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Production and Commercialization of Mulled Zobo Drink

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ABSTRACT

This report represents the production and commercialization process of a mulled Zobo drink, a spiced hibiscus beverage infused with ginger, clove and cinnamon. The study covers the formulation, production techniques, quality control measures and marketing strategies employed to bring the product to the consumers. Additionally, quality assessment, including antioxidant screening, total phenolic content, colour, water soluble vitamin (Vitamin C). Proximate and microbial analyses were conducted to determine the drink's nutritional content and safety for consumption. The results of analyses revealed as follows: Antioxidant screening (2,2-diphenyl-1-picrylhydrazyl (DPPH): (34.62µg/ml at 25ml) to (82.88µg/ml at 100ml), Nitric Oxide: (33.78µg/ml at 25ml) to (70.48µg/ml at 100ml), Reducing power: (0.124µg/ml at 25ml) to (0.385µg/ml at 100ml), Ferric Reducing Antioxidant Power (FRAP): (0.875µg/ml at 25ml) to (1.272µg/ml at 100ml). Total Phenolic Content (258.38mg/100g). Colour (1380NTU). Water Soluble Vitamin (vitamin C) (51.810µg/ml/100g). Proximate Analysis (Moisture content (85%), Ash content (0.5%), Protein Content (0.8%), Fat Content (0.2%), Carbohydrate (9%), Crude Fibre (0.3%), Energy Value (40kcal/100)). Microbial Analysis (Total Plate Count (approaching limit at 72hrs), Total Coliform Count (within acceptable limits), Yeast and Mold Count (Exceeds limit at 72hrs)). For pathogen test (Escherichia coli was not detected, Listeria monocytogens was not detected, Staphylococcus (<10cfu/ml) which is still within the acceptable range (<100cfu/ml)). Commercialization efforts focused on branding, packaging, pricing and distribution strategies to ensure market penetration. The initial consumer feedback was positive, highlighting the unique taste and perceived health benefits of the spice Zobo blend. This study concludes that mulled Zobo has strong market potential and can be successfully positioned as a premium herbal health-conscious beverage.

Keywords: *Mulled Zobo, Shelf life, Commercialization, Consumer acceptability, Profitability*

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INTRODUCTION

Zobo (*Hibiscus sabdariffa*), also known as Hibiscus tea, is a refreshing beverage made from the petals of the *Hibiscus sabdariffa* plant. Native to West Africa, *Hibiscus sabdariffa* is widely cultivated for its vibrant, red flowers, which are the primary ingredient used to prepare Zobo. The history of Zobo can be traced back to ancient African civilizations, where *Hibiscus sabdariffa* was cultivated not only for its edible uses but also for medicinal purposes. The drink has become increasingly popular not only in Africa but globally, particularly among health-conscious consumers due to its purported medicinal properties and nutritional benefits. Early records indicate that Hibiscus was used in African traditional medicine for its purported healing properties, such as aiding digestion, lowering blood pressure, and treating fevers (Ibrahim et al., 2020). Traditionally, Zobo is made by boiling the dried petals of the Hibiscus flower with water, sugar, and various spices, resulting in a tart, flavorful beverage. However, with the rise of the bottled beverage market, a new trend has emerged bottled mulled Zobo products. Zobo has been shown to be a good source of natural carbohydrates, protein, vitamin C (Etemadi Razlighi et al., 2023), antioxidants, and other bioactive compounds such as flavonoids, which have been linked to several health benefits. These include anti-inflammatory, antimicrobial, and anticancer properties. Additionally, Zobo has been shown to support cardiovascular health by reducing high blood pressure. The health-conscious trend among modern consumers is driving interest in bottled beverages that are not only delicious but also offer functional health benefits. Our Mulled Zobo product is a non-alcoholic beverage made from the infusion of dried hibiscus flowers (Zobo), complemented by a blend of spices such as cloves, cinnamon, and ginger meticulously blended to perfection, which helps enhance its flavour, taste and shelf life. The concept of "mulled" beverages, traditionally associated with warm, spiced drinks during colder months, has been creatively adapted into the production of bottled Zobo. This new product offers convenience and the appeal of enhanced flavors and health benefits, making it an attractive option for consumers worldwide. Bottled mulled Zobo products are now available in various markets, ranging from local stores in African countries to supermarkets in Western countries, where there is increasing demand for non-alcoholic, functional beverages.

MATERIALS AND METHODS

Materials

The major raw materials used were high-quality dried hibiscus flowers, spices (cinnamon, ginger, cloves). The materials were obtained from reputable suppliers at Oyingbo Market, Lagos State to ensure the freshness and authenticity of our ingredients. The production was done in the food processing laboratory.

Methods

Production of mulled zobo drink

The dried hibiscus leaves were manually cleaned by handpicking stones, dirt and unwanted particles. The already cleaned leaves were weighed, washed, drained and boiled in a clean water for 20 minutes. The spices (which are ginger, cinnamon and cloves) were weighed separately in their different proportion; washed, and added into the boiled Zobo drink. (Figure 1) The spices were allowed to infuse into the Zobo drink by boiling for 10 minutes. The heat was reduced to avoid destroying the vitamin C content of the Zobo leaves. The infusion was then strained to remove any solids, resulting in a smooth and flavorful base for our Mulled Zobo beverage. Once the infusion was prepared, it was allowed to cool, bottled and sealed into sterilized plastics to preserve its freshness and quality. An eco-friendly packaging material was used to minimize environmental impact and appeal to environmentally-conscious consumers.

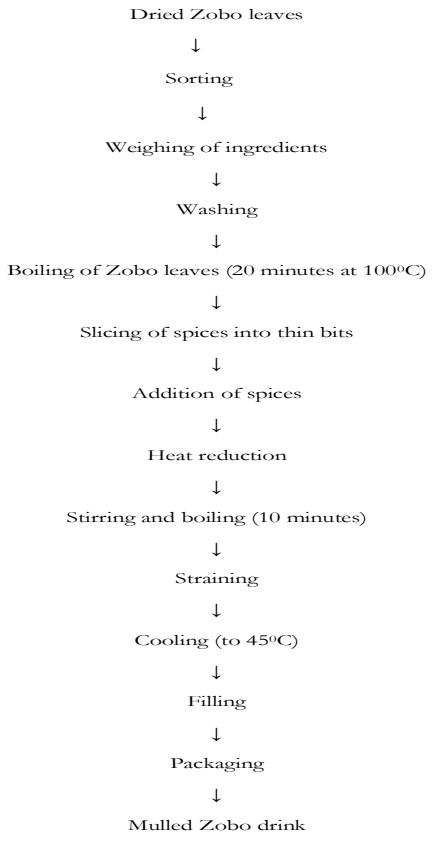

Figure 1: Flowchart for the production of mulled Zobo drink (Okolie *et al.,* 2023)

