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Review

Natural spices and flavour substitution in *zobo* tea and drink production: A review

[1]*Nwankwo, C. S., [1]Ulu, F. O., [2]Okpomor, E. O., [3]Mbachiantim, J. T., [4]Okoyeuzu, C. F., [1]Carew, I. E., [5]Belay, D., [6]Ike, D. C. and [7]Teshome, A.

[1]*Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Benue, Nigeria*
[2]*International Centre for Biotechnology under the Auspices of UNESCO, University of Nigeria, Nsukka, Nigeria*
[3]*Department of Nutrition and Dietetics, Federal University of Agriculture, Makurdi, Benue, Nigeria*
[4]*Department of Food Science and Technology, University of Nigeria, Nsukka, Nigeria*
[5]*Department Food Process Engineering, Wolkite University, Ethiopia*
[6]*Department of Chemistry, Federal University of Agriculture, Makurdi, Benue, Nigeria*
[7]*Department of Chemical Engineering, Kombolcha Institute of Technology, Wollo University, Wollo, Ethiopia*

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Abstract

This paper reviews the effects of natural spices and flavour on the overall quality of *zobo* tea and drinks. *Zobo* is made from the calyces of *Hibiscus sabdariffa*, also known as roselle. *Zobo* tea and drinks are rich in nutrients (carbohydrate, fibre, ash, vitamins A and C), minerals (potassium, magnesium, sodium, calcium, and iron), and bioactive compounds (organic acids, anthocyanins, flavonoids, and phenolic acids). These components make them predisposed to microbial proliferation. However, nutrient composition and product quality vary with the variety of raw materials used and methods of production. *Zobo* can be consumed unsweetened or sweetened with honey, maple syrup, sugar cane, or fruit juice extracted from orange, pineapple, apple, or strawberry. Spoilage activities of microorganisms can be minimised with spices when they are used in place of chemical flavouring and preservatives. Several natural preservatives or spices of organic or natural origin could improve the quality attributes of *zobo* tea and drinks, and reduce both microbial diversity and density. Some of the widely utilised and potential spices include garlic, ginger, mixture of garlic and ginger, lime, clove, cinnamon, nutmeg, kola nut, and pepper. These spices, along with the dried calyces of *H. sabdariffa*, have antimicrobial properties, and are rich in phytonutrients, including vitamins and minerals. The overall effects are highly dependent on the biological composition of natural additives and the synergistic or antagonistic effects between roselle calyces and the additives used. If properly packaged, *zobo* tea can be kept longer than *zobo* drinks, thus resulting in a product with longer shelf life. The use of natural additives in *zobo* production would go a long way towards enriching both the *zobo* tea and drinks, and subsequently replenishing the lack of nutrients.

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Introduction

In Nigeria, *zobo* drinks are locally made as non-alcoholic beverages that are consumed by people of all socioeconomic strata (Olayemi *et al.*, 2011; Izah *et al.*, 2016). The name *zobo* is of Hausa origin from the northern parts of Nigeria, where it gained its popularity. *Zobo* drink is also called "yakwua" or *zoborodo*" in Hausa, *"aukan"* in Igbo, "*ishapa*" in Yoruba, "*bissap*" in Senegal, "*karkade*" in Sudan, "*da Bilenni*" in Mali, "*drink of the Pharaohs*" in Egypt, "*karkanji*" in Chad, "*wonjo*" in Gambia, "*meiguiqie*" in China, "*krajeap*" in Thailand, "*rosella*" in Australia, "*flor de Jamaica*" in Mexico, "*cranberry*" in Florida, and "*sorrel drink*" in other western parts of the world (Olayemi *et al.*, 2011; Izah *et al.*, 2015; Mohammed *et al.*, 2017; Salami and Afolayan, 2020; Adebayo *et al.*, 2021). The various names of the drink reflect its popularity and worldwide acceptance (Adebayo *et al.*, 2021).

Roselle, which is known scientifically as *Hibiscus sabdariffa*, belongs to the family Malvaceae (Izah *et al.*, 2015; Adeoye *et al.*, 2018). *Zobo* is obtained by steeping, boiling, and extraction or

*Corresponding author.
Email: toteupstar@yahoo.com

filtration of the flower's calyx in water (Braide *et al.*, 2012; Adeoye *et al.*, 2018). It could be consumed as hot liquor or cooled, refrigerated, and served chilled for consumption as a refreshing drink. Due to the numerous benefits of *zobo*, lots of research exist on its nutritional values, medicinal properties, microbial quality, and is sensory characteristics. *Zobo* is consumed as an alternative to carbonated drinks and alcohols and considered a healthy drink, especially when consumed without sugar (Obi, 2015) because it is rich in vitamins, minerals, and antioxidants. Vitamins A and C, phenols, potassium, sodium, and phosphorus were all found to be abundant in *zobo* (Egbere *et al*., 2007). *Zobo* is also believed to have medicinal properties, and has low glycaemic index when not sweetened with sugar (Adeniji, 2017; Salami and Afolayan, 2020). *Zobo* contains dietary fibres and phytochemicals necessary for maintaining an ideal body weight, and also for optimal digestion (Akujobi *et al.,* 2018).

Roselle calyces are a good source of phenolic compounds and anthocyanins and are used to make juice, wine, herbal tea, and as colouring for jellies, jams, beverages, and meals (Gbadegesin *et al.*, 2017). According to Salami and Afolayan (2020), *zobo* contains major bioactive components, including anthocyanins and flavonoids, which have several pharmacological functions. Daphniphylline has been discovered as the primary pigment formerly known as hibiscin. There are also trace amounts of delphinidin 3-monoglucoside (myrtillin), cyaniding-3-monoglucoside (chrysanthemin), and delphinidin (Gbadegesin *et al.*, 2017). The two principal anthocyanin compounds found in *zobo* calyx are delphinidin-3-sambubioside and cyaniding-3-sambubioside (Adebayo *et al*., 2021). Some of the beverage's acclaimed medicinal properties include antihypertensive, diuretic, cough, scurvy, and cancer treatment (Obi, 2015). Other than its nutritional and health benefits, dried roselle flowers are easily accessible in most communities, and *zobo* drink is easy to produce and affordable (Izah *et al.*, 2015; Adeniji, 2017). However, the drink's rapid deterioration rate at ambient temperature without preservation is the greatest drawback and limitation of large-scale production of the drink (Braide *et al.*, 2012). If not refrigerated, *zobo* drink has a shelf life of 24 - 26 h after manufacturing at ambient temperature, and approximately three days post-production if refrigerated (Omemu *et al*., 2006; Izah *et al.*, 2015; Adeoye *et al.*, 2018). The drink's short shelf life diminishes its capacity to impact long-term wellness for consumers. Most of the deterioration that occurs may be attributed to nutritional deterioration induced by spoilage microorganisms and fermentation of the drink (Izah *et al*., 2016). Although the majority of the isolates detected in *zobo* drinks relate to food, the high acidity of the juices may give an explanation for the low quantity and few species of microorganisms isolated (Omemu *et al.*, 2006). Several fungal and bacterial groups (*Aspergillus flavus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Bacillus subtilis*, and *Staphylococcus aureus*) that have been associated with *zobo* drink during storage, have devastating health implications ranging from food intoxication / poisoning to death (in worst case scenarios). *Zobo* tea, a formulation of dehydrated *zobo* extract, could be kept longer if properly packaged (Mohammed *et al*., 2017).

Various spices have been used in food preparation due to their aroma and certain preservative qualities (Obi, 2015). In *zobo* production, ginger (*Zingiber officinalis*), garlic (*Allium sativum*), and clove (*Syzigium aromaticum*) are the most popularly used spices (Izah *et al.*, 2016). Other spices that have been used include nutmeg (*Myristica fragrans*), cinnamon (*Cinnamomum zeylanicum*), pepper (*Dennettia tripetala*), lime (*Citrus aurantifolia*), and kola nut (Ezearigo *et al.*, 2014; Izah *et al.*, 2016). Obi (2015) experimented on the preservative effect of Nigerian local spices like *uziza* (*Piper guineense*), *uda* (*Xylopia aethiopica*), *ehuru* (*Monodora myristica*), and *ehu* (*Aistonei boonei*) on *zobo*. Overall, the use of spices improves the nutrient content, flavour, and health benefits of *zobo* (Izah *et al.*, 2015).

