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Antioxidant and Sensory Properties of New Beverage Formulations Composed of Palm Sugar, *Aframomum melegueta*, and Citric Acid

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Abstract: Non-alcoholic still beverages were prepared from palm sugar, *Aframomum melegueta* pepper, and citric acid, and their physico-chemical, nutritional, antioxidative, and sensory properties were examined in order to determine their suitability as functional refreshing drinks of good nutritional value. Results for titrable acidity, pH, 5-hydroxymethylfurfural (5-HMF), and antioxidant capacity (total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and reducing power), vitamin C, and carbohydrate content indicate that the beverage formulations had suitable chemical, nutritional, and antioxidant characteristics, and may be functional. Sensory evaluation of the formulations showed that they were acceptable and refreshing, thus presenting attractive ways of delivering the health benefits of oil palm sugar and *Aframomum melegueta* pepper.

Keywords: palm sugar; *Aframomum melegueta* seed extract; citric acid; beverage formulations; antioxidant capacity

1. Introduction

Beverages are refreshing drinks. They include the carbonated non-alcoholic, and the non-carbonated or "still" beverages, such as fruit drinks and fruit juices. Another important group of beverages is characterised by a common property of having an initially stimulating effect. These include alcoholic beverages, tea, coffee, and cocoa [1]. Beverages provide water, an important nutrient which is essential for good health and the prevention of dehydration; some contain carbohydrates to provide a sweet taste and as a source of calories to meet the body's energy requirements, and either natural or added vitamins, especially vitamin C, which are required daily for good health [1].

Palm sugar is the brown sweetener derived from palm sap, the white semi-translucent, sugary liquid obtained by tapping the stalk of the immature inflorescence of palm trees, the upper stem, or by tapping the felled trees. In Nigeria, palm sap is obtained mainly from the African oil palm (*Elaeis guineensis* Jacq) and the raffia (*Raphia* spp) palms [2] and is usually left to ferment, and drunk as such (palm wine—fresh, or pasteurised and bottled), or distilled to yield a strong liquor.

Palm sugar has been used as a traditional sweetener for thousands of years [2]. It is now gaining popularity globally because it is considered to be natural and healthy. One of the major health claims is its low glycemic index (GI) [3,4]. Low GI foods play an important role in the dietary management of diabetes, weight reduction, peak sport performance, and the reduction of risks associated with heart disease and hypertension [4–8]. Other findings are the high in vitro α-amylase, α-glucosidase, and ACE (angiotensin I-converting enzyme) inhibitory activity of brown sugar preparations (notably palm sugar), with potential for low cost dietary management of type 2-diabetes and hypertension [9]. Oil palm sugar is rich in the minerals Ca, Fe, and K, and phenolic compounds; it exhibits considerable

antimicrobial activity against clinical stains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* [10].

The spice plant, *Aframomum melegueta* (Roscoe) K Schum (Zingiberaceae), is indigenous to the west coast of Africa from Guinea to Angola [11]. In Nigeria, its spicy seeds, known as alligator pepper, are commonly used, along with other spices, as ingredients in 'pepper soup', a peppery sauce, which may contain fish, chicken, beef, goat meat, or 'bush meat' (game). They are also chewed along with kolanut (*Cola spp*), a stimulant, and served along with the latter and alcoholic drinks to entertain guests [12]. In the food industry, alligator pepper forms an ingredient in non-alcoholic drinks, ice creams, and confectionery, and is used as flavouring in alcoholic beverages such as beer, wine, and gin [11]. The spice is rich in the minerals Ca, Mg, Fe, Zn, K, and Mn, with a modest content of protein and carbohydrate [13–15], and phytochemicals of medicinal value [16]; it features in traditional medicine [17–20]. However, although rich in minerals, with a modest content of protein and carbohydrate, alligator pepper is not usually considered to be important from the nutritional point of view, but is widely used as a spice and flavoring in food, and in ethno-medicine throughout the world for health benefits [21].