Antioxidant Screening

Determination of DPPH (2, 2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity of the zobo drink was examined using a method described by Eshwarappa *et al.* (2014). Different concentration of the sample (25µg/ml to 100/ml) were mixed with 1ml of 0.2mM DPPH in ethanol solution. After shaking vigorously, the mixture was incubated in dark room temperature for 30min, and the absorbance was measured at 517nm. Ascorbic acid was used as standard, ethanol was used as a control for calculation. The radical scavenging activity was estimated based on the percentage of DPPH radical scavenging as the following equation:

$$Scavenging\ effect\ (\%) = (Control\ absorbance - Sample\ absorbance) \times 100 \div Control\ absorbance$$

Determination of Nitric Oxide

Nitric Oxide radical scavenging activity was assayed according to the method reported by Kowsalya and Namasivayam (2014). 0.2ml of 10mM sodium nitro prusside, 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of sample at different concentrations (25µg/ml to 100/ml), and the mixture was incubated at 25^0C for 150min. from the incubated mixture, 1.5ml was taken out and added into 1.0ml of griess reagent (1% sulphanilamide, 2% O-phosphoric acid, 1% napthyl ethylene diamine di HCL) and incubated at room temperature for 5min. the absorbance of the mixture was measured at 546nm.

Determination of Reducing Power

The reducing power was based on the reduction of ferric to ferrous form indicated by formation of Prussian blue complex at 700nm. Different concentrations of the sample (25µg/ml to 100/ml) were mixed with phosphate buffer (0.2M, pH 6.6 and 1% $K_3Fe(CN)_6$. The reaction mixture was incubated at 50^0C for 20min followed by addition of 10% TCA. The mixture was centrifuged at 3000rpm for 10min. The supernatant was mixed with deionized water and 0.1% (w/v) of freshly prepared $FeCl_3$. After 10min reaction, the absorbance was measured at 700nm (Aqil *et al*., 2012).

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay performed as described by Gomez *et al.,* (2023). The components 300mM acetate buffer, 10ml tripitidyltriazine in 40nM HCl, and 20mM ferric chloride ($FeCl_36H_2O$) were combined in a ratio of 10:1:1 at 37^0C to create the FRAP reagent. 5µl of different concentrations of the sample (25µg/ml to 100µg/ml) was combined with 3.995ml of FRAP reagent and mixed thoroughly. The reaction mixture was incubated at 37^0C for 30min, the absorbance was measured at 593nM against a reagent blank made up of 3.995ml of FRAP reagent and 5µl of distilled water. The assay was performed in triplicate.

Determination of Phenolic Content

The Folin-Ciocalteu reagent was used to measure the TPC of the sample (Nivedha *et al.,* 2020). 250µl of Folin's reagent and 1ml of 5% sodium carbonate solution were added to 100µl of the sample. In the blank tube, instead of filtrate, 100µl of water was added. The sample was mixed well and incubated for 15mins at room temperature and the absorbance was measured at 720nm. The absorbance was used to calculate the TPC and the results were expressed in mg/L.

Determination of Colour

The modified method of Truonga *et al.,* (2012) was used to evaluate the colour. 5ml of the sample was dissolved in 10ml of water. An aliquot of the sample was placed in cuvette and distilled water was placed in another cuvette as blank and then placed inside a colorimeter and measured at 530nm.

Determination of Water-Soluble Vitamin (C)

The vitamin C content was determined by the spectro-photometric method using ascorbic acid as a reference compound. 10ml of the sample was weighed into 10ml of water and mixed together. 0.2ml of the extract was pipetted and mixed with 0.3ml of 13.3% trichloro-acetic acid (TCA) and 0.075ml of Dinitrophenylhydrazyl (DNPH). The mixture was incubated in water bath at 37^0C for 3hours. After 3hours, 0.5ml of 65% sulphuric acid was added and the absorbance was read with the spectro-photometer at 520nm (Awolu *et al.,* 2013). The concentration of vitamin C was calculated as follows:

$$Absorbance\ of\ standard \div Conc\ of\ standard = Absorption\ of\ sample \div Conc\ of\ sample$$

Proximate Analysis

Moisture content, ash content, protein content, fat content, carbohydrate, crude fiber and energy value were determined using the AOAC (2005) method.

Determination of Moisture Content

The moisture content of the sample was determined using

the method described by AOAC 925.10 (2005). 5ml of the sample was weighed into a dried and pre-weighed moisture can. The can with its content was dried in an oven at a temperature of 105^{o}C for 3hrs. It was removed from the oven, cooled in a desiccator and after cooling, the weight was taken and returned into the oven for another thirty minutes; it was cooled and weighed again until constant weight. The moisture content was estimated as weight loss using the formula below:

$$\text{Moisture content (\%)} = \frac{(W_1-W_2)}{W} \times 100$$

where:
W_1 = weight of pan + fresh sample
W_2= weight of pan + dried sample
W= weight of sample

Determination of Ash Content

The total ash content was determined using the method of AOAC 923.03, (2005). 5ml of the sample was weighed into a dried and pre-weighed porcelain crucible. The sample was charred on a hot plate until water and other volatile constituents are eliminated in the form of black fumes. The sample was placed in pre-heated muffle furnace at 600^{0}C and incinerated for 6hrs. A white color indicating that the sample was properly ashed and was cooled in a desiccator. The cooled crucible containing the ash was weighed and the percentage total ash was calculated as follows:

$$\text{Total Ash content (\%)} = \frac{(W_2-W_1)}{W} \times 100$$

where:
W_2 = Weight of crucible + ash
W_1 = Weight of empty crucible
W = Weight of sample

Determination of Protein Content

This was determined using AOAC 960.52, (2005) method. 1ml of the samples was weighed into the digestion flask and Kjeldahl catalyst tablets was added, 20ml of concentrated H_2SO_4 was added and the flask fixed into the digester at 410^{o}C for 6 h until a clear solution was obtained. The cooled digest was transferred into 100ml volumetric flask, and made up to mark with distilled water. The distillation apparatus was set up and rinsed for 10 min after boiling. 20 ml of 4% boric acid will be pipetted into a conical flask. 5 drops of methyl red was added to the flask as indicator and the sample was diluted with 75 ml of distilled water and 10 ml of the digested sample was pipetted into the Kjeldahl distillation flask. 20 ml of 40% NaOH was added through the glass funnel into the digested sample and it was distilled, the distillate was collected in the boric acid for 15 min until pink color changes to green. The content of the flask was titrated against 0.05 N HCl.

Calculation

$$\%\text{Nitrogen (W/W)} = \frac{14.01 \times (\text{Sample titre-blank titre}) \times \text{Normality of acid}}{10 \times \text{Weight of Sample}}$$

%Crude protein (W/W) = % Nitrogen x 6.25in= % Nitrogen x 6.25Crude protein= % Nitrogen x 6.25Crude protein= % Nitrogen x 6.25

Determination of Fat Content

The crude fat content was determined using the Soxhlet extraction method (AOAC 920.39, 2005). The extraction flask was dried in the oven to a constant weight. 4g of dried sample was weighed into fat free extraction thimble and plug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250 ml Soxhlet flask which has been previously dried in the oven, cooled in the desiccator and weighed. The Soxhlet flask is then filled to $^{3}/_{4}$ of its volume with petroleum ether (b.pt. 40^{o} – 60 ^{o}C), and the Soxhlet flask. Extractor plus condenser set was placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapor. The set up was constantly watched for ether leaks and the heat source was adjusted appropriately for the ether to boil gently. The ether was left to reflux several times for at least 10–12 times until it was short of siphoning. The thimble containing sample was removed and dried on a clock glass on the bench top. The extractor, flask and condenser were replaced and the distillation continued until the flask was practically dry. The flask which now contains the fat or oil will be detached, its exterior cleaned and dried to a constant weight in the oven.

