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**Research Paper**

# Functional and storage stabilities of functional beverages developed from crude extracts from tropical spices (*Zingiber officinale, Monodora myristica and Tetrapluera tetraptera*)

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## ABSTRACT

**This study envisaged the development of functional beverages from the extracts of tropical spices using pineapple juice as a carrier and the determination of the functional properties and the shelflife studies of the developed beverages. Crude extracts (essential oils) were extracted using a microwave-assisted extraction from dried Ginger, *T. tetraptera*, and *M. myristica*, and subsequently stored for further processing. Standard protocols were adopted for the processing of different extracts as well as in the beverage formulations. The physicochemical properties and the shelf life studies of the formulated beverage samples were determined using standard analytical procedures. The physicochemical properties revealed that the TTA values ranged from 1.92 to 3.28 and were recorded by the control and AGM, respectively. The pH values ranged from 3.66 to 4.83 and were recorded by the control and sample AGM. The viscosity values also ranged from 69.45 to 90.41, and values were ascribed to the control and sample AGT. The control had the lowest TSS value, while ATY gave the highest mean score of 4.60 for the TSS, the total sugars (brix) is highest for the control (7.17°B) and least for sample ATM with a mean score of 2.94°B. The shelf life studies of the formulated beverages revealed that the product containing no preservatives (citric acid) can stay on a shelf at room temperature for about four weeks, while it will last for more than eight weeks in refrigerated conditions.**

***Key words*:** Ginger, Microwave assisted extraction, Functional beverage, *Monodora myristica, Tetrapluera tetraptera,* Tropical spices, Physicochemical

## INTRODUCTION

Stress is a feeling of emotional or physical tension or an intrinsic/extrinsic stimulus that evokes a biological response. It can come from any event or thought that makes you feel frustrated, angry or nervous. Stress is your body's reaction to a challenge or demand. In short bursts, stress can be positive such as when it helps you avoid danger or meet a deadline. The compensatory responses to these stresses are known as stress responses (Habib *et al.* 2017). In response to the above stress-associated problems, people resort to the use of pills, drugs, synthetic drinks (often called energy drinks) and herbal preparations which some of whose clinical integrities are questionable. Nevertheless, most people do not prefer the use of synthetic drugs owing to some of their associated side effects, but rather prefer natural substances from roots, herbs and some tropical crops (spices) such as teas, coffee, ginger, turmeric, thyme, ginger as well as some fruits and cereals (Martin 2016). However, antistress drinks originate primarily from fruit and vegetable sources, but also include those from other plants such as ginger, tea, coffee, cocoa, soybean as well as animal products like milk and dairy-based and alcoholic drinks (Ekeledo *et al,* 2013). The health benefits of such beverages are rendered via different mechanistic pathways and are used to ward off stress-related ailments, as earlier stated. Thus, formulation of high-quality beverages containing extracts from natural sources (spices) such as ginger, *Tetrapleura, and Monodora myristica,* which contain antistress components for promotion of health and disease prevention has not received critical attention. The phytochemicals present in plant foods including fruits and vegetables are responsible for the health benefits of such foods and the mode of action of phytochemicals present as well as their chemical composition is varied. However, compounds with antioxidant potentials

such as terpenes, Isoflavones, phenolics and polyphenolics appear to be important (Bryer 2005). These developments have intensified attention to the extraction of the bioactive compounds from most of the tropical spices.

The bioactive compounds are however, one of the major constituents of nutraceutical beverages. In addition, the development of biomarkers, the study of synergistic and antagonistic effects of nutraceutical-drug interactions deserves particular attention. More so, some bioactive compounds, which have been confirmed to contain varying degrees of phytochemicals, antioxidants, stimulants and antistress components may also be incorporated in soft drinks to perform some specific health roles. Some studies have shown that about 80% health health-related problems and cardiac arrest are often associated with stress (Bone *et al.* 1999). Sequel to that, there are various natural components basically of plant origins that can be used to arrest stress in humans aside from being drug-based. Several foods and beverages that contain components of plant origin such as wines, fruit juices, etc, contain various essential nutrients ranging from carbohydrates, amino acids, vitamins, minerals, lipids, essential oils, etc., which play vital health roles in the body (Ofoedu *et al*. 2021). Also tropical spices such as ginger, thyme, curry, onion, garlic, etc. are said to contain various levels of phytochemicals (bioactive compounds) such as carotenes, flavonoids, phenols, polyphenols, terpenes, mycerene, limonene, pinene, steroids, triterpenes and many other antioxidants and essential oils numerous to mention. These tropical spices are sometimes used in herbal preparations but mostly in food preparations due to their health and therapeutic potentials (Ofoedu *et al*. 2021). However, little or no studies have been conducted on most of these tropical spices in the areas of their use in pharmaceutical and other health formulations hence, the current study on the extraction and evaluation of phytochemical potentials of tropical spices.