Several experiments have shown that *Aframomium melegueta* seed extracts may exert antioxidant and antibacterial effects [22,23], antidiarrheal action [24], anti-inflammatory properties [25], neuroprotective potentials due to the presence of quercetin and kaempferol [26], and manage erectile dysfunction [27]. The alkaloid fraction of the seeds exhibited ACE, acetyl cholinesterase (AChE), phosphodiesterase-5 (PDE-5), and arginase inhibitory activity; gas chromatographic analysis of the fraction revealed the presence of alkaloids, including senkirkine, angustifoline, undulatine, myristicin, safrole, lupanine, powelle, and indicine-N-oxide. The inhibition of these enzymes, according to the authors, could be the mechanism for the utilisation of the seeds for the management of erectile dysfunction in folk medicine [28]. The seed extracts have also been shown to contain four alpha-amylase and alpha-glucosidase inhibitory compounds, three arylalkanes (6-paradol, 6-gingerol, 6-shagaol), and a pentacyclic triterpene (oleanolic acid); of these, 6-gingerol and oleanolic acid exhibited higher inhibitory activity against these enzymes than the antidiabetic drug ascarbose [29,30]. Lawal et al. [31] reported the hypotensive and antihypertensive effects of *A. melegueta* seeds in both normotensive and hypertensive human subjects and suggested that these may be due to a central effect linked to peripheral vasodilatation. Gestational weight gain, which is one of the major causes of pre-eclampsia (elevated blood pressure during pregnancy), was reported to have been reduced by an aqueous extract of *A. melegueta* seeds [12].

Due to their medicinal properties, palm sugar and *A. melegueta* seeds may possess great potential for utilisation as a sweetener and flavouring in functional food and beverage formulations, thereby giving rise to value-added products and improving the income from their production and processing. In this study, oil palm sugar-sweetened, and *A. melegueta* pepper and citric acid-flavoured beverages, were prepared and their physico-chemical, antioxidant, and sensory properties were examined in order to determine their suitability as refreshing drinks of good nutritional value that may, in addition, provide other health benefits.

2. Materials and Methods

2.1. Materials

2.1.1. Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), 5-HMF, gallic acid, and β-carotene were obtained from Sigma-Aldrich Co. St. Louis, MO, USA. Citric acid, thiobabituric acid, 2,6–dichlorophenol indophenol, Folin-Ciocalteu reagent, methanol, potassium ferricyanide, ascorbic acid, BHT (butylated hydroxytoluene), trichloroacetic acid, linoleic acid, Tween 20, sodium hydroxide, monosodium phosphate, disodium phosphate, phosphoric acid, and phenolphthalein were obtained from Merck, Darmstadt, Germany. All reagents were analytical grade.

2.1.2. Palm Sap

Oil palm sap was obtained from the Nigerian Institute for Oil Palm Research near Benin City, Nigeria. The sap on analysis had a pH value of 3.73 and a titrable acidity value of 0.77%.

2.1.3. Alligator Pepper

Dried *A. melegueta* fruits were obtained from the kolanuts section of Oliha Market, Benin City, Nigeria. The seeds were extracted from the dried fruits and further dried to a constant weight at 40 °C in a ventilated oven (UNISCOPE SM 9053 Laboratory oven, Surgifriend Medicals, Essex, England).

2.2. Methods

2.2.1. Preparation of Palm Sugar

Palm sugar was prepared as follows: Oil palm sap with a pH of 3.73 and titrable acidity of 0.77% was filtered through a cheese cloth. The filtrate was then boiled in an open pan at around 100 °C until it turned brown and viscous; the syrup was cooled and its volume was measured. One and a half litres (1.5 L) of the sap gave 150 mL of syrup. On cooling, the thin syrup turned into a viscous gel.

2.2.2. Preparation of *A. melegueta* Pepper Cold Aqueous Extract

Dried seeds (50 g) were crushed in a mortar and the pieces were further reduced to a fine powder using a laboratory blender. Twenty g of the powder was placed in a beaker and made up to 100 mL with distilled water. The mixture was stirred for 10 min and kept in the refrigerator at 4 °C for three days. It was then stirred again for 10 min and filtered through a cheese cloth; the extract was collected in a conical flask, covered with aluminium foil, and stored at 4 °C in the refrigerator.

2.2.3. Beverage Formulation

Formulations (A, B, C, and D) containing palm sugar, citric acid, alligator pepper extract, and water were prepared as stated in Table 1.

Table 1. Beverage formulations.