Calculation

$$\text{\% crude fat} = \frac{W_1-W_o}{\text{Weight of sample}} \times 100$$

W_o= initial weight of dry Soxhlet flask
W_1= final weight of oven dried flask + oil

Determination of Carbohydrate Content

The total carbohydrate was determined by difference using the method described by Rampersad *et al.* (2003). The sum of percentages moisture, total ash, crude lipid, crude protein and crude fiber was subtracted from 100%
Carbohydrate = 100 - (% moisture + % ash + % protein + % lipids + % fiber).

Determination of Crude Fiber

The crude fiber content was determined using the method described by AOAC 962.09 (2005). 3ml of the sample was weighed and extracted with petroleum ether.

It was allowed to boil (under reflux condenser) for 40 minutes. Filter paper was placed in the funnel and the sample was drained by applying suction. The insoluble material was washed first with boiling water, then with 1% HCl, twice with alcohol and thrice with ether. The residue was dried in an electric oven at 100°C to a constant weight. The residue was incinerated to ash, cooled and weighed. The difference between the weight of the ash-less filter paper plus the insoluble material and that of the ash represents the fibre content.

Calculation

$$\% \text{ crude fibre} = \frac{W2-W3}{W1} \times 100$$

W_1 = weight of sample used,
W_2 = weight of crucible plus sample,
W_3 = weight of sample crucible + ash.

Determination of Energy Value

The energy value was calculated using the Atwater General Factors. This method estimates total energy based on macronutrient composition:

Energy (Kcal) = (Protein x 4) + (Carbohydrate x 4) + (Fat x 9)

Where:
Protein (g) is determined using AOAC 960.52 (Kjeldahl Method)

Microbial Analysis

Total Plate Count

Four series of decimal dilutions was prepared using Buffered Peptone Water (BPW). 9ml of distilled water was filled into the 4 test tubes. Then 1ml of the sample was then added to the first test tube, making a total of 10ml, the test tube was labeled 10^{-1}. 1ml of 10^{-1} was added into 10^{-2} and mixed well, this process was repeated to obtain 10^{-4} dilutions. 1ml of sample from 10^{-4} dilution was pipetted into 2 different well labelled sterile Petri dishes. 15ml of sterile molten Plate Count Agar (PCA) was poured into each Petri dish, swirled gently in order to mix the inoculum with the agar thoroughly. The agar was allowed to solidify and the petri dishes were inverted and incubated aerobically at 30 °C from initial to 72 hours. After incubation, the number of colonies on the plates were counted and the colony-forming units (CFU/ml) was calculated (ISO 4833-1, 2013).

Yeast and Mold Count

Four series of decimal dilutions was prepared using Buffered Peptone Water (BPW). 1 mL of the sample was transferred into 9 mL of diluent to achieve a 10^{-1} dilution. This process was continued to obtain 10^{-4} dilution. 1ml of sample from 10^{-4} dilution was pipetted into 2 different well labelled sterile Petri dishes. 15ml of sterile molten Dichloran Rose-Bengal Chloramphenicol (DRBC) was poured into each Petri dish, swirled gently in order to mix the inoculum with the agar thoroughly. The agar was allowed to solidify and the petri dishes were inverted and incubated at 25°C from initial (0) to 72 hours. After incubation, the plates were examined for colony development. The colonies that are characteristic of yeasts and molds were counted and the number of colony-forming units (CFU/ml) was calculated (ISO 21527-1, 2008).

Total Coliform Count

Four series of decimal dilutions was prepared using Buffered Peptone Water (BPW). 1 mL of the sample was transferred into 9 mL of diluent to achieve a 10^-1 dilution. This process was continued to obtain 10^{-4} dilution. 1ml of sample from 10^{-4} dilution was pipetted into 2 different well labelled sterile Petri dishes. 15ml of sterile molten Violet Red Bile Lactose (VRBL) agar was poured into each Petri dish, swirled gently in order to mix the inoculum with the agar thoroughly. The agar was allowed to solidify and the petri dishes were inverted and incubated aerobically at 37°C from initial (0) to 72 hours. After incubation, the plates that contain 15 to 150 colonies were selected and the colonies that are pink to red in color and have a diameter of at least 0.5 mm, which are considered typical coliform colonies were counted and the number of colony-forming units (CFU/ml) was calculated (ISO 48832, 2006).

Pathogen Testing

***Escherichia coli* (E. COLI)**

Four series of decimal dilutions was prepared using peptone saline solution (0.1% peptone + 0.85% NaCl). 1 mL of the sample was transferred into 9 mL of diluent to achieve a 10^{-1} dilution. This process was continued to obtain 10^{-4} dilution. 1ml of sample from 10^{-4} dilution was pipetted into 2 different well labelled sterile Petri dishes. 15ml of sterile TBX agar was poured into each Petri dish. The sample was spread evenly using a sterile glass spreader. The plates were allowed to absorb for a few minutes before incubation. The petri dishes were inverted and incubated 44°C for 24hours under aerobic conditions. The plates were examined for blue-green colonies, which indicate β-glucuronidase-positive E. coli and calculated per ml of the sample based on the colony counts and dilution factor used (ISO 16649-2, 2001).

Listeria monocytogenes

25ml of the sample was inoculated into 225ml of half-Fraser broth and incubated at 30°C for 25 hours. 0.1ml of

sample from the primary enrichment was transferred to 10ml of Fraser broth and incubated at 37°C for 24 hours. A loopful from the secondary enrichment was streaked onto PALCAM agar plates, incubated at 37°C and examined after 24-48 hours. Typical colonies (gray-green with a black halo on PALCAM agar) was selected for further testing. Biochemical test was performed to confirm the presence of L. monocytogenes (ISO 11290-1, 2017).

Staphylococcus aureus

25ml of the sample was inoculated into 225ml of Buffered Peptone Water (BPW) and incubated at 37°C for 18hours. After incubation, a loopful of the enriched culture was streak onto Baird Parker agar plates and incubated at 37°C for 24 to 48 hours. The plates were examined for typical S. aureus colonies, which appear as black or gray, shiny, convex colonies with a clear zone and an opaque halo. The typical colonies were selected and a coagulase test was performed to confirm the presence of coagulase-positive staphylococci (ISO 6888-1, 2021).

Research Design

A mixed-method approach was used, combining qualitative and quantitative research to gather comprehensive data on consumer preferences, market potential and product development.