Stress has become a silent killer of individuals all over the world. Stress-induced health conditions such as ulcers, diabetes, cancers, etc., are on the increase globally. The fight against stress has assumed wider dimensions, ranging from the creation of leisure activities, drugs, and the production of food and drinks that can help individuals to overcome stress. Tropical spices are rich in essential oils with components such as antioxidants, phenols, carotenes, terpenes and terpenoids, among others, which are potent against physical and mental stress. However, these spices, which have enormous health and therapeutic benefits are not fully tapped/efficiently utilized for the production of nutraceutical beverages so as to solve the health needs of the populace ( Nwokenkwo *et al.* 2020 and Olawuni 2024).

The main objective of this research work is to determine the physicochemical attributes and shelf life stability of the functional beverages developed from the inclusion of crude extracts from tropical spices.

Understanding the types of bioactive compounds present in some tropical spices will inaugurate a new frontier in the effective utilization of these tropical spices. It is expected that the findings of this study will help in advancing the industry, aid future research and be of benefit to the farmers, as well as promotenutraceutical and allied industries and commerce also. Therefore, the scope of the work covers the sourcing, extraction and identification of the bioactive substances (photochemical) in tropical spices such as ginger (*Zingiber officinale*, Oshiokirisho (*Tetrapluera tetraptera)*, Ehuru (*Monodora myristica*).

## MATERIALS AND METHODS

### *Sources of materials*

### *Raw material collection*

Mature and freshly harvested ginger (*Zingiber officinale*) and dried Ehuru (*Monodora myristica*) seeds and fresh ripe pineapple fruits were purchased directly from the farmers in Owerri, Imo State. Mature dry *Oshiokirisho (Tetrapluera tetraptera)* fruits were obtained from the Federal University of Technology, Owerri, among the trees planted as windbreaks and ornamental plants within the university. The ginger *(Z. officinale), Oshiokirisho (T. tetraptera*) fruits, and *Ehuru (M. myristica)* were identified by a crop scientist at the Department of Crop Science, FUTO and then taken to the Department of Food Science and Technology Laboratory, Federal University of Technology, Owerri, where the sample preparations were carried out. The pictorial views of the tropical materials were shown in Plates 1 and 2.

### *Reagents, laboratory wares and equipment*

The various chemical reagents and laboratory wares/equipment used were of analytical grades. They were obtained from the Department of Food Science and Technology, Department of


Plate1. Freshly harvested Ginger and *Tetrapleura tetraptera.*

**Plate 2**. Left. Roasted seeds of *Monodora myristica*; Right, Deshelled roasted *Monodora myristica*.

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***Sample preparation and phytochemical screening for the bioactive compounds***

***Processing and extraction of ginger (Z. officinale) extracts***

The processing and extraction of wild ginger (*Z. officinale*) extract was done according to the method described by Jayashree *et al.* (2014). About 10 kg of freshly harvested ginger rhizomes were processed for extraction. The ginger was cleaned and washed with alcohol to decontaminate the surfaces from microbial loads. The washed ginger rhizomes were peeled to remove the bark. The peeled fingers of ginger rhizomes were sliced into smaller sizes 5, 10, 15, 20 and 30 mm long and dried under the sun for about five days before finishing-drying in an oven at an initial temperature of 55°C before it was elevated to 65 °C for 10 hours, which brought the moisture content from 88.7% down to 11.56%. The dried materials were milled into powdered forms (37µm) and stored in airtight containers for subsequent processing. Afterwards, it was followed by weighing 400 g of the milled samples into a flask and microwave-assisted extraction to generate the essential oils (crude extracts) required for the beverage formulation.