Beverage Ingredients	Formulations			
	A	**B**	**C**	**D**
Citric acid	-	0.5 g	-	0.5 g
A. melegueta seed extract	-	-	10 mL	10 mL
Palm sugar	20 g	20 g	20 g	20 g
Final volume (made up with distilled water)	100.0 mL	100.0 mL	100.0 mL	100.0 mL

2.3. Analytical Procedure

2.3.1. Titrable Acidity

Titrable acidity of sap and beverage preparations was determined by the titration of 5 mL samples with 0.1 N NaOH, using phenolphthalein as an indicator, to a definite pink end point. Oil palm syrup (20 g) was made up to 100 mL with previously neutralised distilled water. Aliquots (5 mL) were then titrated with 0.1 N NaOH using phenolphthalein as an indicator. Titrable acidity was calculated as % lactic acid.

2.3.2. pH

The pH values of palm sap, palm sugar, and beverage formulations were measured using a Jenway Model 3505 pH meter (Camlab, Over, Cambridge, UK). Palm sap or beverage formulation (10 mL) was

read in triplicate. Palm syrup (2.0 g) was dissolved in 10.0 mL of distilled, deionised water and the pH of the solution was read in triplicate.

2.3.3. Total Carbohydrate Content

The total carbohydrate content of the oil palm syrup was determined using the anthrone method [32]. The syrup sample (0.5 mL, 20% aqueous syrup solution) was hydrolysed in a boiling water bath for 3 h with 5 mL, 2.5 N HCl. The mixture was cooled to ambient temperature and neutralised with Na_2CO_3 until the effervescence stopped. The volume was made up to 100 mL and centrifuged. Aliquots of the supernatant (0.5 mL) and 0.2, 0.4, 0.6, 0.8, and 1 mL of the glucose standard (0.1 mg/mL) were measured into test tubes and made up to 1 mL with distilled water; aliquots (4 mL) of the anthrone reagent were added to each. A blank was prepared using distilled water instead of sample or glucose standard. The tubes were heated for 8 min in a boiling water bath (Thermo Scientific Precision, General Purpose, Thermo Fisher, Loughborough, UK), and then cooled rapidly. Absorbance was read at 630 nm using a uv-visible spectrophotometer (GENESYS 10S, Thermo Fisher Scientific, Madison, WI, USA) and the carbohydrate content was calculated from the standard graph.

2.3.4. Glucose Content

Glucose content of the syrup was determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., Antrim, UK.), according to Buba et al. [33]. The sample or standard (20 µL) was allowed to react with 2.0 mL of the reagent, mixed well, and incubated for 10 min at 37 °C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read at 540 nm against a reagent blank within 60 min. Glucose was calculated as follows:

$$\text{Glucose content (mg / dL)} = (A_{sample} / A_{standard}) \times 100$$

2.3.5. Fructose Content

This was determined using the resorcinol reagent method [34]. Resorcinol reagent was prepared by dissolving 1 g resorcinol and 0.25 g thiourea in 100 mL glacial acetic acid, and 1.0 mL of this was added to each of the 2 mL aliquots of a 20% solution of the syrup and the standard solutions (containing 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL fructose) and mixed thoroughly; dilute HCl (7 mL) was then added. A reagent blank was also prepared along with the standard and treated in the same manner. The solutions were heated in a water bath at 80 °C for 10 min, cooled by immersion in tap water for 5 min, and their absorbance was read at 520 nm within 30 min. The fructose content was calculated from the standard graph.

2.3.6. Sucrose Content

Sucrose content was calculated as the difference between total carbohydrate content and the sum of the glucose and fructose content:

$$\text{Sucrose content} = \text{Total carbohydrate content} - (\text{glucose content} + \text{fructose content})$$

2.3.7. 5-Hydroxymethylfurfural (5-HMF) Content

Hydroxymethylfurfural (5-HMF) concentration was determined spectrophotometrically according to Rattanathanalerk et al. [35]. Palm sugar (10 g) was dissolved in 50 mL deionized water and centrifuged at 5000 rpm for 15 min. Aliquots (2 mL) of the supernatant (or 2 mL of each beverage formulation) were placed in test tubes and 2 mL of 12% trichloroacetic acid (TCA) and 2 mL of 0.025 M thiobabituric acid (TBA) were added and mixed thoroughly. The test tubes were placed in a water bath at 40 °C for 50 min and their contents were cooled to ambient temperature under water. The absorbance of the solutions was measured at 443 nm. A calibration graph plotted using different concentrations of 5-HMF was used to quantify the 5-HMF concentration. Determinations were done in triplicate.