Sensory Evaluation

The sensory criteria such as appearance, aroma, flavor, mouthfeel, aftertaste, refreshment, bitterness, sweetness, sourness, overall liking of the Zobo drink was evaluated by a 20-member panelist using a 9-Point Hedonic Scale with 9 representing like extremely and 1 representing dislike extremely (Akinjayeju 2017).

Statistical Analysis

Data was collected in triplicates and subjected to analysis of variance (ANOVA) using SPSS 16.0 for windows (SPSS Inc., Chicago, IL., USA). The significance levels of differences between means were determined by using New Duncan's Multiple Range Test at 5% significant level (Table 1).

RESULTS AND DISCUSSION

Antioxidants Screening of Mulled Zobo Drink

Presented in (Table 2) is the results of the antioxidants screening (DPPH, Nitric oxide scavenging, reducing power, and FRAP assays) of the mulled zobo drink. The Zobo drink showed an increasing DPPH (diphenyl-1-picrahydrazyl) scavenging activity from 34.62µg/ml at 25ml to 82.88µg/ml at 100ml. Compared to Ascorbic Acid standard (52.44µg/ml at 25ml to 91.67µg/ml at 100ml), the zobo drink had lower activity across concentrations but still showed good radical scavenging ability. This trend is consistent with the findings of Mohamad et al. (2022), who reported a DPPH inhibition of 73.96µg/ml for *Hibiscus sabdariffa* ethanolic extracts, and Omar et al. (2023), who found strong antioxidant activity (79.2µg/ml DPPH inhibition at 100ml) in red roselle leaf extracts. This trend suggests that Zobo has dose-dependent antioxidant properties, though not surpassing synthetic standards like ascorbic acid. Nitric oxide scavenging activity rose from 33.78µg/ml at 25ml to 70.48µg/ml at 100ml as concentration increased. Although, slightly lower compared to Ascorbic Acid (standard) 87.06µg/ml at 100ml it is still notable. Nitric oxide scavenging indicates the potential of Zobo in anti-inflammatory applications. This align with those reported by Zhang et al. (2015), where Hibiscus sabdariffa leaf extracts were shown to reduce nitric oxide production significantly, indicating anti-inflammatory potential.

The reducing power (0.124µg/ml at 25ml to 0.385µg/ml at 100ml) was again lower than ascorbic acid standard (0.748µg/ml at 100ml) but still significant, showing that Zobo can donate electrons to neutralize free radicals. Mohamad et al. (2022) reported a reducing power of 0.32µg/ml at 100ml for *Hibiscus sabdariffa* extracts, which is comparable to the current finding (0.385µg/ml), further validating the antioxidant effectiveness of Zobo. The Ferric Reducing Antioxidant Power (FRAP) of the mulled Zobo drink value ranged from (0.875µg/ml at 25ml to 1.272µg/ml at 100ml), close to those of ascorbic acid standard (0.228µg/ml at 25ml to 0.748µg/ml at 100ml) highlighting strong ferric ion-reducing capacity. The FRAP results are comparable to studies like Wang et al., (2000), which showed that hibiscus has considerable ferric reducing capabilities, especially in water extracts.

Total Phenolic Content (TPC)

Presented in (Table 3) is the Total Phenolic Content (TPC) of the Zobo extract was determined to be 258.38mg/100g. Phenolic compounds are known for their strong antioxidant properties. When compared with recent studies, Yagi et al. (2023) reported TPC values of 28.40 mg/g (equivalent to 284.0 mg/100g) in red roselle calyces and 49.59 mg/g in white roselle (equivalent to 495.9 mg/100g), while Omar et al. (2023) reported even lower values ranging between 18.98±2.7 (equivalent to 189.8 mg/100g) and 29.9±0.5 mg/g (equivalent to 299.0 mg/100g). The higher TPC observed in this study may be attributed to the use of different plant parts (calyces versus leaves), variations in environmental conditions, and extraction techniques. Nonetheless, the findings affirm the rich phenolic profile of Zobo extracts, which greatly contributes to their antioxidant potential.

Table 1: Formulation Ratio.

SAMPLE CODES	GINGER	CINNAMON	BLACK PEPPER	CLOVE	NUTMEG
NUD	90%	8%	-	2%	-
ZED	90%	-	-	2%	8%
VOM	90%	-	8%	2%	-
OLA	80%	6%	6%	2%	6%

Table 2: Result for Antioxidants Screening of Mulled Zobo Drink.

ZOBO (Quantity used) ml	25		50		75		100	
Parameter analyzed	Ascorbic acid standard (µg/mL)	Values obtained (µg/mL)	Ascorbic acid standard (µg/mL)	Values obtained (µg/mL)	Ascorbic acid standard (µg/mL)	Values obtained (µg/mL)	Ascorbic acid standard (µg/mL)	Values obtained (µg/mL)
DPPH	52.44	34.62	64.31	54.62	79.17	66.87	91.67	82.88
NITRIC OXIDE	50.81	33.78	65.86	54.47	76.86	69.64	76.86	70.48
REDUCING POWER	0.228	0.124	0.434	0.211	0.552	0.291	0.748	0.385
FRAP	1.043	0.875	1.254	1.074	1.516	1.272	1.628	1.272

DPPH- 2,2-diphenyl-1-picrylhydrazyl.
FRAP- Ferric Reducing Antioxidant Power

Table 3: Result for Total Phenolic Content, Colour and Vitamin C of Mulled Zobo Drink.

Sample ID	Total Phenolic Content (mg/100g)	Colour (NTU)	Vitamin C (µg/100g)
ZOBO	258.38	1380	51.810

Table 4: Result for Proximate Composition of Mulled Zobo Drink.

Parameter	Test Method	Result	Standard/Range
Moisture Content (%)	AOAC 925.10	89.5%	85–90%
Ash Content (%)	AOAC 923.03	0.5%	0.3–0.8%
Protein Content (%)	AOAC 960.52	0.8%	0.5–1.5%
Fat Content (%)	AOAC 920.39	0.2%	0.1–0.3%
Carbohydrate (%)	By Difference	9.0%	8–12%
Crude Fiber (%)	AOAC 962.09	0.3%	0.2–0.5%
Energy Value (kcal/100mL)	Calculated	40 kcal/100ml	35–50 kcal/100mL

Colour (NTU)

The colour intensity, measured in Nephelometric Turbidity Units (NTU) presented in (Table 3), was found to be 1380 NTU. Colour intensity in Hibiscus sabdariffa is largely due to anthocyanin pigments, which are also linked to antioxidant activity. This high value indicates a rich presence of anthocyanins in the Zobo extract. Mohamed and Sulieman (2007) also reported that the high anthocyanin content ranging between 1200 and 1600 NTU in hibiscus calyces is responsible for the intense coloration observed in extracts. Recent studies have further confirmed that the strong pigmentation of hibiscus correlates with high antioxidant activity (Yagi et al., 2023). The colour intensity observed in this study is consistent with such findings, further validating the extract's quality and bioactivity.