***Processing and extraction of bioactive compounds from Ehuru (Monodora myristica)***

The method described by Onwuka (2006) was adopted with slight modification. 2 kg of matured and dried Ehuru (*Monodora myristica*) seeds were sorted and gently roasted to enhance ease of dehulling. The dehulled seeds were further dried using a drying oven (at 105 °C) to ensure that the moisture contents were drastically reduced. The dried seeds were ground stored in airtight containers and subsequently, 400 g of the dried milled samples were prepared to extract the bioactive components/ essential oils (crude extracts).

***Processing and extraction of crude extracts from Oshiokirisho (Tetrapluera tetraptera) fruits***

The method described by Jayashree *et al.* (2014) was adopted for this extraction with slight modifications. About 20 pieces of matured and dried fruits of Oshiokirisho (*Tetrapluera tetraptera*) were used for this process. However, the fruits were cleaned and washed with an alcohol solution to disinfect the surface microorganisms. The washed fruits were dried in an electric oven (EUROSONIC, Model No. ES-9080, China) at 60°C. The dried pods were cut into pieces for easy grinding into powders. The ground samples were stored in an air-tight container and subsequently, 400 g of the *T. tetrapluetra* powder was subjected to microwave-assisted extraction to generate the bioactive component in the *T. tetraptera* fruits.

***Microwave-assisted extraction of the crude extracts***

Spigno and Faveri (2009) described the method

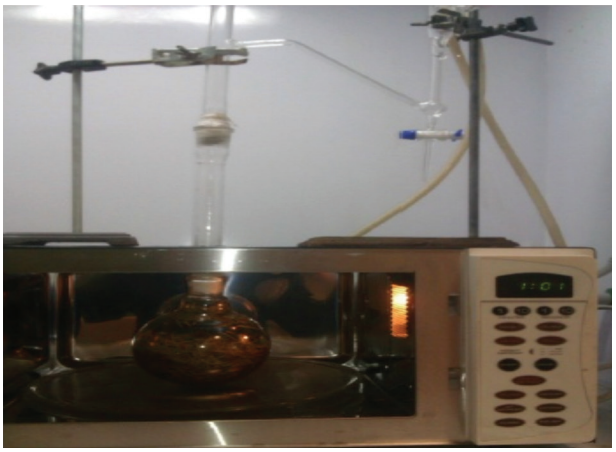

**Fig. 1.** A microwave-assisted extraction set-up (containing, retort stands, condensers and flat-bottomed flask holding the samples under extraction).

to extract the bioactive compounds. A 200 g of the milled, dried sample of the plant materials in each case was transferred into a flat-bottomed flask of 500 ml capacity and 250 ml of appropriate organic solvent (ethyl ether) was transferred into the flask containing the sample to be extracted. The flask was placed inside the microwave, stoppered and fitted to a condenser (a cooling system designed with a water inlet and outlet).

The microwaveis electric powered, taking into cognizance the predetermined temperature of the solvent, extraction time (30 minutes) and power (300W), the basic operating conditions for the MAE. The microwave generated from the magnetron is directed by the waveguide onto the sample/solvent system, thus causing the solvent to boil and rise within the vessel. However, the evaporating solvent was cooled by the water-cooled reflux condenser. This made the solvent condense and return to the holding vessel. This process was repeated for a short time, between 30 minutes, enabling the organic compounds to be desorbed from the sample matrix into the organic solvent. As the boiling process continued for about 30 minutes, the crude extracts (essential oil) were recovered by separating the solvent from the pool. This procedure was repeated for all the different dried (powdered) samples of the plant materials (*Zingiber officinale, M. Myristica,* and *T. tetraplegia*). The extract yield was determined and recorded in each of the cases.

***Production of pineapple juice***

The pineapple juice production process used for the functional beverage formulation was prepared according to a method described by Ojukwu *et al.* (2015) and in line with FDA (2022) guidelines, with slight modifications. A 20 kg of fully ripe and matured pineapples were graded, washed, and peeled. Then they were crushed in the crusher (juice extractor) to obtain the juice, and the juice extracted was transferred to a kettle and boiled. The extracted fruit juices were filled in clean plastic cans and pasteurized at 68$^{o}$C for 30 minutes. The cans containing the pasteurized juice were cooled quickly after sealing and stored for subsequent use.