There has been research on using sodium benzoate and acetic acid to make *zobo* drinks last longer (Braide *et al*., 2012; Izah *et al*., 2016). However, with growing concerns about the adverse effects of chemical preservatives (Mohammed *et al*., 2017; Adeoye *et al.*, 2018), the use of natural spices as potential antimicrobials and preservatives has been investigated (Adeoye *et al.*, 2018; Salami and Afolayan, 2020). Microorganisms associated with *zobo* drinks are bacteria (*Staphylococcus, Escherichia, Proteus, Pseudomonas, Salmonella, Shigella, Enterobacter, Klebsiella, Serratia, Bacillus, Streptococcus, Lactobacillus, Clostridium, Corynebacterium, Aeromonas, Micrococcus, Stenotrophomonas*, *Leucobacter*, *Pantoea*) and fungi (*Aspergillus*, *Saccharomyces*, *Penicillium*, *Candida*, *Rhizopus, Fusarium, Mucor, Geotrichum)* (Izah *et al.*, 2015; Adeoye *et al.*, 2018). Salami and Afolayan (2020) found that a *zobo* drink made with lime extract had a lower microbial load than a *zobo* drink without it. This could be due to its acidic nature, which inhibits the survival of many pathogens while promoting the growth of acidophiles. Izah *et al.* (2016) reported that both ginger and garlic had antibacterial properties, but that combining the two spices in *zobo* had a greater effect than using them separately, while Adeoye *et al.* (2018) reported that despite the fact that ginger extracts are effective against both Gram-positive and Gram-negative bacteria, the combination of clove and ginger is more effective in suppressing microbial activity than either of the spices when they are used alone in *zobo*. Braide *et al.* (2012) stated that lime was the most effective in reducing microbial activity in *zobo* as compared to garlic, ginger, and clove. The ability of natural spices to inhibit pathogenic growth was found to be in this ascending order: nutmeg, cinnamon, kola nut, clove, garlic, ginger, ginger + garlic, lime (Izah *et al.*, 2016). Obi (2015) reported that *zobo* drinks treated with *ehuru* had no microbial growth, and were better than those treated with ginger, after six days. Due to the numerous benefits of *zobo* drinks, this paper reviews the effects of fruit and spice extracts on the overall quality of *zobo* tea and drinks.

Zobo drink

The calyx of the red variety of *Hibiscus sabdariffa* is used in *zobo* drink and tea production, while the green variety is used to prepare stews, soups, and sauces (Izah *et al.*, 2015). According to Olayemi *et al.* (2011), the three types of *Hibiscus sabdariffa* used in *zobo* drink preparation are the dark red, bright red, and wine-coloured varieties, while the leaves are used in soup preparations, and the oil from the seeds is considered a source of nutritional minerals. The leaves could also be used to produce syrup, jelly, jam, and gelatine, while the seeds are a good source of oil (Akujobi *et al.*, 2018). Although *zobo* tea and drinks are rich in nutrients, minerals, and bioactive compounds, which make them predisposed to microbial proliferation, their nutrient composition and quality vary with the variety of raw materials used and methods of production (Olayemi *et al.*, 2011). Generally, *zobo* drink has a tangy / sour profile, with low pH as the plant is naturally acidic, and contains glycosides, alkaloids, tannins, polyphenols, saponins, sterols, and phenols, as well as oxalic, tartaric, malic, and succinic acids (Bola and Aboaba, 2004; Builders *et al.*, 2010; Olayemi *et al.*, 2011; Salami and Afolayan, 2020).

Zobo drink is made using locally available technology from the acid-succulent roselle plant calyx (Adeniji, 2017) by aqueous extraction in preferred solid-solvent ratios (Izah *et al.*, 2015). Production processes for *zobo* production are very crude and not mechanised. It needs to be fully standardised and regulated (Braide *et al.*, 2012; Obi, 2015). Experimentation with various plant materials included as a blend in *zobo* production is now frequent, with the goal of improving the drink's nutritional quality, flavour, and shelf life. Honey, maple syrup, or sugar cane are used to sweeten the harsh sour flavour of *Hibiscus sabdariffa* raw extract (Salami and Afolayan, 2020), or with fruit juice of orange, pineapple, apple, or strawberry (Fasoyiro *et al.*, 2005; Egbere *et al.*, 2007; Adeniji, 2017; Akujobi *et al.*, 2018).

Zobo tea

In an effort to increase the shelf life, convenience, and acceptance, and overcome the limitations of large-scale production and distribution of *zobo*, novel *zobo* tea is being investigated and developed. Infusion, dehydration, and size reduction processes were used to create an instant *zobo* drink / tea combination by Mohammed *et al*. (2017). The calyx was cleaned, sorted, and infused with water at about 72°C for 90 min, then dehydrated at 72°C for 5 h and 45 min before being cooled and pulverised to instant powder, which was subsequently mixed with pulverised granulated sugar. To obtain the beverage, the instant powder was directly mixed with water and reconstituted. Other methods suggested by the researchers for *zobo* tea production include extraction, concentration, and dehydration by freezing or spray-drying to reduce the loss of nutrients. Ibeabuchi *et al.* (2019) formulated a *zobo* mix recipe of dried roselle calyx powder, moringa leaf powder, dried ginger, and clove powder, together with orange flavouring. The *zobo*-moringa mix was packaged in teabags and reconstituted by extraction using hot water. *Zobo* tea, a formulation of dehydrated *zobo* extract, could be kept for longer, thus giving the product an extended shelf life if properly packaged. The use of tea bags in *zobo* tea packaging would reduce quality loss, contamination, and discoloration while allowing for large-scale production and storage with optimal nutritional value retention (Ibeabuchi *et al*., 2019). The International Organization for Standardization (ISO) has established processes for infusing calyces to make *zobo* tea (Salami and Afolayan, 2020).

Natural spice and its importance in zobo tea and drink production

As a result of the search for natural sources of antimicrobial additives that are harmless and effective when used in foods, extracts and essential oils from spices, herbs, and other plants have been studied, and their antimicrobial activities have been reported (Youssef *et al*., 2013). Common everyday spices have been used to enhance the flavour and aroma of foods for centuries. Spices are aromatic or strong-tasting plant substances that are native or exotic in origin, and are used to enhance the flavour of foods. Leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stems (cinnamon), rhizomes (ginger), and other plant parts can be used as spices (Pundir *et al*., 2010; Izah *et al*. 2016). Obi (2015) also reported that apart from spices being used as flavour enhancers, some spices are traditionally used as food preservatives. Food preservatives are any chemicals or ingredients added to food to aid the retention of its physicochemical and microbial quality over a longer period of time than is naturally possible. With growing concerns about the adverse effects of chemical additives, the use of natural spices as potential antimicrobials and preservatives has been investigated (Egbere *et al*., 2007; Nwachukwu *et al*., 2007; Omoruyi and Emefo, 2012; Braide *et al*., 2012; Adesokan *et al*., 2013; Adeoye *et al.,* 2018; Mahomoodally *et al*., 2018; Salami and Afolayan, 2020). Alkaloids, phenolics, flavonoids, tannins, and saponins are secondary metabolites that are accountable for herbal plants' medicinal and antimicrobial properties (Youssef *et al*., 2013).

Fruit juice extract has also been used in place of artificial flavours in food production (Fasoyiro *et al.*, 2005; Akujobi *et al.*, 2018). Moreover, spices and fruits are readily available and inexpensive; also, some synthetic / chemical food flavourings contain compounds that are toxic, mutagenic, carcinogenic, and genotoxic (Izah *et al.*, 2016). Some plant additives with applications in *zobo* drink production are highlighted in the present review, giving bearing to expectations for their application in *zobo* tea powder production.

