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Physicochemical and Shelf Life Studies of Functional Beverages Developed from Crude Extracts from Tropical Spices (*Zingiber officinale, Monodora myristica* and *Tetrapluera tetraptera*)

A.F. Ofoedum[1], C.I. Owuamanam[1], J.O. Iwouno[1], C.E. Ofoedu[1,2]
E.C. Chikelu[3], E.N. Odimegwu[1], S.O. Alagbaoso[1], N.E. Njoku[1], E.J. Anaeke[1]

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ABSTRACT

Background: This study envisaged the development of functional beverages from the extracts of tropical spices using pineapple juice as a carrier and determination of the functional properties and the shelf life studies of the developed beverages. A functional beverage can be defined as "any non-alcoholic drink that provides additional health benefits due to the inclusion of any bioactive component from a plant, animal, marine or microorganism source. Crude extracts (essential oils) were extracted using a microwave assisted extraction from dried Ginger, *T. tetraptera* and *M. myristica* and subsequently evaluated. Quantitative phytochemicals and antioxidant activities were determined in the crude extracts.

Methods: Crude extracts 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.

Result: 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 and more than eight weeks in refrigeration condition.

Key words: Functional beverage, Microwave assisted extraction, Physicochemical, Shelf life, Tropical spices.

INTRODUCTION

Stress is a feeling of emotional or physical tension or intrinsic/extrinsic stimulus that evokes 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), herbal preparation which some of their 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 substance 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 fruits and vegetables 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,

[1]Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria.
[2]School of Agriculture, Food and Wine, Faculty of Sciences, Engineering and Technology, University of Adelaide, Australia.
[3]School of Chemistry and Material Science, Nanjing University of Information Science and Technology, China.

Corresponding Author: A.F. Ofoedum, Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria. Email: ofoedum.arizona.edu@gmail.com

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Tetrapleura, Monodora myristica, which contain antistress components for promotion of health and disease prevention have not received critical attention. The phytochemicals present in plant foods, including fruits and vegetables, have been shown to be responsible for 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 and Odimegwu *et al.,* 2024). These developments have intensified attention to 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, development of biomarkers, study of synergistic and antagonistic effects of nutraceutical-drug interactions deserve 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.

Sequel to that, there are various natural components basically of plant origins that can be used to boost health in humans aside 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). However, little or no studies have been conduction on most of these tropical spices in the areas of their use in functional beverage formulations; hence the current study on extraction and evaluation of phytochemical potentials.

Tropical spices are rich in essential oils with components such as antioxidants, phenols, carotenes, terpenes, terpenoids, among others which are potent against physical and mental stress (Nwokenkwo *et al.*, 2020 and Olawuni *et al.,* 2024).

The main objective of this research work is to determine the physicochemical attributes and shelf life stability of the functional beverages developed from inclusion of crude extracts from tropical spices. It is expected that the findings of this study will help in advancing the industry, aid future researches and be of benefit to the farmers, as well as promote nutraceutical 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.

MATERIALS AND METHODS

Sources of materials

Raw material collection

Mature and freshly harvested *Zingiber officinale* and dried *Monodora myristica* seeds and fresh ripe pineapple fruits were purchased directly from the farmers in Owerri, Imo State. Mature dry *Tetrapluera tetraptera* fruits were obtained from the Federal University of Technology, Owerri, among the trees planted as wind-breaks and ornamental plants within the university.

Place and duration of the research

The research was conducted at Department of Food Science and Technology Laboratory, Federal University of Technology, Owerri, covering a period of 15 months.

Period of research

The period taken for the research was 15 months which commenced from August, 2023 to October, 2024.

Reagents and equipment used

They were obtained from the Department of Food Science and Technology, in the Federal University of Technology, Owerri, Nigeria and were of analytical grades.

Processing and extraction of ginger extracts

The processing and extraction of wild ginger (*Z. officinale*) extract was done according to the method described by Martin (2016). About 10 kg of freshly harvested ginger rhizomes were processed for extraction. The ginger was cleaned and washed with 98% alcohol to decontaminate the surfaces from microbial loads. The washed ginger rhizomes were manually peeled to remove the peels using knife.