***Development of the functional beverage***

The method, as described by FDA (2022), was used for the beverage formulation with slight modifications. A 5 ml of the crude extracts of Ginger (*Zingiber officinale*), Oshiokirisho (*Tetrapluera tetraptera*), and Ehuru (*Monodora myristica*) were accurately measured out, respectively, using a pipette. A 500 ml of the extracted pineapple juice was measured and transferred to each crude extract alongside other ingredients such as 500 ml of water, 3 drops of flavourings, 1 gram of acidity regulator (sodium citrate), 1 gram of binders (carboxyl methyl cellulose; CMC), etc. However, for the beverage containing the blends of the extracts, the ratio of formulation was 50:50 (i.e., 50% of each sample of crude extract, mixed with 50 % of another, with a combination of pineapple juice that was extracted). The mixture was continuously stirred until a homogenous solution was obtained. The resultant mixture was transferred into a kettle and sterilized at 65 $^{o}$C for 30 minutes. The resultant beverages were filled and packaged in clean, PET bottles and pasteurized at 60 $^{o}$C for 30 minutes before cooling. The crude extracts were also used in different combinations (proportions/ratios) to produce beverages using the same protocols. The beverage developed was stored at room temperature or refrigeration temperature.

***Determination of physicochemical properties of the beverage***

***Determination of total solids***

The method of Iwouno *et al.* (2019) was used for this determination. A 25 ml sample was weighed into a silica dish of known weight using an electronic balance (Gold Tech Precision Electronic Instrument Co. G.TET024, Delhi, India), and the value was recorded. The silica dish containing the sample was placed over a boiling water bath to evaporate excess water, and the evaporated sample was dried in an oven (EUROSONIC; Model No. ES-9080, China) at 100$^{o}$C for 2 hours. The dried residue was cooled and weighed, and returned to the oven. It was continuously dried until a constant weight was obtained. The percentage of dried matter was calculated as the percentage of total solids as follows:

$$Percentage\ total\ solids\ (TSS) = \frac{Mass\ of\ dried\ residue}{Mass\ of sample} X\ 100$$

***Determination of specific gravity***

The AOAC (2017) method was used to determine the specific gravity of the beverage samples. Thus, the specific gravity of the beverage samples was determined using a density bottle as stated below:

A clean and previously dried specific gravity

bottle of 50ml capacity was weighed with the stopper, and the readings obtained were recorded. The distilled water of 50 ml volume was weighed, and 50 ml of the test sample was measured using a measuring cylinder and substituted with water after drying the bottle. It was then weighed afterwards. The weight of the sample and the bottle was determined and recorded. The specific gravity of the beverage samples was calculated using the density of a reference sample (water). Thus, the Specific gravity was calculated as follows:

$$Specific\ gravity = \frac{Density\ of\ Sample}{Density of equal\ volume of\ water}$$

***Determination of pH***

The beverage pH was determined by Nwokenkwo *et al.* (2020) and AOAC (2019) procedure. The analysis was conducted using a portable (hand-held) pH meter or potentiometer (Tecnal model TEC-3MP, China) calibrated in pH 4.0 and pH 7.0 as indicated below:

The pH meter electrode was thoroughly rinsed with distilled water, and the reading was adjusted to zero mark. A 50 ml test sample was poured into a beaker, and the pH meter (previously calibrated) was immersed into the test samples. The sample was allowed to cover the pH meter electrode (probe), and the readings were taken and recorded.

***Determination of total titratable acidity***

The total titratable acidity was determined by the method of AOAC (2017). The acidity was determined by titrimetric analysis using a sample of 1 ml of the formulated beverage diluted in 49 ml of distilled water. This diluted solution was neutralized with 0.1 N NaOH, using a solution of 1% phenolphthalein as an indicator.

***Determination of brix (sugar content)***

The apparent degrees brix ($^oB$) of the beverage samples was determined according to the method described by Iwouno *et al.* (2019) using a Milwaukee Digital refractometer. The glass prism of the digital Milwaukee refractometer was cleaned with distilled water and blotted with a clean wiper. A few drops of the test sample at 20$^o$C were placed on the refractometer's optical (sensitive) region using a micro-pipette, and the readings were noted from the visual display and thus, recorded.

***Viscosity determination***

The method used for this determination is described by Onwuka (2005) and Iwouno *et al.* (2019) using a viscometer. The Ostwald viscometer model-LVT (Brook field Engrg. Lab. Inc., M.A.0217 USA) was used to check the viscosities of the various beverage samples. That was achieved by measuring 200 ml of the sample in a beaker of 250 ml capacity. The viscometer was set to revolve at 250 revolutions per minute (rpm), and the readings were determined Sand recorded.