Clove

Clove (*Syzigium aromaticum)* contains several metabolites, including terpenoids, glycosides, phylobatannin, tannins, saponins, sugars, steroids, flavonoids, and coumarins (Youssef *et al*., 2013; Izah *et al.*, 2016). Clove oil, which is produced from the plant's flowers, contains ß-caryophyllene, which represents 14 - 21% of its compounds and 10 - 13% of tannins, as well as sesquiterpenes and phenols. Apart from eugenol, which is a major component of clove oil, and responsible for the plant's scent, phenylpropene is the most important component (Izah *et al.*, 2016). Clove buds and essential oils have been known to have antimicrobial and antioxidant properties, with the major constituents being eugenol acetate, eugenol, and caryophyllene, with the latter two having antibacterial and antifungal properties (Pundir *et al*., 2010). Clove has diuretic, odontalgic, stomachic, tonicardiac, and aromatic condiment activities, as well as carminative, stimulant, antibacterial, and anti-inflammatory properties (Izah *et al*., 2016). Clove has antibacterial effects against *Escherichia coli*, *Escherichia cloacae*, *Klebsiella pneumoniae*, *Salmonella* Paratyphi, *Citrobacter* sp., and *Staphylococcus aureus* ATCC 25923 (Ayoola *et al*., 2008), and antifungal effects against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ostianus*, *Fusarium solani* and *Candida albicans* (Youssef *et al*., 2013). Pundir *et al.* (2010) reported that clove has effects against *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus sphaericus*, *Bacillus polymyxa*, *Staphylococcus aureus*, *Escherichia coli*, *Penicillium oxalicum*, *Aspergillus flavus*, *Aspergillus luchuensis*, *Rhizopus stolonifer*, *Scopulariopsis,* and *Mucor* sp.

Braide *et al*. (2012) studied clove as a preservative for *zobo* drinks. They reported that bacterial density was higher than control after 24 h of production, dropped between 48 - 72 h, and then increased again after 96 h. After 24 h, no fungal growth was observed, and the population was lower than the control throughout the 24 - 36 h period, according to the authors. Clove appeared to have a better effect on the *zobo* fungal population than on the bacterial population. Clove significantly lowered the density of *Micrococcus roseus*, *Staphylococcus aureus*, and *Enterococcus faecalis* bacteria; moderately lowered the growth of *Rhizopus stoloifer*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, and *Saccharomyces ellipsoideus* fungi; only slightly lowered the growth of *Mucor* spp. and *Penicillium* *caseicolum* fungi, while entirely eliminating *Fusarium poae* fungus (Braide *et al*., 2012). Adeoye *et al.* (2018) also reported that 0.25% clove and above in *zobo* drink had inhibitory effects for up to 16 days of storage at ambient temperature after pasteurisation, with better inhibition against fungal colonies observed. Reports from Youssef *et al.* (2013) showed very high antifungal activity of clove oil extracted with *n*-hexane on *Aspergillus ostianus* and *Alternaria alternata* after 72 h of inoculation. In comparison to other medicinal plants evaluated, ethanol-extracted clove oil had better inhibitory effects against the tested fungi but had the most impact against *Alternaria alternata.* The use of *Moringa oleifera* extract for the enrichment of *zobo* drink produced with ginger, garlic, and clove impacted on its overall sensory acceptability. The contents of the enriched *zobo* drinks improved. They contained vitamins A and C, iron, manganese, magnesium, and phosphorus contents. However, *Moringa* enhanced the growth of microorganisms in *zobo* drinks, hence the need to identify alternative natural preservative methods to increase the shelf life of *zobo* drinks if the goal of commercialisation is to be achieved (Bassey *et al*., 2020).

Ginger

Ginger (*Zingiber officinale*) is one of the most widely used herbs for food flavouring and health benefits, as well as a key ingredient in *zobo* production, and belongs to the family Zingiberaceae (Izah *et al*., 2016). Bioactive compounds in ginger include tannins, flavonoids, alkaloids, phlobotannins, glycosides, saponins, and terpenoids. Bello and Osho (2012) reported 42.31% tannin, 5.04×10^{-5}% saponin, 28.60% alkaloid, and 0.78% total phenol in ginger. Ginger's ability to reduce inflammation helps to stimulate the heart and circulatory system; it also has hepatoprotective, nephroprotective, antioxidant, larvicidal, antidiabetic, antidiarrheal, anti-inflammatory, antifungal, antibacterial, antihelminthic, cytotoxic, and analgesic properties (Izah *et al*., 2016). As such, ginger is commonly used to treat ailments such as arthritis, rheumatism, sprains, muscular aches and pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases, and helminthiasis. According to Izah *et al.* (2016), *Zingiber officinale* has been found to have antimicrobial properties against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Enterobacter aerogenes*, and *Proteus mirabilis*. Braide *et al*. (2012) reported the high microbial effects of ginger against *Micrococcus roseus*, *Staphylococcus aureus*, and *Enterococcus faecalis* bacteria, and *Rhizopus stoloifer*, *Aspergillus flavus*, *Mucor* spp., and *Penicillium caseicolum* fungi. The presence of secondary metabolites in ginger is thought to be responsible for its ability to influence the microbial quality of *zobo*.

Adeoye *et al.* (2018) reported that 0.25% and above ginger in *zobo* drink had inhibitory effects for up to 16 days of storage at ambient temperature after pasteurisation, with better inhibition against bacterial colonies observed. As compared to when ginger and garlic were used separately, the combined effect on bacterial density of *zobo* was superior (Adesokan *et al*., 2013; Izah *et al.*, 2016). Also, a blend of ginger and clove had higher inhibitory effects than when used individually (Adeoye *et al.*, 2018). Ogiehor *et al*. (2008) reported that extracts of local spices (ginger), alone or in combination with low temperature storage (refrigeration), extended the shelf life of *zobo* beverages for a minimum of six weeks, and contributed to the overall quality and acceptability. In addition, the findings are useful in developing measurable and reliable indices for the production, processing, and handling of *zobo* beverages.

Garlic

Garlic (*Allium sativum*), like ginger, belongs to the family Alliaceae, and is a common spice used to flavour meals. Garlic is a bulbous, erect herb that grows 30 - 60 cm tall, and has a strong odour when crushed, thanks to aromatic sulphuric chemicals that are responsible for its odour and flavour (Pundir *et al*., 2010). Garlic has been shown to reduce the risk of heart disease, stroke, and high blood pressure (Izah *et al.*, 2016). Terpenoids, glycosides, flavonoids, saponins, tannins, and hydrocynaides are bioactive substances found in garlic (Youssef *et al*., 2013; Izah *et al.*, 2016). Bello and Osho (2012) reported 40% tannin, 4.87×10^{-5}% saponin, 26.60% alkaloid, 0.68% phenol, and 124.95% phytate in garlic.

The antimicrobial properties of garlic are largely attributed to allicin, a volatile compound responsible for garlic's pungent odour (Pundir *et al*., 2010). It has antiviral and antibacterial properties, and recent applications against congestion, bronchitis, and cold symptoms, and protection against re-infection (Bello and Osho, 2012). Garlic has traditional and medicinal applications as an anti-infective agent. Garlic has antimycotic properties against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ostianus*, *Alternaria alternata*, *Fusarium solani*, and *Candida albicans* (Youssef *et al*., 2013), *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus sphaericus*, *Bacillus polymyxa*, *Staphylococcus aureus*, *Escherichia coli*, *Penicillium oxalicum*, *Aspergillus flavus*, *Aspergillus luchuensis*, *Rhizopus stolonifer*, *Scopulariopsis*, and *Mucor* sp. (Pundir *et al*., 2010). At various times, garlic has been utilised as a spice in the production of *zobo*. According to the literature, *zobo* drink treated with garlic had lower microbial counts than a control sample (Braide *et al*., 2012; Adesokan *et al*., 2013). Braide *et al.* (2012) reported a reduction in the population of *Micrococcus luteus*, *Micrococcus roseus*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis* bacteria, and the complete elimination of *Rhizopus stoloifer*, *Aspergillus flavus*, *Penicillium caseicolum*, and *Fusarium poae* fungi, in *zobo* treated with garlic. Reports from Youssef *et al.* (2013) show high inhibition of garlic essential oil extracted with *n*-hexane on *Aspergillus* sp., *Alternaria alternata*, and *Fusarium solani*, while Pundir *et al*. (2010) reported maximum activity of the spice against *B. subtilis*.