2.3.8. Determination of Ascorbic Acid Content

Ascorbic acid content was determined titrimetrically by the 2,6-dichlorophenol indophenol method [36]. Briefly, standard indophenol solution was prepared by dissolving 50 mg 2,6-dichlorophenol indophenol in 100 mL water and standard ascorbic acid solution was prepared by dissolving 50 mg ascorbic acid in 60 mL 20% metaphosphoric acid and diluting to 250 mL with distilled water. The dye was then standardized by titration with the standard ascorbic acid solution. To 50 mL sugar solution alone or with citric acid and/or *A. melegueta* extract, 25 mL of 20% metaphosphoric acid was added and the solution was diluted to 250 mL with distilled water. Aliquots (10 mL) were titrated with standard indophenol until a pink colour persisted for 15 s. Vitamin C content was calculated as follows:

$$\text{Vitamin C (mg /100 mL)} = \text{Titre value} \times \text{Vitamin C/ mL indophenol solution} \times 100$$

2.3.9. Qualitative Analysis of Phytochemicals

The palm sugar and *A. melegueta* pepper extract were screened for the presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, and tannins according to Evans [37].

2.3.10. Determination of Antioxidant Capacity

The antioxidant capacity of palm sugar, the *A. melegueta* pepper extract, and formulations was evaluated by determination of their total phenolic content, β-carotene bleaching antioxidant activity, 2,2-diphenyl-1-picrylhyderazyl (DPPH) radical scavenging activity, and reducing power.

Total Phenolic Content

Total phenolic content (TPC) was determined spectrophotometrically according to the method described by Singleton et al. [38]. Aliquots (100 µL each) of sample (20% sugar solution or alligator pepper extract) and gallic acid standards (50, 100, and 150 up to 500 mg/L) were oxidized with 500 µL, 10% (v/v) Folin–Ciocalteu reagent and neutralised with 400 µL, 7.5% aqueous sodium carbonate. The reaction mixture was incubated in the dark for 40 min at ambient temperature and the absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration graph and expressed as mg gallic acid equivalent (GAE)/mL of sample. Determinations were carried out in triplicate.

β-Carotene Bleaching Antioxidant Activity

The β-carotene bleaching antioxidant activity of palm sugar, *A. melegueta* pepper extract, the formulations, and BHT, was determined as follows [39]: Briefly, β-carotene solution (0.2 mg β-carotene/mL of chloroform) was added to 0.02 mL of linoleic acid and 0.2 mL of Tween 20 in a round bottom flask. The chloroform was removed in a rotary evaporator at 40 °C. The resultant mixture was immediately diluted with 100 mL distilled water and mixed for 2 min to form an emulsion. A mixture prepared similarly without β-carotene was used as a blank. A control containing 0.2 mL of 70% ethanol instead of the sample was also prepared. Aliquots (5 mL) of the emulsion were transferred into test tubes, each of which contained 0.2 mL of each sample alone, and incubated at 50 °C in a water bath for 2 h. Absorbance was read at 470 nm and the percentage antioxidant activity was calculated as follows:

$$\text{Antioxidant activity } (\%) = 1 - [(A_0 - A_t)/(A_{0c} - A_{tc})] \times 100$$

where A_{0s} and A_{ts} are the absorbance values for the sample measured at time t = 0 and t = 120 min, respectively; and A_{0c} and A_{tc} are the absorbance values for the control at t = 0 and t = 120 min, respectively.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH radical scavenging activity of the various preparations and BHT was evaluated according to the method of Tang et al. [40]. One mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl in absolute ethanol was placed in a test tube containing 4 mL of the sample. A control was prepared by adding 1 mL of DPPH solution to 4 mL of 70% ethanol. Following storage in the dark for 30 min, the absorbance was read at 517 nm.

Percentage free radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity } (\%) = 1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}$$

Reducing Power

The reducing power of the palm sugar solution, BHT, and the formulations was determined according to the method of Oyaizu [41]. Aliquots (100 µL) of these were mixed with 250 µL, 200 mM sodium phosphate buffer (pH 6.6), and potassium ferricyanide (250 µL, 1%). The mixtures were incubated at 50 °C for 20 min. Tricholoroacetic acid (250 µL of a 10% solution) was added to each mixture and 250 µL of this was mixed with 250 µL distilled water and 0.5 mL, 0.1% $FeCl_3$. Absorbance was measured at 700 nm. Ascorbic acid was used as the standard and results were expressed as mg % Ascorbic Acid Equivalent [42].