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 finish-drying in an oven at 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 (37 µm) and stored in air-tight 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. The flow chart is represented in Fig 1.

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

The method described by Onwuka (2006) and Odimegwu, *et al.* (2024) was adopted with slight modification. A 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 which

is the used of microwave technique at varying temperature and frequency. 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 microor ganisms. The washed fruits were dried in an electric oven (EUROSONIC; Model No. ES-9080, China) at 60°C for 3 hours. The dried pods were cut into pieces and ground into powders then stored in an air-tight containers prior to extraction.

Microwave-assisted extraction (MAE) of the crude extracts

The method of Martin (2016) was adopted. 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 microwave is 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 was recovered by separating the solvent from the pool. This procedure was repeated for all the different samples of the plant materials.

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 kettle and boiled for 15 minutes. The extracted fruit juices were filled in clean glass bottles and pasteurized at 68°C for 30 minutes.

Development of the functional beverage

The method, as described by FDA (2022) and Ofoedum *et al*. (2024), used for the beverage formulation with slight modifications. A 5 ml of the crude extracts of *Zingiber officinale*, *Tetrapluera tetraptera* and *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 water, 3 drops of pineapple flavour, 1 gram of acidity regulator, 1 gram of 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 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 stainless vessel and sterilized at 65°C for 30 minutes. The resultant beverages were filled and packaged in clean, PET bottles and pasteurized at 60°C for 30 minutes before cooling (Fig 1). The beverage developed was stored at room and refrigeration temperature respectively.

Determination of physicochemical properties of the formulated beverage

Determination of total solids

The method of Iwouno *et al.* (2019) and Mohanapriya (2024) were 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 percentage of total solids as follows:

$$\text{Percentage total solida (TSS)} = \frac{\text{Mass of dried residue}}{\text{Mass of sample}} \times 100$$

Determination of specific gravity (S.G)

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. The specific gravity of the beverage samples was calculated using the density of a reference sample (water) as follows:

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

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.

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 (°B) of the beverage samples was determined according to the method described by Iwouno *et al.* (2019) using a Milwaukee Digital refractometer.

Viscosity determination

The method used for this determination is described by Onwuka (2005) and Uzoukwu *et al.* (2024) using a viscometer. The Ostwald viscometer model-LVT (Brook field Engrg. Lab. Inc., M.A.0217 U.S.A) was used to check the viscosities of the various beverage samples.

Shelf-life study

Chukwu *et al.* (2017) reported the method to determine product shelf-life 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°C) and ambient temperature for a period of 60 days and were taken out at intervals; 0 day, 7th day, 14th day, 21st day, 28th day, 35th day, 42nd day, 56th day, 63rd day, 70th day, 80th and 90th day and the changes in the beverage samples (*e.g.* 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 by varying the storage durations.

Sedimentation rate

Sedimentation rate was determined according to the method of Gad *et al.* (2013) with few modifications. The sedimentation value was indicated as a percentage of the total fluid weight by using the following equation according to Babosa *et al.* (2014):