Tamarind

Dialium guineense, the African black velvet tamarind, is a huge tree that can be found throughout Africa, including west Africa, the Central African Republic, and Chad. The tree belongs to the family Fabaceae - Caesalpinioidaea, and can reach a height of 30 meters. It has a dense, leafy crown, is typically shrubby, and its leaves are broadly elliptic, blunt at the tip, leathery, and have a depressed midrib. It has pale flowers on horizontally spread branches that grow wild fruits (Osanaiye *et al*., 2013). Tamarinds are typically round and flattened in shape, black in colour, with a 6 mm long stalk, a small collar near the apex, and a bristle shell enclosing a dry, brownish / orange edible pulp. It is locally referred to as "*tsamiyarkurmi*" in Hausa (Abd El-Ghani, 2016), and as "*icheku*" in Igbo. Wild tamarind is used as a dietary supplement for Nigerians living in rural areas, and in medicinal cures, as a source of vitamin C, and as flavouring for snacks and non-alcoholic beverages (Osanaiye *et al.*, 2013). The bark and leaves of the tree are used to treat a variety of diseases, including malaria, bronchitis, cough, and as a diuretic (Abd El-Ghani, 2016). The leaves can be crushed and used on wounds, while the pulp is used as a traditional cure for diarrhoea and stomach discomfort. Based on a study carried out on the proximate composition of African tamarind by Osanaiye *et al.* (2013), the fruit pulp was found to have a high level of micronutrients such as sodium, magnesium, and potassium, as well as high amounts of vitamin C. The values obtained by the researchers are as follows: moisture, 10.53%; dry matter, 88.40%; ash, 12.52%; organic matter, 41.55%; crude fat, 5.34%; crude fibre, 1.05%; carbohydrate, 58.65%; protein, 3.94%; nitrogen, 0.65%; magnesium, 0.40 mg/L; sodium, 2.88 mg/L; iron, 1.43 mg/L; calcium, 0.35 mg/L; and potassium, 1.21 mg/L. Despite its nutritional composition and potential as a beverage additive, there is currently no research on tamarind-flavoured *zobo*.

Lime

Citrus aurantifolia is a member of the family Rutaceae. Nigeria and numerous other tropical countries have a large population of this species. *C. aurantium* (bitter orange), *C. sinensis* (sweet orange), *C. reticulata* (tangerine), *C. limon* (lemon), *C. grandis* (pomelo), *C. paradisi* (grape), *C. indica* (India wild orange), and *C. tachibans* (mandarin) are other species of this genus (Izah *et al.,* 2016). Lime juice, together with its peels, has been used in *zobo* production (Salami and Afolayan, 2020). Akujobi *et al.* (2018) experimented with substituting *zobo* with sweet orange. Lime has a variety of therapeutic characteristics that are used to treat a variety of skin disorders, as well as antioxidants, throat, and mouthwash. Lime is high in phytonutrients which provide numerous health advantages. Alkaloids, flavonoids, phenols, saponins, and tannins are among the bioactive substances found, as well as vitamins such as ascorbic acid, thiamine, and riboflavin, also minerals such as potassium, phosphorus, magnesium, sodium, and calcium (Izah *et al*., 2016). The acidic nature of lime may contribute to its ability to reduce the microbial load of *zobo*. Many pathogens are inhibited by acidic foods, whereas acidophiles, such as *Bacillus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* thrive in them. Lime's acidity is organic, making it less damaging to the gastrointestinal tract (Salami and Afolayan, 2020). In a study by Braide *et al*. (2012), lime had a lower microbial load than other organic spices / preservatives like garlic, ginger, and clove. At the same concentration of preservatives added for 24 h of preservation, the authors found a lower microbial population than sodium benzoate. However, from 24 to 36 h, the bacterial density was comparable to that of other preservatives. Higher fungal density was reported for other organic preservatives as compared to lime in *zobo* drink from 24 to 48 h, while from 12 to 336 h of lime completely eliminated *Staphylococcus aureus* bacterium and *Fusarium poae* fungus in *zobo* drink (Braide *et al*., 2012).

Pineapple

Pineapple (*Ananas comosus*) is a tropical fruit from the family Bromeliaceae with a fruity flavour and taste. Pineapple is rich in nutrients such as calcium, vitamin C, potassium, carbohydrates, dietary fibres, and phytochemicals that are vital for the maintenance of an ideal body weight and a functional digestive system (Akujobi *et al.*, 2018). It is common for pineapple peels to be used as natural flavouring in *zobo* production, subsequently reducing wastage (Gbadegesin *et al*., 2017). Akujobi *et al.* (2018) showed the effect of artificial flavour substitution with pineapple and orange juice in *zobo* drink production. The authors reported that *zobo* with orange flavour + pineapple juice and orange + pineapple juice had higher ash, crude fibre, and natural carbohydrates with a significant difference, than *zobo* produced with artificial flavour. Proximate composition for *zobo* with flavour was given as moisture (93.53%), ash (0.24%), crude fibre (0.08%), fat (0.05%), protein (0.76%), carbohydrate (5.34%), iron (0.85 mg/100 g), potassium (23.45 mg/100 g), zinc (0.84 mg/100 g), calcium (12.27 mg/100 g), vitamin C (17.87 mg/100 g), and vitamin A (28.57 µg/100 g); while proximate composition for *zobo* with orange flavour + pineapple juice was given as moisture (91.77%), ash (0.32%), crude fibre (0.2%), fat (0.06%), protein (0.82%), carbohydrate (6.91%), iron (1.05 mg/100 g), potassium (35.73 mg/100 g), zinc (0.92 mg/100 g), calcium (13.94 mg/100 g), vitamin C (24.16 mg/100 g), and vitamin A (41.42 µg/100 g); while *zobo* substituted with only natural juices (pineapple and orange) was given as moisture (91.19%), ash (0.35%), crude fibre (0.22%), fat (0.07%), protein (0.87%), carbohydrate (7.29%), iron (1.14 mg/100 g), potassium (40.98 mg/100 g), zinc (0.97 mg/100 g), calcium (14.42 mg/100 g), vitamin C (28.93 mg/100 g), and vitamin A (49.44 µg/100 g). An increase in flavonoids, phytate, and phenol was reported for naturally flavoured *zobo*. High tannin and alkaloid levels were observed in *zobo* produced with an artificial flavour additive.

Uziza

Piper guineense is the most pungent and flavourful of all the leafy vegetables, and its fruits are used in preparing soups for women post-partum. The leaf consists of alkaloids (0.86%) and tannins (1.19%), which are of very high pharmacological value; saponin (1.88%), which is used to some extent as an expectorant and emulsifying agent; flavonoids (0.72%), which have antibacterial functions; and phenolic compounds (0.66%), which are known to have antimicrobial effects (Chinwendu *et al.*, 2016). Research carried out by Obi (2015) showed that *uziza* had a moderate preservative effect on *zobo* drinks with a microbial count of 29 CFU/mL after six days.