***Shelflife study***

Chukwu *et al.* (2017) reported the method to determine product shelflife, and it was also used in this research. The samples were placed in Scotch flasks with screw lids and stored at refrigeration condition (3-4$^o$C) and ambient temperature for a period of 60 days and were taken out at intervals; 0 day, 7$^{th}$ day, 14$^{th}$ day, 21$^{st}$ day, 28$^{th}$ day, 35$^{th}$ day, 42$^{nd}$ day, 56$^{th}$ day, 63$^{rd}$ day, 70$^{th}$ day, 80$^{th}$ and 90$^{th}$ day and the changes in the beverage samples (eg. colour, odour, pH, precipitates, phase separations, and sedimentations) were determined.

However, analysis of liquid phase separation of the beverages was carried out according to Baccouche *et al.* (2013) with little modifications. In brief, the samples in 10 ml screw caped glass tubes were kept at 4 °C to assess liquid separation under the natural gravity force. The sample tubes were regularly inspected several times at one-week intervals for 56 days. A layer of clear supernatant was found at the top of the tube when the sedimentation occurred, and the volume was recorded as an indication of the instability of the product. The liquid separated was represented as a percentage volume. The colour, phase separations, and off flavours were determined subjectively.

Sedimentation was determined according to the method of Gad *et al.* (2013) with a few modifications. An aliquot of 5 ml of the beverage samples was centrifuged at 3000×$g$ for 30 min at 25°C. The sedimentation value was indicated as a percentage of the total fluid weight by using the following equation according to Babosa *et al.* (2014):

$$\text{Sediment \%} = \frac{\text{Sediment formed}}{\text{Fluid total weight}} \times 100$$

## RESULTS AND DISCUSSION

***Physicochemical properties of the beverage***

The mean scores of the physicochemical properties of the beverages formulated are shown in Table 1. The total titrable acid (TTA), pH, Viscosity,

total suspended solids, and total sugars (Brix) values are stated in the table. From the results obtained, sample AGM recorded the highest mean score but was not statistically higher than ($p \geq 0.05$) samples ATY, AMY, and AGT, which recorded mean values of 3.21, 2.90, and 2.92, respectively. Similarly, sample AGY recorded a mean score of 2.60 for TTA and is not significantly higher than ($p \geq 0.05$) sample ATM with a mean score of 2.56. However, the latter shows no statistical difference ($p \geq 0.05$) between samples AMY and AGT. That implies that they are closely related. Not with standing, the control (CTY) had the lowest mean score of 1.92 for total acidity and was preceded by sample GTM with a value of 2.08. Thus, statistical analysis suggested that the control sample is not significantly lower than the sample GTM. Omobuwajo *et al.* (2003) reported a lower total titrable acidity in research conducted using *Monodora myristica.*

The pH of the formulated beverages clearly showed that the samples were acidic. Scientifically, pH is the measure of the acidity or alkalinity of a solution or substance. Sample CTY (control) recorded the lowest pH value of 3.66 and differed significantly ($p \leq 0.05$) from all other samples. Sample AGM recorded the highest mean pH score and differed significantly from all other samples analyzed. More so, sample AGY had a mean score of 4.50, significantly different from other samples. Samples ATM, AMY, ATY, AGT, and GTM scored 4.25, 4.28, 4.23, 4.16, and 4.18, respectively, thus, statistical analysis suggested they were not significantly higher than each other ($p \geq 0.05$). Therefore, the results showed that the control was more acidic than all the samples. The high acidity of the beverage samples could be attributed to the nature of the constituents of the carrier upon which the extracts were incorporated. The implications of the pH levels obtained in this work are that the formulated beverage is moderately acidic and can be suitable for consumption, as there might not be feelings of astringency or unpleasant aftertaste. It could also have some preservative effects on the beverage samples. However, the values obtained in this work suggested high acidity, as reported in the work already established by Ekeledo *et al.* (2013).