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

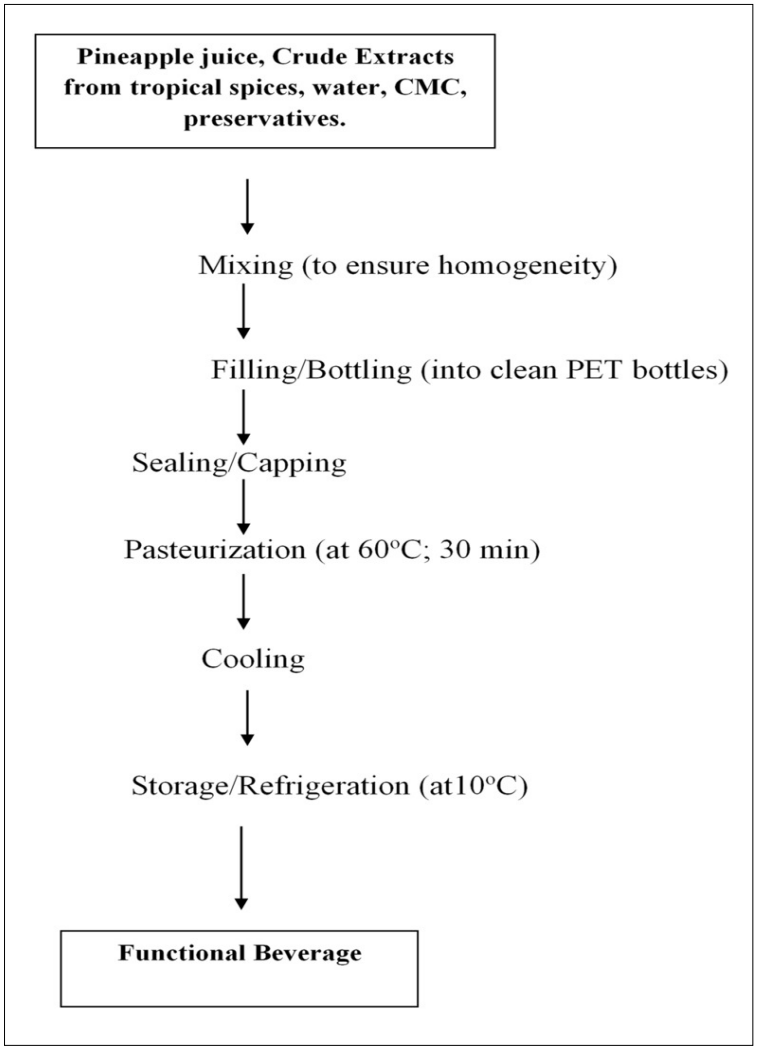


Fig 1: Formulation of functional beverage.

RESULTS AND DISCUSSION

Physicochemical properties of the beverage samples developed

The mean scores of the physicochemical characteristics of the beverages formulated were 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 for TTA 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. Notwithstanding, the control (CTY) had the least 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 was not significantly lower than sample GTM. Omobuwajo *et al.* (2003) and Ofoedum *et al.* (2024) reported a lower total titrable acidity in a research conducted using *Monodora myristica* when its quality was evaluated.

The pH of the formulated beverages clearly showed that the samples were acidic. Scientifically, pH is the measure the acidity or alkalinity of a solution or substance. Sample CTY (control) recorded the least pH value of 3.66 and differed significantly ($p \geq 0.05$) 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 sample 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 is 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 Iwouno *et al.* (2019).

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 least score of 60.71. As mentioned earlier, the two samples are significantly different and differ greatly from all other samples ($p \geq 0.05$). Sample ATM with a value of 85.62 varied significantly from all other samples, 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 are not significantly different from each other, with each scoring 70.29 and 69.45, respectively.

The total soluble solids can be regarded as the total suspended solids, otherwise referred to as Brix. 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

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

Samples	TTA (mg lactic acid)	pH	Viscosity (mPas)	TSS (^{o}Brix)	Total sugars (mg/l)
CTY	$1.92^{c}\pm0.001$	$3.66^{d}\pm0.04$	$69.45^{e}\pm0.66$	$2.93^{f}\pm0.04$	$7.17^{a}\pm0.25$
AGY	$2.60^{b}\pm0.02$	$4.50^{b}\pm0.19$	$70.29^{e}\pm0.04$	$5.38^{a}\pm0.06$	$6.22^{b}\pm0.19$
ATY	$3.21^{a}\pm0.01$	$4.23^{c}\pm0.03$	$60.71^{f}\pm0.52$	$4.60^{b}\pm0.03$	$4.20^{cd}\pm0.00$
AMY	$2.90^{ab}\pm0.58$	$4.28^{c}\pm0.02$	$77.47^{d}\pm0.51$	$2.94^{f}\pm0.02$	$4.10^{cd}\pm0.10$
AGM	$3.28^{a}\pm0.00$	$4.83^{a}\pm0.02$	$77.71^{d}\pm0.84$	$4.48^{c}\pm0.04$	$3.40^{e}\pm0.10$
ATM	$2.56^{b}\pm0.00$	$4.25^{c}\pm0.03$	$85.62^{b}\pm0.42$	$4.33^{d}\pm0.03$	$2.94^{f}\pm0.04$
AGT	$2.92^{ab}\pm0.00$	$4.16^{c}\pm0.03$	$90.41^{a}\pm0.24$	$4.38^{d}\pm0.01$	$3.94^{d}\pm0.04$
GTM	$2.08^{c}\pm0.010$	$4.18^{c}\pm0.03$	$81.45^{c}\pm0.68$	$3.46^{e}\pm0.02$	$3.46^{e}\pm0.02$
LSD	0.43	0.15	1.15	0.07	0.26

The means 'abc…' with different superscripts within the same column are significantly different ($p \leq 0.05$), while the means with the same superscripts are not significantly different.