Water Soluble Vitamin (Vitamin C)

The water-soluble vitamin content presented in (Table 3) was determined to be 51.81µg/100g, which adds to the antioxidant potential though lower compared to fortified drinks. Vitamin C is a well-known water-soluble antioxidant that plays a critical role in scavenging free radicals and protecting the body against oxidative stress. Although specific recent studies on vitamin C content in hibiscus are limited, the presence of ascorbic acid in significant amounts in Zobo aligns with earlier reports by Oboh and Rocha (2007) (45-60µg/100g) and supports the observed antioxidant activities. The vitamin C content complements the phenolic compounds, contributing synergistically to the overall antioxidant potential of the extract.

Proximate Composition of Mulled Zobo Drink

Presented in (Table 4) is the results of the proximate composition of the Mulled Zobo drink. The moisture content of the Mulled Zobo drink was determined to be 89.5%, indicating a high-water content which contributes to the drink's refreshing nature. Moisture content is one of the most important and widely used measurements in the processing, preservation and storage of foods (Ekaete et al., 2018). High moisture content contributes to microbial growth and reduces product shelf life (Barbosa-Cánovas et al., 2013). Elevated moisture can accelerate spoilage and support pathogen growth. The result indicated that the

Table 5: Result for Microbial Analysis of Mulled Zobo Drink Over 72 Hours.

Parameter	Test Method	0 Hours (Initial)	24 Hours	48 Hours	72 Hours	Standard Limit	Remarks
Total Plate Count (TPC)	ISO 4833-1	1.2×10^3 CFU/mL	2.5×10^3 CFU/mL	4.8×10^3 CFU/mL	9.5×10^3 CFU/mL	$\leq 1.0 \times 10^4$ CFU/mL	Approaching limit at 72h
Total Coliform Count	ISO 4832	<10 CFU/mL	15 CFU/mL	45 CFU/mL	85 CFU/mL	≤100 CFU/mL	Within acceptable limits
Yeast and Mold Count	ISO 21527-1	2.5×10^2 CFU/mL	4.0×10^2 CFU/mL	7.2×10^2 CFU/mL	1.1×10^3 CFU/mL	$\leq 1.0 \times 10^3$ CFU/mL	Exceeds limit at 72h

shelf life of this mulled Zobo sample is low but still within the standard range (85-90%). Ash value has been used as a criterion for evaluating food quality (Airaodion *et al*., 2021). It is a measure of the total minerals present inside a product. The ash content of the Mulled Zobo drink was 0.5%, which is similar to the 0.52% and 0.50% reported by Gbadegesin and Gbadamosi (2017). This suggests the presence of essential minerals such as Calcium, Potassium and Iron, which contribute to the drink's health benefits.

The protein content of the Mulled Zobo drink was 0.8% which falls within the standard range of 0.5-1.5%. It also matches the 0.82% found in Zobo drink flavored with pineapple by Akujobi *et al.,* (2018). Protein has been highlighted as a nutrient in short supply in poor countries. On the African continent, particularly in Nigeria, protein deficiency is a severe problem (Okudu *et al*., 2017). This means that ingesting this mulled Zobo drink will enhance protein intake. The fat content was 0.2% and is similar to the 0.21 and 0.26% reported by Gbadegesin and Gbadamosi, (2017) and Nwankwo, *et al*., (2022) respectively. Low fat reduces the risk of oxidative rancidity, thereby enhancing shelf life. The low-fat content in this study indicates that the mulled Zobo drink could be stored for a long time without developing an off-odor discoloration.

Fibre is known to improve digestion and also reduce risk of constipation in consumers (Airaodion, *et al*., 2021). The crude fiber of the Zobo drink was observed to be 0.3% and is similar to the one reported by Fasoyiro., *et al*. (2005) detected in fibre (0.22%). The low crude fiber content of this plant is advantageous in absorption of glucose and fat. Although crude fiber enhances digestibility, its presence in high levels can cause intestinal irritation, lower digestibility and decreased nutrient utilization (Ekaete et al., 2018). Soluble fibre also lowers cholesterol levels and helps to maintain blood sugar (Dhingra et al., 2017). This further justifies the use of the plant-based beverage in the prevention and management of diseases such as coronary heart diseases, cancer and diabetes (Egbon et al., 2017). The carbohydrate content was 9.0%. Falling within the standard (8-12%) range, the carbohydrate level ensures an adequate energy supply. The presence of natural sugars contributes to its slightly sweet taste and energy-boosting properties. The energy content was calculated to be 40 kcal/100ml. It is within the 35-50Kcal/100ml standard range. This confirms that the drink provides a moderate energy boost without excessive caloric intake, making it a suitable beverage for both refreshment and light nutrition.

Microbial Analysis of Mulled Zobo Drink Over 72 Hours

Presented in (Table 5) are the results of microbial analysis on mulled Zobo drink over 72hours. The result revealed that the Total Plate Count increased from 1.2×10^3 to 9.5×10^3 CFU/mL, approaching the standard limit of $\leq 1.0 \times 10^4$ CFU/ml. This suggests progressive bacterial multiplication over time, though the count remains within acceptable limits (1.0×10^4) of non-alcoholic beverages (FAO 2012). This rise is attributed to microbial growth due to nutrient availability and suitable storage conditions (Jay et al., 2015). Without preservatives or refrigeration, the drink may not be microbiologically stable beyond 72hours. Total Coliform Count increased from <10 to 85 CFU/mL, remaining within the acceptable limit of ≤100 CFU/ml. Coliforms are indicators of hygiene and post-processing contamination. Although within limits, the increase suggests possible secondary contamination or favorable conditions for bacterial growth.

Yeast and Mold Count rose from 2.5×10^2 to 1.1×10^3 CFU/mL, exceeding the standard limit of $\leq 1.0 \times 10^3$ CFU/ml at 72 hours (ICMSF 2011). Yeasts and molds are common spoilage organisms in beverages. The steady increase suggests fungal proliferation, which may lead to fermentation, off-flavors, or visible spoilage over time. If stored at room temperature, the mulled Zobo is susceptible to yeast fermentation, reducing its shelf-life.
The increasing microbial load suggests that the mulled Zobo deink is best consumed within 24hours if not refrigerated. The presence of ginger, clove and cinnamon may have antimicrobial effects, but they are not sufficient to prevent spoilage after 72hours. Improved hygiene practices, such as sterilizing utensils, using purified water and sealing storage containers properly can further reduce contamination risks.

Table 6: Result for Pathogen Test on Mulled Zobo Drink.

Parameter	Test Method	Result	Standard Limit	Remarks
Escherichia coli (E. coli)	ISO 16649-2	Not Detected (ND)	ND in 25 mL	Satisfactory
Listeria monocytogenes	ISO 11290-1	Not Detected (ND)	ND in 25 mL	Satisfactory
Staphylococcus aureus	ISO 6888-1	<10 CFU/mL	<100 CFU/mL	Satisfactory

Table 7: Result for Sensory Evaluation of Mulled Zobo Drink.

