alkaloids, flavonoids, terpernoids, glycosides, coumarins, tannins (Youssef *et al*., 2013). The seed, leaf, root-bark and stem-bark of *C. zeylanicum* contain phytosterols, phenols, tannins, saponins and terpernoids (Bernard *et al*., 2014). Reji and Rajasekaran (Reji and Rajasekaran, 2015) also reported that *C. zeylanicum* contain alkaloids, killer killani, quinine, steroids, tannins, terpernoids, carbohydrate and saponin as bioactive constituents. The authors further reported that the plant can be used against *E. coli*, *P. mirabilis* and *K. pneumoniae.* Youssef *et al*. (2013) reported that *C. zeylanicum* has antimycotic properties against *A. flavus*, *A. niger*, *A. ostianus*, *Alternaria alternata* and *Fusarium solani* and *Candida albicans*. Shareef (2011) also reported that cinnamon has antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *Brucella* and *Proteus* sp.

The potentials of cinnamon as a preservative for zobo drink has been studied (Table 4). Ezearigo *et al*. (Ezearigo *et al*., 2014) have demonstrated that the cinnamon has effects on the reduction in the microbial counts of Zobo which is similar to other preservatives such as ginger, garlic, nutmeg after 24 hours of preparation. The role of cinnamon in the reduction of microbial counts in zobo drink could be due to the presence of phytochemical ingredients found in the plant.

Kola nut

Kola nut has wide range of traditional uses in Nigeria especially during ceremonies. Kola nut has antioxidant properties. Typically Kola nut belongs to the *Sterculiaceae* family. Kola nut trees could reach 20 - 40 feet tall. The seed of kola nut are used as a condiment by Western and Central tropical African natives, as such it constitute essential commodity for trade in West Africa possibly due to their caffeine content (Bankole *et al*., 2013). Several species of Kola nut exist. Cola is sometimes spelled as Kola. In Nigeria about three species are edible including *Kola acuminate*, *Kola nitida* and *Kola verticilata*. The *K. nitida* and *K. acuminate* is often referred to red and white kola respectively (Muhammad and Fatima, 2014). Typically, Kola nuts constituents include caffeine (with tinge of theobromine), kolanin (combination of kola red and caffeine), glocuside, starch, sugar, fatty matter, tannins, oils, some anti-oxidants like phenolics, anthrocyanin and protein (Theimcgroup, Undated). Odebunmi *et al.* (2009) reported the nutritional and proximate composition of *C. nitida* as 66.0%, 33.60%, 5.71%, 2.63%, 1.50%, 7.13%, 28.56%, 3480.67 mg/kg, 124.40mg/kg, 392.00mg/kg, 16.43mg/kg, 5.24mg/kg, 411.43mg/kg for moisture, dry matter, crude fat, crude protein, ash, crude fibre, total carbohydrate, potassium, calcium, magnesium, iron, zinc and phosphorus respectively.

Kola nut products are energy boosters because of the caffeine content, but excess consumption could be deleterious to humans, which produces the following side effects, stomach upset**,** insomnia**,** high blood pressure (Theimcgroup, Undated). Despite the adverse effects it also have some benefits including helps in digestion**,** decrease high blood pressure**,** helps in weight loss, acts as pain reliever (Theimcgroup, Undated). Muhammad and Fatima (2014) reported that *C. nitida* possesses chemotherapeutic and antibacterial activities that could be useful against odontopathogens. Sonibare *et al.* (2009) reported that *C. acuminate* is effective against *S. albus, K. pneumonia, A. niger* and C. *albicans; C. ntida* effective against *C. albicans, A. niger and S. albus.* The Secondary metabolites found in fresh leaves *Cola acuminate*, *Cola nitida,* include alkaloids, saponins, tannins and cardenolides. Muhammad and Fatima (2014) reported that alkaloid, saponin, and glycosides, steroids, flavonoids, tannins, volatile oil and balsam as the bioactive constituents of *C. nitida*. Kanoma *et al.* (2014) also reported secondary metabolites found in *C. nitida* and *C. acuminate* such as alkaloid, tannins, glycoside, steroids and saponins glycoside.