Ehuru

Monodora myristica is a plant in the family Araceae. It can be found all over the world, from Africa to Asia, to central and south America, to Australia. Its range extends from Sierra Leone through Uganda, Kenya, Congo, and Angola in west, central, and east Africa. In west Africa's evergreen forest, it is one of the most important spice trees, with a strong presence in the southern half of the country. Almost every part of the tree is useful economically. It is known locally in Nigeria as "*abo-lakoshe*" in Hausa, "*ehuru*" or "*ehiri*" in Igbo, "*ariwo*" in Yoruba, Jamaica nutmeg, or Calabash nutmeg (Enwereuzoh *et al.*, 2015; Abd El-Ghani, 2016). Its seeds are used in treating constipation, lice, and guinea worms (Abd El-Ghani, 2016). The nutritional value of *ehuru* is centred on its use as a beverage additive due to its aromatic qualities, with the seeds embedded in the white, sweet-smelling pulp of the sub-spherical fruit being the section of interest (Enwereuzoh *et al.*, 2015). According to the literature, the proximate composition of *ehuru* seed was obtained as: moisture, 6.0%; ash, 4.9%; crude fat, 24.33%; crude fibre, 3.3%; crude protein, 18.69%; and carbohydrate, 42.78%. Obi (2015) assessed the preservative effects of local spices on *zobo* drink. It was observed that *zobo* spiced with *ehuru* had no colony growth after six days of production.

Uda

Xylopia aethiopica is a spice in the family Annonaceae. It is locally known as "*uda*" or "*eeru"* in Nigeria, and is commonly referred to as "*Negro pepper*" or "*grains of Selim*". Its roots, bark, seeds, and leaves are traditionally used in the treatment of fever and jaundice (Abd El-Ghani, 2016). According to Obi (2015), *uda*-flavoured *zobo* had the highest microbial count of 90 CFU/mL. In comparison to other locally spiced *zobo*, it showed the least preservative effect.

Effect of natural spices on nutritional composition of zobo tea and drinks

Macronutrients required by humans are measured in gram amounts daily. Since *zobo* drink is deficient in macronutrients like protein, the drink should be substituted with protein-rich plant parts to enhance its quality (Adeniji, 2017). The effects of various spices and added plant extracts on the nutritional composition of *zobo* tea and drinks vary (Izah *et al.*, 2015). The effects are highly dependent on the composition of additives and the synergistic or antagonistic effects between the *zobo* and the additives used (Adeoye *et al.*, 2019). Generally, an overall increase in carbohydrate, ash, fibre, and crude protein has been reported with added fruit juice extracts, while a decrease in moisture and fat, depending on the spice or fruit extract added, has also been recorded (Adelekan *et al.*, 2014; Akujobi *et al.*, 2018).

Carbohydrate

Carbohydrates are macronutrients that are essential for life processes. Carbon, hydrogen, and oxygen make up the compound, which is divided into monosaccharide, disaccharide, oligosaccharide, and polysaccharide, depending on the number of carbon atoms. Carbohydrates serve as an immediate energy source for cellular function. Vegetables, whole fruits, legumes, nuts, seeds, whole grains, and tubers are considered good sources of carbohydrates. The carbohydrate content of *zobo* tea and drinks has been reported as high, and more so with the addition of sweeteners. Ezearigo *et al.* (2014) showed ginger-spiced *zobo* had 11.12% carbohydrate, while garlic-spiced *zobo* had 10.98% carbohydrate. Akujobi *et al.* (2018) reported an increase in the carbohydrate content of *zobo* produced with pineapple juice and artificial orange flavour. The carbohydrate content was 6.91% as opposed to the *zobo* produced with orange juice and pineapple flavours only, which had 5.34% carbohydrate. The researchers further showed that *zobo* with orange and pineapple juice extract had the overall highest carbohydrate content of 7.29%. Fasoyiro *et al.* (2005) enriched the roselle beverage with fruit juice in various ratios. The resulting roselle-pineapple beverages of ratios 1:1, 1:2, and 1:3 had 8.70, 8.30, and 10.40% carbohydrate, respectively. *Zobo* enriched with ginger and pineapple juice had 21.60% carbohydrate (Adelekan *et al*., 2014).

Fat

Fats are esters of fatty acids, which could be saturated or unsaturated. Plant oils, dairy products, meats, nuts, and baked goods are sources of fatty acids. The fat content of most fruits and vegetables, as with *zobo* calyx, is generally low, making them suitable for weight loss due to their low glycaemic index. Spices, on the other hand, are packed with aromatic oil compounds. The fat content of *zobo* produced with pineapple juice and orange flavour was 0.06%, in contrast to *zobo* tea and drinks produced with pineapple and orange flavour of 0.05% (Akujobi *et al.*, 2018). Fasoyiro *et al.* (2005) found that roselle-pineapple beverages of ratios 1:1, 1:2, and 1:3 had 0.67, 0.93, and 0.98% fat, respectively, showing that fat content increased with increasing fruit ratio, thus implying higher fat content in pineapple. Adelekan *et al*. (2014) reported 1.92% fat for pineapple- and ginger-spiced *zobo*, while Ezearigo *et al.* (2014) reported 0.38 and 0.40% fat for ginger- and garlic-spiced *zobo*, respectively.

Protein

Crude protein is calculated as the amount of nitrogen mineral multiplied by 6.25, following that proteins contain about 16% nitrogen on an average. Proteins consist of amino acids joined together by peptide bonds. Amino acids are categorised into essential and non-essential based on those that can be synthesised in humans. Sources of protein are mostly from meat, poultry, and sea foods. Seeds and nuts are good plant sources of protein. *Zobo* tea and drinks have been considered as good source of natural protein; therefore the supplementation increases the protein content. Akujobi *et al.* (2018) reported a higher protein content of 0.82% in *zobo* drinks enriched with pineapple juice than *zobo* drinks with artificial flavours (0.76%), while Fasoyiro *et al.* (2005) reported 0.93, 0.92, and 0.94% crude protein for roselle-pineapple beverages of ratios 1:1, 1:2, and 1:3, respectively. Adelekan *et al*. (2014) reported 4.13% protein for pineapple- and ginger-flavoured *zobo*. Ezearigo *et al.* (2014) reported 8.00 and 8.13% crude protein for garlic- and ginger-enriched *zobo*, respectively. This indicated increased protein with the addition of spices rather than fruits.

Fibre

Crude fibre is necessary in the diet because it increases faecal bulkiness due to its water retention properties, thus softening stool, promoting peristalsis within the intestinal muscles, and subsequently reducing intestinal and rectal health problems. Fruits and vegetables contain high fibre content, and *zobo* calyx is not left out. *Zobo* tea contains a high level of fibre content, and supplementing it with fruits further increases the fibre content. *Zobo* enriched with pineapples had 0.20% fibre, while that enriched with both orange and pineapple juice had 0.22%, in comparison with *zobo* produced with artificial flavour which only had 0.08% fibre (Akujobi *et al.*, 2018). Fasoyiro *et al.* (2005) reported 0.64, 0.66, and 0.72% fibre in roselle-pineapple beverages of ratios 1:1, 1:2, and 1:3, respectively; 0.52, 0.55, and 0.57% fibre in roselle-orange beverages of ratios 1:1, 1:2, and 1:3, respectively; and 1.44, 1.93, and 2.02% fibre in roselle-apple beverages of ratios 1:1, 1:2, and 1:3, respectively, thus indicating increased fibre with increased fruit ratio.

Mineral

Minerals and vitamins are referred to as micronutrients, as they are needed in small quantities of less than 100 mg daily. They are major components of vegetables and fruits. Mineral content in food refers to the inorganic residue left after the meal has been ignited or completely oxidised. Fruit-enriched *zobo* tea and drinks had higher ash values than non-enriched *zobo*. This is most likely attributed to the high mineral content of fruits, and the synergistic effects of various *zobo* tea and drink combinations. Ezearigo *et al.* (2014) reported a 0.44% ash content for garlic-enriched *zobo*, and a higher content of 1.33% ash in ginger-enriched *zobo*. Adelekan *et al*. (2014) reported 2.51% ash for *zobo* produced with pineapple and ginger. Akujobi *et al.* (2018) reported an ash content of 0.32% for pineapple juice enriched *zobo*, and 0.35% for pineapple and orange juice enriched *zobo*, as opposed to 0.24% ash for *zobo* not enriched with fruit juice extract. Fasoyiro *et al.* (2005) found 0.32, 0.33, and 0.31% crude ash in roselle-pineapple beverages with ratios of 1:1, 1:2 and 1:3, respectively.