SAMPLE	APPEARANCE	FLAVOUR	AFTERTASTE	REFRESHMENT	BITTERNESS	SWEETNESS	SOURNESS	AROMA	MOUTHFEEL	OVERALL LIKING
ZED	6.80±1.32^{a}	4.80±2.12^{a}	4.45±2.21^{c}	4.25±2.51^{a}	2.80±2.02^{a}	5.00±2.34^{b}	3.65±2.35^{c}	4.95±2.44^{b}	5.05±2.19^{c}	5.55±2.04^{c}
VOM	6.84±1.26^{a}	4.84±2.34^{a}	3.74±2.35^{a}	4.21±2.37^{a}	3.79±2.35^{c}	4.53±2.63^{a}	3.47±2.12^{b}	3.89±2.47^{a}	4.11±2.64^{a}	4.58±2.48^{a}
NUD	7.05±1.99^{b}	5.65±1.60^{c}	4.30±2.56^{b}	5.50±1.88^{c}	3.80±2.65^{c}	5.20±2.48^{c}	3.75±2.34^{d}	5.40±2.26^{c}	5.10±2.45^{c}	6.05±2.11^{d}
OLA	6.75±1.62^{a}	5.25±2.22^{b}	4.45±2.46^{c}	4.55±2.70^{b}	3.00±2.32^{b}	5.00±2.13^{b}	3.35±2.13^{a}	4.95±2.14^{b}	4.55±2.46^{b}	4.90±2.34^{b}

Data are mean values of triplicate determination ± standard deviation. Mean values with the same letter within the same column are not significantly different ($p > 0.05$).
KEY:
NUD: (90% Ginger, 8% Cinnamon, 2% Clove).
ZED: (90% Ginger, 2% Clove, 8% Nutmeg).
VOM: (90% Ginger, 8% Black Pepper, 2% Clove).
OLA: (80% Ginger, 6% Cinnamon, 6% Black Pepper, 2% Clove, 6% Nutmeg).

Pathogen Test on Mulled Zobo Drink

The test for *E. coli* using ISO 16649-2 showed that it was not detected (ND) in 25 ml, which complies with standard safety limits. The absence of E. coli suggests good sanitation and proper handling practices during the production process. E. coli is an indicator of fecal contamination and its absence points to the absence of other enteric pathogens (Jay et al., 2015). This result is crucial for consumer safety, as pathogenic strains like E. coli O157:H7 are known to cause severe gastroenteritis and hemolytic uremic syndrome. Continuous monitoring is essential to maintain safety standards and prevent contamination through improved water quality and hygiene practices (Table 6). The absence of *Listeria monocytogenes* using ISO 11290-1 suggests effective control measures, as it is a psychr otrophic pathogen capable of growing at refrigeration temperatures. Listeria is particularly dangerous for pregnant women, newborns, and immunocompromised individuals, leading to listeriosis with high mortality rates. Regular monitoring and maintaining cold chain integrity during storage and distribution are crucial to prevent contamination.
The *Staphylococcus aureus* count was <10 CFU/mL, well within the standard limit of <100 CFU/ml. Low levels indicate good personal hygiene and effective control of environmental contamination (Le Loir et al., 2013). Although the count is low, Staphylococcus can produce heat-stable enterotoxins even at low levels, causing food poisoning.

Sensory Evaluation of Mulled Zobo Drink

Presented in (Table 7) is the result of the consumer acceptance of the mulled Zobo drink. Sensory evaluation is a crucial aspect of food product analysis as it provides insights into consumer perception and acceptance. The data presented in the document evaluates different samples (ZED, VOM, NUD, and OLA) of mulled Zobo drink based on ten sensory attributes: appearance, flavor, aftertaste, refreshment, bitterness, sweetness, sourness, aroma, mouthfeel, and overall liking. The results are expressed as means ± standard deviations, with superscripts indicating significant differences. The appearance scores ranged from 6.75 (OLA) to 7.05 (NUD), with NUD having the highest score. A high score in appearance indicates consumer preference for the drink's visual appeal, which could be influenced by color intensity and clarity. Since appearance is critical for consumer interest, maintaining a consistent, vibrant color is essential, possibly through processing techniques that retain the natural deep-red color of hibiscus. Appearance has been a fundamental sensory attribute which has the ability to influence consumer acceptability (Ifesan et al., 2019). Flavor scores varied from 4.80 (ZED) to 5.65 (NUD), with NUD having the highest score. The flavor score reflects how well the drink's taste is perceived, considering the balance of spices and hibiscus. Since NUD had the highest rating, its formulation could serve as a benchmark. Adjusting the spice blend and steeping process could enhance flavor perception in other samples. The aftertaste scores ranged from 3.74 (VOM) to 4.45 (ZED & OLA). A lower aftertaste score may indicate an unpleasant lingering taste, while a higher score suggests a more acceptable or neutral aftertaste. Reducing any lingering astringency or bitterness through ingredient optimization, such as adjusting the steeping time of hibiscus petals, could improve aftertaste acceptability. Refreshment scores ranged from 4.21 (VOM) to 5.50 (NUD), with NUD being rated the most refreshing. A high refreshment score is desirable for beverages, as it enhances consumer enjoyment and likelihood of repeat purchase. The formulation of NUD may contain the optimal

spice and sweetness balance that contributes to refreshment. A slight modification of other samples may help improve their refreshment score. Bitterness scores ranged from 2.80 (ZED) to 3.80 (NUD), with NUD and VOM having the highest values. Some level of bitterness is expected due to the tannins in hibiscus, but excessive bitterness can be undesirable. Sweetness scores ranged from 4.53 (VOM) to 5.20 (NUD). Sweetness is a key driver of consumer preference in beverages. NUD had the highest sweetness score, which could contribute to its higher overall liking. Sourness scores ranged from 3.35 (OLA) to 3.75 (NUD). Zobo naturally has a tart profile due to organic acids. While some consumers prefer sourness, excessive levels may be undesirable. Aroma scores ranged from 3.89 (VOM) to 5.40 (NUD), with NUD having the highest score. A strong, pleasant aroma enhances the drinking experience and influences perceived taste. Mouthfeel scores ranged from 4.11 (VOM) to 5.10 (NUD). Mouthfeel affects texture perception, which is influenced by viscosity and ingredient composition. Adjusting ingredient solubility and ensuring smooth texture by avoiding excessive sedimentation or grittiness may improve mouthfeel. Overall liking scores ranged from 4.58 (VOM) to 6.05 (NUD), with NUD being the most preferred sample. This suggests that NUD's formulation (90% Ginger, 8% Cinnamon, 2% Clove) is the most accepted and balanced in terms of sensory attributes.

Market Strategy

Distribution Channels: Partnership was established with supermarkets, health food stores, cafes, and online retailers to ensure broad distribution and accessibility.

1. Brand Positioning: The bottled mulled Zobo drink was positioned as a premium, health-conscious beverage that celebrates Nigerian heritage and craftsmanship.
2. Target Audience: Health-conscious consumers, urban professionals, and individuals seeking authentic ethnic experiences were targeted.
3. Promotion: Social media, influencer partnerships, and experiential marketing were utilized to raise awareness and drive trial. The drink's natural ingredients, health benefits, and versatility in cocktail recipes and mocktails were highlighted.