**Table 1.** The mean scores of the physicochemical properties of the beverages produced

| Samples | TTA (mg lactic acid) | pH | Viscosity (mPas) | TSS (mg/l) | Total sugars ($^o$Brix) |
|---|---|---|---|---|---|
| CTY | $1.92^{e} \pm 0.001$ | $3.66^{d} \pm 0.04$ | $69.45^{e} \pm 0.66$ | $2.93^{f} \pm 0.04$ | $7.17^{a} \pm 0.25$ |
| AGY | $2.60^{b} \pm 0.02$ | $4.50^{b} \pm 0.19$ | $70.29^{e} \pm 0.04$ | $5.38^{a} \pm 0.06$ | $6.22^{b} \pm 0.19$ |
| ATY | $3.21^{a} \pm 0.01$ | $4.23^{c} \pm 0.03$ | $60.71^{f} \pm 0.52$ | $4.60^{b} \pm 0.03$ | $4.20^{cd} \pm 0.00$ |
| AMY | $2.90^{ab} \pm 0.58$ | $4.28^{c} \pm 0.02$ | $77.47^{d} \pm 0.51$ | $2.94^{f} \pm 0.02$ | $4.10^{cd} \pm 0.10$ |
| AGM | $3.28^{a} \pm 0.00$ | $4.83^{a} \pm 0.02$ | $77.71^{d} \pm 0.84$ | $4.48^{c} \pm 0.04$ | $3.40^{e} \pm 0.10$ |
| ATM | $2.56^{b} \pm 0.00$ | $4.25^{c} \pm 0.03$ | $85.62^{b} \pm 0.42$ | $4.33^{d} \pm 0.03$ | $2.94^{f} \pm 0.04$ |
| AGT | $2.92^{ab} \pm 0.00$ | $4.16^{c} \pm 0.03$ | $90.41^{a} \pm 0.24$ | $4.38^{d} \pm 0.01$ | $3.94^{d} \pm 0.04$ |
| GTM | $2.08^{c} \pm 0.010$ | $4.18^{c} \pm 0.03$ | $81.45^{e} \pm 0.68$ | $3.46^{e} \pm 0.02$ | $3.46^{e} \pm 0.02$ |
| LSD | 0.43 | 0.15 | 1.15 | 0.07 | 0.26 |

**Note:** CTY= Control (Eviron Health drink), AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts, AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* crude Extracts, AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts, GTM= 1:1:1 Ginger, *T. tetraptera and M. myristica* Crude Extracts.

**Table 2.** Results of the shelf life studies for the formulated beverage

| Parameters | Days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 35 | 42 | 56 | 63 | 70 | 80 | 90 |
| pH | 4.25 | 4.23 | 4.20 | 4.15 | 4.00 | 3.97 | 3.92 | 3.87 | 3.82 | 3.78 | 3.60 |
| Sedimentation (%) | 7.01 | 7.12 | 7.57 | 7.04 | 7.02 | 7.52 | 7.09 | 7.88 | 7.9 | 8.54 | 8.60 |
| Off flavours | - | - | - | - | + | + | + | ++ | ++ | ++ | +++ |
| Off colour | - | - | - | - | - | - | - | + | + | + | ++ |
| Precipitate | - | - | - | - | - | + | + | ++ | ++ | +++ | +++ |
| Phase Separation | - | - | - | - | - | + | + | ++ | ++ | +++ | +++ |

Note: (-) =Absent; (+)=Present

The viscosity of the beverage samples was also recorded and from Table 1, it is observed that sample AGT had the highest mean score of 90.41, while ATY had the lowest score of 60.71. As mentioned earlier, the two samples are significantly different and differ greatly from all other samples ($p \leq 0.05$). Sample ATM with a value of 85.62 varies significantly from all other samples ($p \leq 0.05$), likewise, sample GTM with a mean score of 81.45. Samples AMY and AGM scored 77.47 and 77.71, respectively. Thus, sample AMY is not significantly lower ($p \geq 0.05$) than sample AGM. In the same manner, sample AGY and CTY (Control) are not significantly different ($p \geq 0.05$) from each other, with each scoring 70.29 and 69.45, respectively.

The total soluble solids can be regarded as the total suspended solids. It is also the measure of the clarity of a solution in the sense that it confers clarity to a beverage mixture or any liquid sample solution. Thus, only samples ATM and AGT with respective means scores of 4.33 and 4.38 mg/l showed similarities in that they are not significantly different ($p \leq 0.05$). Samples AMY with a value of 2.94 mg/l were not significantly higher than the control, which recorded a mean score of 2.93 mg/l and the lowest value. Nevertheless, sample ATY had the highest mean score of 5.38, followed by ATY with a value of 4.60 mg/l. However, sample AGM and GTM scored 4.48 and 3.46°Brix respectively. As such, they differ significantly from each other ($p \leq 0.05$). The means recorded here compared favourably with the work reported by (Prasad and Tyagi 2015). The total sugar measures the Brix level of any liquid beverage. The means scores recorded showed that the Brix levels for the different samples vary from each other. The control sample gave the highest mean value of 7.17 °Brix, significantly different from all other samples. Statistical analysis also revealed that sample AGY differs significantly from every sample with a Brix value of 6.22 °Brix. Sample ATY and AMY are related to sample AGT in that they are not significantly different ($p \geq 0.05$), scoring 4.20, 4.10 and 3.94 °Brix, respectively.