Potassium

Potassium assists nerve function and muscle contraction, aids a regular heartbeat, acts as a cell electrolyte, and is also essential in co-regulating ATP with sodium. *Zobo* enriched with pineapples had 35.73 mg/100 g of potassium, while that enriched with both orange and pineapple juice had 40.98 mg/100 g of potassium, which was higher than *zobo* produced with artificial flavour (23.45 mg/100 g) (Akujobi *et al.*, 2018). Ezearigo *et al.* (2014) reported 9.87 mg/100 g potassium in garlic-enriched *zobo*, and a higher content of 10.08 mg/100 g in ginger-enriched *zobo*.

Phosphorus

Phosphorus is found in abundance in bones and teeth, bodily cells, DNA, and ATP, and is necessary for energy processing. It is needed for the body to synthesise protein, and to adequately utilise carbohydrates and fats. Fasoyiro *et al.* (2005) reported 2.40, 2.62, and 2.63 mg/100 g of phosphorus in roselle-pineapple beverages of ratios 1:1, 1:2, and 1:3, respectively; 1.71, 1.80, and 1.82 g/100 g of calcium in roselle-orange beverages of ratios 1:1, 1:2, and 1:3, respectively; and 1.60, 1.80, and 1.87 g/100 g of phosphorus in the resultant roselle-apple beverages of ratios 1:1, 1:2, and 1:3, respectively.

Calcium

Calcium is needed for strengthening bones and teeth, for heart, muscle health, as well as digestive system health, bone growth, and blood cell production and function. Other than fish and meat, green vegetables and dairy are good sources of calcium. *Zobo* enriched with pineapples had 13.94 mg/100 g of iron, while that enriched with both orange and pineapple juice had 14.42 mg/100 g of iron, which was higher than *zobo* produced with artificial flavour (12.27 mg/100 g) (Akujobi *et al.*, 2018). Fasoyiro *et al.* (2005) found 1.54, 1.66, and 1.71 mg/100 g of calcium for roselle-pineapple beverage with ratios of 1:1, 1:2, and 1:3, respectively; 2.11, 2.42, and 2.34 mg/100 g of calcium for roselle-orange beverage with ratios of 1:1, 1:2, and 1:3, respectively; and 2.51, 2.62, and 2.63 mg/100 g of calcium for roselle-apple beverage with ratios of 1:1, 1:2, and 1:3, respectively. Adelekan *et al.* (2014) reported 0.54 and 1.68 mg/100 g of calcium for *zobo* produced with pineapple + ginger, and pepper, respectively.

Iron, zinc, and copper

Iron is required by many enzymes and proteins, notably haemoglobin to prevent anaemia. The conversion of beta-carotene to vitamin A is also aided by iron. Zinc is necessary for many enzymes such as carboxypeptidase, carbonic anhydrase, and liver alcohol dehydrogenase, while copper is required in the production of redox enzymes, including cytochrome oxidase. These minerals are needed by various enzymes, and are necessary for cell division and growth. *Zobo* enriched with pineapples had 1.05 mg/100 g of iron, while that enriched with both orange and pineapple juice had 1.14 mg/100 g iron, which was higher than *zobo* produced with artificial flavour (0.85 mg/100 g) (Akujobi *et al.,* 2018). Adelekan *et al*. (2014) reported 0.54, 1.25, and 1.68 mg/100 g of iron in *zobo* produced with pineapple + ginger, *zobo* produced with pineapple + pepper, and that produced with pepper only, respectively. *Zobo* enriched with orange juice had 0.94 mg/100 g, that enriched with pineapples had 0.92 mg/100 g of zinc, while that enriched with orange + pineapple juice had 0.97 mg/100 g of iron, in comparison with *zobo* produced with artificial flavour (0.84 mg/100 g) (Akujobi *et al.,* 2018). Adelekan *et al*. (2014) reported 0.15, 0.18, and 0.42 mg/100 g copper for *zobo* produced with pineapple + ginger, *zobo* produced with pineapple + pepper, and that produced with pepper only, respectively.

Vitamin

Olayemi *et al.* (2011) and Salami and Afolayan (2020) both agree on the high vitamin content of *zobo* beverages, especially vitamin C, which was found to be nine times higher in *zobo* than in orange. The role of vitamins, including combating oxidative damage, preventing some DNA-damaging free radicals, and reducing the likelihood of major respiratory diseases like asthma and so on, cannot be overemphasised. There is therefore a need for supplementation of *zobo* tea and drinks to ensure a ready supply of necessary vitamins.

Ascorbic acid

Vitamin C (ascorbic acid) is a water-soluble, essential vitamin necessary for the repair of tissue, and the enzymatic production of certain neurotransmitters. It acts as an antioxidant which prevents cell oxidation, and is necessary for the prevention and alleviation of scurvy. The United States' recommended daily allowance (RDA) of ascorbic acid is 90 mg. Citrus fruits, berries, and green leafy vegetables are good sources of the vitamin C (Babalola *et al*., 2001; Wong *et al*., 2002; Salami and Afolayan, 2020). *Zobo* tea and drink enriched with pineapples had 24.16 mg/100 g of vitamin C, while that enriched with orange + pineapple juice had 28.93 mg/100 g of vitamin C, which was higher than *zobo* produced with artificial flavour (12.27 mg/100 g) (Akujobi *et al.,* 2018). Fasoyiro *et al.* (2005) reported 35.21, 34.63, and 35.10 mg/100 g of vitamin C for roselle-pineapple beverages of ratios 1:1, 1:2 ,and 1:3, respectively; 46.21, 47.14, and 48.25 mg/100 g of vitamin C in roselle-orange beverages of ratios 1:1, 1:2, and 1:3, respectively; and 35.63, 36.33, and 36.11 mg/100 g of vitamin C in roselle-apple beverages of ratios 1:1, 1:2, and 1:3, respectively. Adelekan *et al.* (2014) reported 29.28 and 33.24 mg/100 g of calcium for *zobo* produced with pineapple + ginger and that enriched with pineapple + pepper, respectively. Adebayo *et al.* (2021) reported that *zobo* drinks enriched with turmeric contained higher vitamin C of 725 µg/100 mL when compared with the control, which had 577 µg/100 mL of vitamin C.

Pro-vitamin A

Pro-vitamin A (beta-carotene) is a precursor, and is converted by the body to vitamin A. Vitamin A, otherwise known as retinol, is a dietary fat-soluble vitamin needed for vision, healthy skin, and optimum immune function. Dairy, fish, meat, red and yellow fruits, and yellow, red, and green leafy vegetables are good sources of vitamin A. United States recommended daily allowance (RDA) of vitamin A is 900 µg, which is not completely met by *zobo* tea and drinks (USDA, 2008). *Zobo* enriched with pineapple had 41.42 µg/100 g of vitamin A, while that enriched with orange + pineapple juice had 49.44 µg/100 g vitamin A, which was higher than *zobo* produced with artificial flavour (28.57 µg/100 g) (Akujobi *et al.*, 2018), while Adelekan *et al*. (2014) reported 28, 68, and 141 RE/L of vitamin A in pineapple + ginger, pineapple + pepper, and enriched *zobo* drinks, respectively.

Folic acid

Folate, also known as folic acid or vitamin B_9, is a water-soluble B-complex vitamin required for red blood cell formation, and for healthy cellular functions. Liver, green leafy vegetables, and peas are sources of vitamin B_9. United States recommended daily allowance (RDA) of vitamin B_9 is 400 µg, which can be sufficiently met with enriched *zobo* tea and drinks (USDA, 2008). Adebayo *et al.* (2021) reported that street-vended *zobo* drinks are generally high in vitamin B_9, but increase considerably with the addition of turmeric spice. The control had 295 µg/100 mL of vitamin B_9 in contrast to the turmeric-enriched *zobo* (301 µg/100 mL for 2% boiled turmeric in *zobo*, and 297 µg/100 mL for 6% boiled turmeric in *zobo*).