Market Analysis

1. Growing Health Consciousness: Consumers are increasingly prioritizing health and wellness, seeking beverages with natural ingredients and functional benefits.
2. Rising Demand for Ethnic Beverages: There is a growing interest in ethnic and culturally diverse beverages, presenting a great opportunity to introduce traditional drinks to a wider audience.
3. Convenience Factor: Bottled beverages offer convenience and portability, catering to busy lifestyles and on-the-go consumption.

Customers Reviews

1. Temidayo: I like the burst of ginger in the Zobo and how the cinnamon is not too much in the Zobo
2. Olamide: I like the way sugar was not added to the Zobo drink and the Zobo is refreshing and healthy
3. Fathia: I like the burst of flavors in the Zobo drink
4. Mrs. Ayodele: I will buy again and again because of the cinnamon taste in it and how it complements other ingredients
5. Victoria: I like the way the ginger in the Zobo drink was not too dominant as it blends in with the other ingredients.
6. Chika: I do not like the taste because there's no sugar in it
7. Motunrayo: I love the deep, rich taste of this mulled Zobo. The spices blended beautifully.
8. Titi: This drink is so nice. I love the way the cinnamon and cloves give it a cozy, comforting feel. Way better than the regular Zobo
9. Oluwaseun: I have had Zobo drink before, but this mulled Zobo is unique. The tartness of hibiscus with the spices is unique.
10. Oluwatoyin: I love the mix of cinnamon, cloves, it's nice. Not overpowering, it lingers with every sip.
11. Deborah: The cloves and cinnamon completely dominated the drink. It felt more like drinking spiced water than Zobo.
12. Ebube: It was not as sweet as I always want my Zobo drink to be.
13. Temilola: I found the tanginess overwhelming and it is not sweet enough.

Packaging Material

The packaging material used had the following characteristics:

1. Eco-friendly: The packaging material had minimal environmental impact throughout their lifecycle, from production to disposal.
2. Resilient: It protects the Zobo from external factors that could compromise its quality, such as physical damage, microbial contamination, and temperature fluctuations.
3. Convenient: Convenient packaging increases the product's accessibility and appeal, enhancing the consumer's overall experience.
4. Transparent: Transparent packaging allows consumers to see the product before purchase, which is especially beneficial for Zobo, given its visually appealing deep red color.
5. Recyclable: The packaging material used was designed to be processed and re-used in the production of new materials, contributing to a circular economy. As it helps reduce waste and resource consumption, aligning with growing consumer and regulatory demands for sustainability.
6. Versatile: Versatile packaging involves adapting to

various uses and formats which enhances marketability and consumer appeal by meeting diverse needs and extending the packaging's utility.

Figure 2: A close-up of the packaged finished product

Conclusion

The commercialization of Mulled Zobo product presents a lucrative opportunity to capitalize on the growing demand for authentic, natural beverages and an exciting opportunity to tap into a growing market of health-conscious consumers seeking innovative and flavorful beverage options. The findings support the viability of mulled Zobo as a commercially successful product. It leverages the increasing consumer preference for natural, functional beverages while offering a unique, culturally rich drinking experience. Overall, the project confirms that mulled Zobo has the potential to become a commercially successful product, leveraging its health benefits, cultural appeal, and innovative formulation. Further consideration of preservation methods and storage conditions to reduce microbial growth over time should be looked into.

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REFERENCES

Airaodion, A. I., Ayita, B. E., Oluba, O. S., Emaleku, A. S., Osunmuyiwa, J. O. and Megwas, U. M., (2021). "Chemical Composition and Neutraceutical Potential of Velvet Tamarind Fruit Pulp". *Asian Journal of Biochemistry, Genetics and Molecular Biology* 3.5: 22-31

Akinjayeju O., (2017). Quality Control for the Food Industry: A Statistical Approach, reprint. Concept Publications, Lagos Nigeria. 261:265.

Akujobi, I. C., Obicheozo, G., Nwokorie, C. U, (2018). Nutrient Composition, Phytochemical and Sensory Properties of Zobo (*Hibiscus sabdariffa*) Drinks Substituted with Pineapple (*Ananas comosus*) and Orange (*Citrus sinesis*) Juices. *Journal of Agriculture and Food Sciences* 16.2: 1-13

AOAC International, (2005). Official Methods of Analysis, Association of Official Analytical Chemists, 18th Edition. Gaithersburg, MD, USA. Method 925.10, Moisture Content Determination

AOAC International, (2005). Official Methods of Analysis, Association of Official Analytical Chemists, 18th Edition. Gaithersburg, MD, USA. Method 923.03, Ash Content Determination.

AOAC International, (2005). Official Methods of Analysis, Association of Official Analytical Chemists, 18th Edition. Gaithersburg, MD, USA. Method 960.52, Protein Content Determination.

AOAC International, (2005). Official Methods of Analysis, Association of Official Analytical Chemists, 18th Edition. Gaithersburg, MD, USA. Method 920.39, Fat Content Determination.

AOAC International, (2005). Official Methods of Analysis, Association of Official Analytical Chemists, 18th Edition. Gaithersburg, MD, USA. Method 962.09, Crude Fiber Content Determination.

Aqil, F., Gupta, A., Munagala R., Jeyabalan, J., Kausar, H., Sharma, R., Singh, I. P., and Gupta, R. C., (2012). Antioxidant and antiproliferative activities of anthocyanin/ellagitannin-enriched extracts from *Syzygium cumini L*. (Jamun, the Indian Blackberry) Nutr Cancer. 2012; 64(3): 428-438. doi:10.1080/01635581.2012.657766

Awolu, O. O., Aderinola, T. A., and Adebayo, I. A. (2013). Physiochemical and Rheological Behaviour of African Star Apple (*Chrysophyllum albidium*) Juice as Affected by Concentration and Temperature Variation. *Journal of Food Processing Technology* 4: 229. doi:10.4172/2157-7110.1000229.

Barbosa-Cánovas, G. V. (2015). Dehydration of Foods. Springer. Dhingra D, Michael M, Rajput R. (2017). Dietary fibre in foods: a review. *Journal of Food Science and Technology*. 49 (3):255–266.

Egbon E. E., Olayioye E. Y., Olayioye S. A., (2017). Phytochemical screening and proximate composition of *Phyllanthus amarus*. *International Research Journal of Plant Science*. 8(1):9–12.

Ekaete D, Umoh Ukana D, Akpabio. (2018). Phytochemical screening and nutrient analysis of *Phyllanthus amarus*. *Asian Journal of Plant Science and Research*. 3(4):116–122.