Keys:

CTY= Control (Eviron health drink).

AGY= 100% Crude ginger extracts.

ATY= 100% *Tetrapluera tetraptera* crude extracts.

AMY= 100% *Monodora myristica* crude extracts.

AGT= 50:50 Ginger and *Tetrapluera tetraptera* crude extracts.

AGM= 50:50 Crude ginger *and Monodora myristica* extracts.

ATM= 50:50 *Tetrapluera tetraptera* and *Monodora myristica* crude extracts.

GTM= 1:1:1 Ginger, *Tetrapluera tetraptera* and *Monodora myristica* crude extracts.

Table 2: Results of the shelf life studies for the formulated beverage under ambient temperature.

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	-	-	-	-	-	+	+	++	++	+++	+++

Keys:

pH = Acidity/Alkalinity.

- = Absent.

+ = Present.

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. Samples AMY with a value of 2.94 mg/l was not significantly higher than the control, which recorded a mean score of 2.93 mg/l and the lowest value. Nevertheless, sample AGY 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 differed significantly from each other. The means recorded here compared favourably with the work reported by (Prasad and Tyagi, 2015). The total soluble solids 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 least mean value of 2.93°Brix, significantly different from all other samples. The results of the total sugars revealed that the control recorded the highest mean score of 7.17 mg/l. Statistical analysis also revealed that sample AGY differs significantly from every sample with total sugars of value 6.22 mg/l. 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 mg/l respectively.

Moreover, sample AGM and GTM recorded mean values of 3.40 and 3.46. Statistically, the former was not significantly higher than ($p \geq 0.05$) the latter. The total 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 2.94 to 7.17°Brix.

Shelf life studies on the beverages developed

The formulated beverage samples were subjected to shelf life studies by storing them on shelves at room temperature and at refrigeration condition for an interval of 3 months. The pH of the samples ranged from 4.25 to 3.60 during the period of storage (Table 2). Therefore, the changes in the pH could be due to the chemical changes within the beverage samples. Thus, there were stability in the pH of the samples between 0 to 35 days of storage with pH range of 4.25 to 4.00.

In addition, there were no colour changes, precipitate formation and off-flavour development observed during the initial day (0-7th 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 periods. On the 80th 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. Therefore, the changes could be attributed to the different constituents of the samples. Furthermore, the sedimentation values for the samples ranged from 7.01% to 8.60%. However, between 0 to 30 days storage, the sedimentation rate ranged between 7.01 % to 7.02%. Thus, it implies that there were stability in the sedimentation rates 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 beverages 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 is that the sample could not be stored under shelf over a prolong period of time. Notwithstanding, under ambient conditions, some of the samples showed no distinct changes while some of the formed separation in 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).

CONCLUSION

The *Monodora myristica* yielded more crude extracts (essential oils) than other spice samples using microwave-assisted extraction.

The physicochemical properties of the samples showed they were moderately acidic, falling within the pH range of 4.16 to 4.83, in relation to the control sample with higher acidity of 3.66. The samples also showed to contain less total sugars than the control. The shelf life studies of the formulated beverages revealed that the product containing no preservatives can stay on a shelf at room temperature for about four weeks while it will last for more than eight weeks in refrigeration condition.

Notwithstanding, further research is needed to optimize the formulation of the functional beverage, including the selection of ingredients, the ratio of ingredients, use of emulsifiers and the addition of other active ingredients to enhance the shelf stability of the beverage.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not stand the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

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Informed consent

This research does not involve any animal studies or human specimens.

Conflict of interest

All authors declared that there is no conflict of interest.

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