Effects of natural spices on biochemical composition of zobo tea and drinks

The phenolic content of *zobo* beverages was found to be a function of the antagonistic or synergistic effect between the natural additive phenolic content and the roselle calyx used in the drink's production. The ability of phenolic compounds to scavenge free radicals and reduce iron determines their antioxidant activity (Adeoye *et al.*, 2019).

Phenolics

Phenolics are aromatic benzene ring compounds comprising one or more hydroxyl groups. Phenols are synthesised by plants only, as an anti-stress protection mechanism. They act as powerful antioxidants when consumed, preventing oxidative damage and chronic diseases like cancers and cardiovascular diseases. *Zobo* enriched with pineapple had 0.043% phenolics, while that enriched with orange + pineapple juice had 0.052%, as opposed to *zobo* produced with artificial flavour, which had 0.038% (Akujobi *et al.*, 2018). According to a study by Adeoye *et al.* (2019), the phenolic content of *zobo* tea and spice drinks increased as the spice concentration increased, implying that the phenolic content of the spices and roselle calyx used in drink production had an additive or synergistic effect.

Flavonoids

Flavonoids are polyphenolic molecules that are plant metabolites, and soluble in water. The six major flavonoids in plants are chalcones, flavones, isoflavonoids, flavanones, anthoxanthins, and anthocyanins. The typical colours of fruits and vegetables rich in flavonoids are yellow, purple, blue, and red, which is the colour of *zobo* calyx. Flavonoids aid in the regulation of cellular activity and the prevention of oxidative stress caused by free radicals. Akujobi *et al.* (2018) reported that *zobo* enriched with pineapple had 0.06% flavonoids, while that enriched with orange + pineapple juice had 0.08% higher than *zobo* produced with artificial flavour, which had 0.05%. Meanwhile, Ezearigo *et al.* (2014) reported 0.6, 0.12, and 0.19 mg/100 g of flavonoid in garlic-, ginger-, and cinnamon-spiced *zobo* drink, respectively. Similar results are expected in enriched dehydrated *zobo* tea.

Scavenging antioxidant activity

Free radical scavenging activities are important for preventing free radical damage, and diphenyl-1-picrahydrazyl (DPPH) free radical scavenging is a commonly used method for testing the antioxidant activity of plant extracts. With an increase in spice concentration, increasing free radical scavenging activity was observed in a study by Adeoye *et al.* (2019), where clove-spiced *zobo* (DPPH) increased from 77.5 to 82.9% for clove concentrations of 0.05 to 0.45%, ginger-spiced *zobo* increased from 73.7 to 78.8% for ginger concentrations of 0.05 to 0.45%, and for mixture of ginger + clove spiced *zobo*, the DPPH increased from 75.7 to 80.1% for concentrations of 0.05 to 0.45%, while the control had a DPPH of 73.2%; all was observed on the first day of *zobo* drink production. The reports prove that spiced *zobo* tea would exhibit higher scavenging antioxidant activity.

Total reducing power

Total reducing power is a direct measure of the total antioxidant activity of compounds. It is a medium that measures the ability of phenols in food to reduce Fe^{3+} to Fe^{2+}. In the reducing power assay by Adeoye *et al.* (2019), they showed increasing total reducing power with the increase in spice concentrated *zobo* drink. It was observed in a study that the total reducing power of clove-spiced *zobo* increased from 1.674 to 1.717 for clove concentrations of 0.05 to 0.45%, ginger-spiced *zobo* increased from 1.655 to 1.674 for ginger concentrations of 0.05 to 0.45%, and for the mixture of ginger and clove spiced *zobo*, the total reducing power increased from 1.656 to 1.662 for concentrations of 0.05 to 0.45%, while the control had total reducing power of 1.628. All were observed on the first day of production.

Effects of natural spices on sensory characteristics of zobo tea and drinks

Various studies showed higher acceptance of *zobo* drinks produced with ginger, clove, and pineapple (Fasoyiro *et al.*, 2005; Obi, 2015; Akujobi *et al.*, 2018). The sensory evaluation was carried out on *zobo* drinks produced with ginger, clove, orange juice, pineapple flavour, orange flavour, and pineapple flavour using 20 panellists. The panellists were aged between 18 - 30 years. The *zobo* drink was served at ambient temperature. The ratings by the panellists suggested that *zobo* drink was likely to be accepted by the public if prepared with pineapple or orange juice (Akujobi *et al.*, 2018). This is most likely attributed to the fact that these natural additives are conventionally used in *zobo* tea and drink production, and also due to the fruity taste of pineapple. Sensory evaluation is significant in the production of novel products in order to gauge consumer acceptance (Akujobi *et al.*, 2018).

Taste refers to a flavour sensation in the mouth and throat. Akujobi *et al.* (2018) reported better acceptance of the taste of conventional *zobo* produced with artificial flavour from sensory panellists (7.87) than *zobo* enriched with orange or pine apple juice, or a combination of both, which scored 7.33, 6.94, and 5.63, respectively. Fasoyiro *et al.* (2005) reported taste acceptance of 6.0, 7.0, and 6.2 for roselle-apple beverages in ratios of 1:1, 1:2, and 1:3; 8.0, 8.0, and 7.7 for roselle-pineapple beverages in ratios of 1:1, 1:2 and 1:3; and 6.1, 5.8, and 6.1 for roselle-orange beverages in ratios of 1:1, 1:2 and 1:3, respectively. *Zobo* enriched with ginger + pineapple juice, pepper + pineapple, and pepper had 6.4, 6.93, and 5.48 scores, respectively, for taste (Adelekan *et al*., 2014). Gbadegesin *et al.* (2017) produced four samples of *zobo* beverage with 100% roselle, 85% roselle and 15% pineapple, 80% roselle and 20% pineapple, and 75% roselle and 25% pineapple. The researchers reported better taste preference in *zobo* drinks with 25% pineapple, scoring 7.9, and the least preference in *zobo* drinks with 100% roselle, scoring 6.2. Obi (2015) spiced *zobo* with Nigerian indigenous spices, and reported taste preferences of 4.9, 6.5, 6.5, 5.4, 4.6, and 5.3 for *zobo* spiced with clove, ginger, *uziza*, *ehu*, *uda*, and *ehuru*, respectively.

The distinctive taste of food is referred to as flavour. Akujobi *et al.* (2018) reported better acceptance of the aroma / flavour of conventional *zobo* drinks produced with artificial flavours from sensory panellists (7.82), than *zobo* enriched with orange or pineapple juice, or a combination of both, which scored 7.75, 7.47, and 6.34, respectively. Fasoyiro *et al.* (2005) reported flavour acceptance of 6.1, 6.3, and 6.2 for roselle-apple beverages in ratios of 1:1, 1:2, and 1:3; 7.6, 7.8, and 7.8 for roselle-pineapple beverages in ratios of 1:1, 1:2 and 1:3; and 6.3, 6.1, and 6.2 for roselle-orange beverages in ratios of 1:1, 1:2, and 1:3, respectively. *Zobo* enriched with ginger and pineapple juice, pepper and pineapple, and pepper had 6.4, 6.4, and 5.06 scores, respectively, for aroma (Adelekan *et al*., 2014). Gbadegesin *et al.* (2017) reported better flavour acceptance in *zobo* drink with 25% pineapple juice, scoring 8.4, and the least preference in *zobo* drinks with 100% roselle scoring 5.8. Obi (2015) reported flavour preferences of 6.1, 6.4, 5.4, 5.3, 4.5, and 5.2 for *zobo* spiced with clove, ginger, *uziza*, *ehu*, *uda*, and *ehuru*, respectively.