Eshwarappa, R. S. B., Iyer, R. S., Subbaramaiah, S. R., Richard, S. A., And Dhanajaya, B. L. (2014). Antioxidant activity of *Syzigium cumini* leaf gall extracts. Bioimpacts. 4(2): 101-107. doi:10.5681/bi.2014.018

Etemadi Razlighi, A., Doroudi, A., Nabeghvatan, N., Jadiri Ghalabi, E. & Smith, A., (2023). Study of the Nutritional, Antimicrobial and Chemical Properties of *Hibiscus sabdariffa:* Towards Findings Novel Natural Substance for Active Film. *Journal of Chemical Health Risks*.https://doi.org/10.22034/jchr.2023.1985978.1743

Fasoyiro, S. B., Olukayode, A. A., Abiodun, A., Folake, O. S, (2005). "Chemical and Storability of Fruit Flavored (*Hibiscus sabdariffa*) Drinks". *World Journal of Agricultural Sciences* 1.2: 161-164.

Food and Agriculture Organization, (2012). Microbiological Risk Assessment Series No. 17: Risk-Based Examples and Approach for Control of Microbiological Hazards in Foods.

Gbadegesin, A. O and Gbadamosi, S. O., (2017). Pineapple Flavoured Roselle Drink Concentrates: Nutritional, Physiochemical and Sensory Properties". *Animals of Food Science and Technology.* 18.2: 1-9

Gomez, S., Anjali, C., Kuruvila, B., Maneesha, P. K., and Joseph, M. (2023). Phytochemical constitution and antioxidant activity of functional herbal drink from Indian gooseberry (*Emblica officinalis* Gaertn.) fruits containing spices and condiments. *Food Production, Processing and Nutrition,*5(1), 1-13. https://doi.org/10.1186/s43014-022-00127-8

Ibrahim, S. A., El-Mansy, H. A., and Hamza, N. H. (2020). Hibiscus sabdariffa: Historical and medicinal perspective. *International Journal*

of Botany, 12(1), 29-38.
Ifesan BOT, Siripongvutikorn S, Hutadilok T.N. (2019). Evaluation of the ability of eleutherin, American crude extract as natural food additives. *Journal of food science.* 74(7):M353-M357.
International Commission on Microbiological Specifications for Foods (ICMSF), (2011). Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. Springer.
ISO 11290-1, (2017). Microbiology of the Food Chain, Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria spp*. — Part 1: Detection Method.
ISO 11290-1, (2017). Microbiology of the Food Chain, Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria spp*. — Part 1: Detection Method.
ISO 16649-2, (2001). Microbiology of food and animal feeding stuffs. Horizontal Method for the Enumeration of β-glucuronidase-positive Escherichia coli Part 2. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.
ISO 21527-1, (2008). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and molds. Part 1: Colony count technique in products with water activity greater than 0.95.
ISO 4833, (2006). Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Coliforms. Colony Count Technique.
ISO 4833-1, (2013). Microbiology of the food chain. Horizontal method for the enumeration of microorganisms Part 1: Colony count at 30 °C by the pour plate technique.
ISO 6888-1, (2021). Microbiology of the food chain. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 1: Method using Baird-Parker agar medium.
Jay, J. M., et al. (2015). *Modern Food Microbiology*. Springer.
Le Loir, Y., et al. (2015). Staphylococcus aureus and food poisoning. *Genetics and Molecular Research*, 2(1), 63-76.
Mohamad, N. R., Abdul Gani, S. S., Abdul Wahab, R., and Darham, W. (2022). Natural Antioxidant from Hibiscus sabdariffa Extract: Assessments on Extraction Yield, Antioxidant Capacity and Total Polyphenol Content of Different Polarities of Solvent Extracts. *Journal of Smart Science and Technology*, 2(2). https://doi.org/10.24191/jsst.v2i2.27
Mohamed, A. I., and Sulieman, A. M. E. (2007). Chemical composition and antioxidant attributes of *Hibiscus sabdariffa* calyces. *Journal of Food Science and Technology*, 44(4), 576–579.
Nivedha, K., Sivasakthi, s., Prakash, A., Devipriya, N., and Vadivel, V., (2020). In vitro studies on antioxidant and cyto-protective activities of poly-phenol-rich fraction isolated from *Mangifera indica* leaf. *South Africa Journal Bot*., 130, pp. 396-406
Nwankwo, C. S., Edozie, P. C., Okpomor, E. O., Mbachiantim, J. T., Okoyeuzu, C. F., Carew, I. E., Belay, D., Abebe, T. (2022). "Quality Evaluation of Zobo Tea Flavoured with Clove and Ginger Packed in Tea Bags". *African Journal of Agriculture and Food Science* 5.1: 1-10
Oboh, G., and Rocha, J. B. T. (2007). Antioxidant in foods: A new challenge for food processors. Leading Edge Antioxidants Research, 35–64.
Okolie, N. P., Anyiam, O. L., Idowu, O. H., Ilesanmi, Y. V. and Olawole, V. (2023). Production and Storage Stability of Mulled Zobo Drink Stored in Different Packaging Materials. *Journal of Nigerian Institute of Food Science and Technology*. NIFOJ 41(2), 1-11.
Okudu, H. O., Umoh, E. J., Ojinnaka., M. C., Chianakwalam, O. F, (2017). "Nutritional, Functional and Sensory Attributes of Jam from Velvet Tamarind Pulp". *African Journal of Food Science Journal* 11.2: 44-49.
Omar, S., Sidik, M. S., Mamat, A., and Sanny, M. (2023). Phytochemical analysis, total phenolic content and antioxidant activity of two varieties of *Hibiscus sabdariffa L*. leaves. *Journal of Tropical Resources and Sustainable Science*, 11(2), 41-48.
Rampersad, R. Badrie, N. and Comissiong, E. (2003). Physio-chemical and sensory characteristics of flavored snacks from extruded cassava/pigeon pea flour. *Journal of Food science.* SO 16649-2, (2001). Microbiology of food and animal feeding stuffs. Horizontal Method for the Enumeration of β-glucuronidase-positive Escherichia coli Part 2. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.
Truonga, V. D., Hua, Z., Thompson, A. R. L., Yenchob, G. C., Pecotab, K. V., (2012). Pressurized liquid extraction and quantification of anthocyanins in purple-fleshed sweet potato genotypes. *Journal of Food Compost Anal.* 26:96-103
Wang, C. J., Wang, J. M., Lin, W. L., Chu, C. Y., Chou, F. P., and Tseng, T. H. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology*, 38(5), 411–416. https://doi.org/10.1016/S0278-6915(00)00004-7
Yagi, S., Uba, A. I., Sinan, K. I., Piatti, D., Sagratini, G., Caprioli, G., Eltigani, S. M., Lazarova, I., and Zengin, G. (2023). Comparative Study on the Chemical Profile, Antioxidant Activity, and Enzyme Inhibition Capacity of Red and White Hibiscus sabdariffa Variety Calyces. ACS Omega, 8(45), 42511–42521. https://doi.org/10.1021/acsomega.3c05217
Zhang, Y., Wang, Y., and Wang, X. (2015). Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves. *Journal of Functional Foods*, 17, 1-10. https://doi.org/10.1016/j.jff.2015.04.027.