Bankole *et al*. (2013) reported that *Kola acuminate* and *Kola nitida* could be used as preservatives in improving the shelf life of zobo drink. The author further reported that extract could reduce the *E. coli* count in Zobo drink from 2.0×10^4 cfu/ml (0 days) to 1.5×10^3 cfu/ml (2 days) and to 1.5×10^2 cfu/ml (4 days) (*Kola nitida*) and 2.0×10^4 cfu/ml (0 days) to

1.4 x 10^4 cfu/ml (2 days) and to 1.0 x 10^3 cfu/ml (4 days) (*Kola acuminate*) (Table 4). The trends in this studies showed that Kola species can be used as preservatives. However, *K. nitida* appear to have superior results than *K. acuminate* in the control of *E. coli* in Zobo drink during storage.

Pepper fruit (*Dennettia tripetala*)

Dennettia tripetala is commonly known as pepper fruit. The plant belongs to the *Annonaceae* family. It is found in West Africa and the fruit is edible. The plant is widely distributed in southern and to a lesser extent in the savanna regions of Nigeria. Typically, the fruit of *D. tripetala* is red and green when ripe and unripe respectively. Most parts of the plants such as leaves, fruit, bark and root possess strong pepperish and pungent spicy taste with aroma and fragrance properties (Ihemeje *et al*., 2013). *D. tripetala* is chewed in different perspectives including fresh (green), ripe (red), dried (black). *Dennettia tripetala* fruits and leaves are used as seasonings in food preparation such as meat, soup, fish etc.

According to Okwu and Morah (2004), *D. tripetala* fruits possess essential nutritive properties such as vitamins (ascorbic acid, thiamine, riboflavin and niacin), minerals (calcium, phosphorus, potassium, magnesium), trace element (iron, copper, zinc etc) and others such as total carbohydrate, moisture, crude protein, lipid, fibre. *D. tripetala* is a spicy indigenous medicinal plant (Elekwa *et al*., 2011). Elekwa *et al*. (2011) studied the nutritional properties of *D. tripetala* and reported the percentage of protein, moisture content, fat, ash, fiber, carbohydrate, calcium, magnesium, iron, phosphorus, potassium, sodium, ascorbic acid, niacin, thiamine, riboflavin and vitamin A contents on wet basis as 6.59%,15.26%, 5.52%, 4.13%, 17.05%, 51.45%,181.69mg/g, 229.78mg/g, 0.2mg/g, 285.8mg/g, 360.8mg/g, 6.12m/mg, 85.65mg/g,0.40mg/g, 0.10mg/g, 0.05mg/g and 65. 58mg/g respectively (unripe) and 4.67%, 18.73%, 5.78%, 3.18%, 14.32%, 53.32%, 138.94mg/g, 173.68mg/g, 0.23mg/g, 243.8mg/g, 324.27mg/g, 5.47mg/g, 115.57mg/g, 0.37mg/g, 0.08mg/g, 0.05mg/g and 388.10mg/g respectively (ripe). Also, *D. tripetala* seed is also rich in phytochemicals including saponins, flavonoids, tannins, cyanogenic glycosides, alkaloids, steroids, terpernoids and phenol (Ihemeje *et al*., 2013; Elekwa *et al*., 2011). Ihemeje *et al*. (2013) studied the potentials of *D. tripetala* seed as possible replacement of ginger in zobo drink. It is reported that there was no significant difference at 95% confidence interval in color, aroma, taste, mouth-fee and general acceptability of both category of the zobo drink blend. But information about the microbial quality of zobo drink blended with *D. tripetala* is scared in literature.

Conclusion

In the quest of overcoming the hurdles associated with the short-life span of zobo and the potential incidence of disease conditions attributed to the zobo drink spoilage microorganisms, studies have discovered several natural preservatives/spices of organic/natural origin that could reduce both microbial diversity and density of zobo spoilage organisms. Some of the widely utilized and potential spices include garlic, ginger, mixture of garlic and ginger, lime, clove, cinnamon, nutmeg, kola nut, pepper fruit. Though, these spices alongside with dried calyces of *H. sabdariffa* have antimicrobial properties and rich in phytonutrients including vitamins and minerals. Hence their ability to improve shelf life of Zobo mostly depends on the concentration added. The study found that the ability of the natural preservatives to wade off microorganisms appears to be in the order; lime > mixture of garlic and ginger > ginger > garlic > clove > kola nut > cinnamon > nutmeg. The activities of the spices due to differences in their biochemistry, nutrition, physiology and metabolism of the microbes. Therefore, attention should be focused on the carbonation of the drink after treatment with natural spices.

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