Colour of food is the first attraction to the food. A study carried out by Akujobi *et al.* (2018) showed better acceptance of pineapple juice enriched *zobo* colour (7.34), followed by *zobo* enriched with orange juice (7.28), conventional *zobo* produced with artificial flavours (7.08), and *zobo* enriched with a combination of orange and pineapple (6.15). Fasoyiro *et al.* (2005) reported colour acceptance of 6.99, 6.60, and 7.2 for roselle-apple beverage in ratios 1:1, 1:2, and 1:3; 8.1, 7.8, and 7.9 for roselle-pineapple beverage in ratios 1:1, 1:2, and 1:3; and 6.7, 7.7, and 7.4 for roselle-orange beverages in ratios of 1:1, 1:2, and 1:3, respectively. *Zobo* enriched with ginger and pineapple juice, pepper and pineapple, and pepper scored 5.93, 7.66, and 7.53, respectively, for colour (Adelekan *et al*., 2014). Gbadegesin *et al.* (2017) reported the most colour preference in *zobo* drinks with 25 and 20% pineapple scoring 7.8, and the least preference in *zobo* drinks with 100% roselle scoring 7.5. Obi (2015) reported colour preferences of 7.9, 8.1, 7.6, 7.6, 7.6, and 7.3 for *zobo* spiced with clove, finger, *uziza*, *ehu*, *uda*, and *ehuru*, respectively.

Overall acceptance of *zobo* in the study of Akujobi *et al.* (2018) in descending order is: *zobo* enriched with orange juice (8.04), pineapple-enriched *zobo* (7.94), conventional *zobo* produced with flavours (7.88), and finally, combination of orange and pineapple in *zobo* (6.03). Fasoyiro *et al.* (2005) reported overall acceptability of 6.5, 6.7, and 6.7 for roselle-apple beverages in ratios of 1:1, 1:2, and 1:3; 7.9, 7.2, and 7.9 for roselle-pineapple beverages in ratios of 1:1, 1:2, and 1:3; and 6.5, 6.0, and 6.3 for roselle-orange beverages in ratios of 1:1, 1:2, and 1:3, respectively. Adelekan *et al*., 2014 reported that *zobo* enriched with ginger and pineapple juice, pepper and pineapple, and pepper had 6.86, 7.2, and 6.53 overall scores, respectively, for acceptability. Gbadegesin *et al.* (2017) reported better overall acceptability in *zobo* drinks with 25% pineapple, scoring 8.5, and the least preference in *zobo* drink with 100% roselle, scoring 6.3. Obi (2015) reported overall acceptance scores of 5.9, 7.1, 6.6, 5.8, 5.5, and 5.9 for *zobo* spiced with clove, finger, *uziza*, *ehu*, *uda*, and *ehuru*, respectively.

Discussion

In the quest to overcome the hurdles associated with the short life span of *zobo*, and the potential incidence of disease conditions attributed to the *zobo* drink / tea spoilage microorganisms, studies have discovered several natural preservatives / spices of organic / natural origin that could improve the quality attributes of *zobo* tea and drinks, and also reduce both the microbial diversity and the density of *zobo* spoilage microorganisms.

Antioxidant contents of five local Nigerian drinks, namely: "*kunu*", palmwine, plantain, soybean, and *zobo* were analysed for flavonoids, phenolics, and vitamin C (Oboh and Okhai, 2012). The antioxidant scavenging abilities were evaluated using four different *in vitro* methods. *Zobo* drink with sugar had the highest phenolic content (16.00 ± 0.26 mg/mL), while *zobo* drink with ginger recorded the highest flavonoids and vitamin C (3.91 ± 0.02 and 2.31 ± 0.01 mg/mL), respectively. The roselle calyces of *Hibiscus sabdariffa* used in preparing *zobo* drink are rich in vitamin C and flavonoids (Wong *et al*., 2002). This is responsible for the high antioxidant content seen in the *zobo* drinks which correlates with the high ability of the drinks to scavenge free radicals. Furthermore, the ferric reducing ability of the drinks was highest in the *zobo* drinks possibly due to its high phenolic or flavonoid contents (r = 0.952 and 0.970, respectively). However, the total phenolic content was significantly different ($p \leq 0.05$) in all the *zobo* drinks. This showed that sugar and ginger affected the total phenolic of the drinks. Z*obo* enriched with ginger had a significantly ($p \leq 0.05$) higher flavonoid and vitamin C content as compared to the other *zobo* drinks (Oboh and Okhai, 2012).

Adesokan *et al*. (2013) demonstrated that the vitamin C contents of *zobo* drinks supplemented with garlic and ginger were higher than those of ordinary *zobo* juice. This means that the vitamin C status of *zobo* beverages can be improved by blending them with these spices. The crude protein content of non-spiced *zobo* juice was 9.1%, but higher values were obtained for the samples containing spices. Adesokan *et al*. (2013) reported that spices were able to reduce the microbial loads of *zobo* samples during 7 days storage. Ogiehor *et al*. (2008) extended the shelf life of *zobo* for 42 days by using 0.2% ginger extract and refrigeration. This method of storage under refrigeration is not practicable because of epileptic public power supply, and the use of power generators is not economical in Sub-Saharan African countries (Adesokan *et al*., 2013). The sensory attributes of the *zobo* samples containing spices were also enhanced. The use of extracts of ginger and garlic in *zobo* preparation should therefore be encouraged as the sensory properties and shelf life of the resulting product will be enhanced (Adesokan *et al*., 2013). Adesokan *et al*. (2013) and Popoola *et al*. (2019) concluded that the incorporation of ginger, garlic, and their extracts into *zobo* drink could be an effective means of improving the quality attributes of this drink.

Hibiscus sabdariffa leaves were extracted by hot water extraction using different local spices namely *Piper guineense* (*uziza*), *Zingiber officinale* (ginger), *Xylopia aethiopica* (*uda*), *Monodora myristica* (*ehuru*), *Syzygium aromaticum* (clove), and *Aistonei boonei* (*ehu*) (Obi, 2015). The drinks were then dispensed into six (2 L) cans, which were cooled in a refrigerator for sensory evaluation, and into six (150 mL) plastic bottles which were stored on the shelf for six days. All samples were pasteurised and allowed to cool before storage. The sensory evaluation results showed the drink with *Zingiber officinale* had the highest overall acceptability, followed by *Piper guineense*, while that with *Xylopia aethiopica* had the lowest acceptability. Results of the microbial counts showed that the *Monodora myristica* sample had the highest preservative effect, showing no visible growth after 48 h, while the *Xylopia aethiopica* sample had the highest microbial count, thus indicating the least preservative effect.

Apart from the spices reported by Obi (2015), other widely utilised and potential spices include garlic, a mixture of garlic and ginger, lime, cinnamon, nutmeg, kola nut, and pepper (Izah *et al*. 2016). These spices, along with the dried calyces of *Hibiscus sabdariffa*, have antimicrobial properties and are rich in phytonutrients, including vitamins and minerals. Hence, their ability to improve the shelf life of *zobo* mostly depends on the concentration added. Izah *et al*. (2016) reported that the ability of the natural preservatives to wade off microorganisms appears to be in this order; lime, mixture of garlic and ginger, ginger, garlic, clove, kola nut, cinnamon, and nutmeg. The activities of the spices were due to differences in the biochemistry, nutrition, physiology, and metabolism of the microorganisms. Therefore, attention should be focused on the carbonation of the drink after treatment with natural spices (Ogiehor *et al*., 2008; Izah *et al*., 2016). *Zobo* can equally be processed in dry form with natural spices, and packaged in tea bags, which could be preserved for longer periods due to low moisture content, and it will still retain its nutritive value and attract acceptance (Mohammed *et al*., 2017).

Conclusion

Zobo is rich in carbohydrates, vitamins C and A, phenols, potassium, sodium, phosphorus, and antioxidants. Consuming and drinking *zobo* tea daily would help to provide the recommended daily allowance of vitamins, and aid in proper health functioning. Due to the adverse effects of chemical additives, the use of plants and natural spices as potential flavour, antimicrobials, and preservatives should be fully adopted. The use of natural additives in *zobo* production would go a long way in enriching *zobo* tea and drink, and supplementing nutrients that may be lacking in health drink, thereby making it a super food. In a bid to extend shelf life, *zobo* tea, which is a formulation of dehydrated *zobo* extract, should be investigated. The use of tea bags in *zobo* tea packaging would reduce loss of quality, and yield maximum retention of nutrients.

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