Moreover, the sample AGM and GTM recorded mean values of 3.40 and 3.46. Statistically, the former is not significantly higher than ($p \geq 0.05$) the latter. The total brix (°Brix) measures the sugar level in a beverage, and it is already known that sugars are one of the major constituents of beverages. Thus, the study recorded mean values for Brix between the ranges of 3.50 to 10.63 °Brix.

***Shelflife of the beverages***

The formulated beverage samples were subjected to shelflife studies by storing them on shelves at room temperature and at refrigeration conditions for an interval of 3 months. However, the pH was determined at intervals. The pH of the samples ranged from 4.25 to 3.60 during the period of storage. Therefore, the changes in the pH could be due to the chemical changes within the beverage samples. Thus, there was stability in the pH of the samples between 0 to 30 days of storage with a pH range of 4.25 to 4.00.

In addition, there were no colour changes, precipitate formation,or off-flavour development observed during the initial day (0-7$^{th}$ day) of production to 21 days of storage. There were noticeable colour changes and slight off-flavour developments on samples ATY, AMY, and AMT within 42 days of storage, while the changes became intensified within 60 to 90 days of storage. Some of the beverages tend to be clearer due to settling under gravity, leading to phase separations, while the ones under refrigeration conditions remained unchanged. There were no phase separations and precipitates noticed within a one-month storage duration, while the changes were observed from 42 days to 90 days of storage period. On the 80$^{th}$ day, the samples stored on the shelf showed more precipitates and phase separations, while for the samples under refrigeration the colour change and layer formation were slightly noticed Figure 2. Therefore, the changes could be attributed to the different compositions as well as the chemical components of the different samples of the beverage. Furthermore, the sedimentation values for the samples ranged from 7.01% to 8.06%. However, between 0 to 30 days of storage, the sedimentation rate ranged between 7.01% to 7.02%. Thus, it implies that the sedimentation rates were stable until the value increased from 42 days storage periods, with a value ranging from 7.52% to 8.60%. At 90 days storage periods, there were increased colour changes, off-flavour formation, and phase separations of the beverage samples. Similar research conducted by Chukwu *et al.* (2017) also revealed that most beverages stored under shelves instead of refrigeration temperature tend to form gas bubbles within 7 to 8 weeks of storage due to microbial spoilage, due to some circumstantial and intrinsic factors such as the nature and the compositions of the samples of the beverage materials. The implications of this are that the sample could not be stored on a shelf over a prolonged period

of time. Notwithstanding, under refrigeration conditions, some of the samples showed no distinct changes, while some of the formed separation of a few samples and the formation of sediments. This implied that the samples, when stored over a prolonged period of time, can settle under gravity (sedimentation), as such, would need clarification and suitable stabilizers and emulsifiers to hold both the solid and liquid/oil phases together.


**Fig. 2.** The samples of the formulated functional beverages.

## CONCLUSION

The Ehuru (*Monodora myristica)* yielded more crude extracts (essential oils) than other spice samples (*Zingiber officinale* and *Tetrapluera tetraptera*) using microwave-assisted extraction. The physicochemical properties of the samples showed they are moderately acidic, falling within the pH range of 4.16 to 4.83, in relation to the control sample (Environmental Health drink), with a higher acidity of 3.66. The samples also showed to contain less total sugar than the control. The shelf-life studies of the formulated beverages revealed that the product containing no preservatives (citric acid), can stay on a shelf at room temperature for about four weeks, while it will last for more than eight weeks in refrigerated conditions. Not withstanding, further research is needed to optimize the formulation of the functional beverage, including the selection of ingredients, the ratio of ingredients, the use of emulsifiers, and the addition of other active ingredients to enhance the shelf stability of the beverage.

## CONFLICT OF INTEREST

No competing interest exist.

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