2.3.11. Sensory Evaluation of Samples

A descriptive sensory analysis using verbal categorical scales was undertaken [43]. A description of colour, taste, odour, and texture of the formulations, based on sensory perception, was carried out by an untrained panel of six judges (three male and three female) selected from the final year biochemistry class of Benson Idahosa University, who apparently had no defect in their ability to perceive the characteristics examined. Labelled samples of the formulations (50 mL of each) in paper cups were placed on a table covered with white cardboard in a well- lit and ventilated room. Prior to inspection of samples, panellists were provided with a sheet containing the following descriptions of characteristics and were asked to record those which were closest to their observations (Table 2).

Table 2. Descriptive sensory evaluation sheet.

Colour	Colourless	Pale Yellow	Light Brown	Brown	Deep Brown
Smell	Bland	Palm sap	Orange juice	Orange juice + palm sap	Spicy, orange juice
Taste	Bland	Sweet	Sweet and sour	Sweet and peppery	Sweet, sour peppery and astringent
Mouth feel (feel between tongue and palate)	Light like water	Light and oily	Light, with friction (slightly abrasive)	Viscous and fatty (sticky)	Viscous andgrainy
Like/dislike	Dislike	Like, just a little	Like averagely	Like much	Like very much
Refreshing (pleasantly new, different and interesting): Yes/no	Different from the familiar, and unpleasant	Different, not unpleasant, but not interesting either.	Interesting, pleasant, but not new	New, pleasant, but just average	Pleasantly new, different and interesting

2.4. Statistical Analysis

Experimental replicates within individual experiments were averaged and expressed as mean ± SD. Comparisons between means were determined by an unpaired Student's *t* test with two-tailed *p*-values reported, employing Microsoft Excel 2007. Each experiment was replicated three times with comparable results. Results with $p < 0.05$ were regarded as statistically significant.

3. Results and Discussion

Lactic acid produced by lactic acid bacteria has been reported as the main organic acid responsible for the acidity of palm sap [44–47], palm syrup, and maple syrup [48]. In their study of the growth of yeast, lactic, and acetic acid bacteria in oil palm (*E. guineensis*) sap during the tapping of felled trees and its fermentation, Amoa-Awua et al. [44] found that *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were the dominant lactic acid bacteria on the first day, while acetic acid bacteria were only isolated after the third day, when levels of alcohol had become substantial. In a more recent study of microbiological and organic acid changes occurring in palm wine tapping of two oil palm varieties (dura and tenera), Karamoko et al. [49] concluded that lactic acid bacteria were largely responsible for the rapid acidification of the product as acetic acid bacteria were not isolated in the palm wine samples on the first days of tapping. Hence, a modest titrable acidity, with a corresponding low pH (such as that obtained in this study), indicates this initial fermentation step of palm sap during tapping.

The physico-chemical characteristics of the oil palm syrup used in the formulation are shown in Table 3.

Table 3. Chemical properties of oil palm syrup.

Characteristic	Value
Total carbohydrate (%)	66.22 ± 4.44
Sucrose (%)	60.25 ± 4.41
Glucose (%)	3.46 ± 0.00
Fructose (%)	2.53 ± 0.17
Titrable acidity (%)	3.60 ± 0.00
pH *	3.48 ± 0.01
Vitamin C (mg %)	170.0 ± 0.05
5-Hydroxymethylfurfural (mmol/kg)	1.009.0 ± 0.002

* Value for a 20% solution of the syrup.

Eze and Ogan [50] determined the sugar composition of unfermented (freeze-tapped) sap from oil palm trees in the plantations of the Nigerian Institute for Oil Palm Research, Benin City, which had a pH of 6.60, total sugar content of 11.61% (with sucrose accounting for 10.59%, and glucose and fructose 0.49 and 0.53%, respectively). Subsequent fermentation of the sap at 36–37 °C resulted in a rapid fall in sugar content to 10.46, and then 6.18%, with a corresponding decrease in pH from 6.60 to 5.14 and then 3.97 in 3 and 9 h, respectively, and a rapid rise in titrable acidity from 2.1 to 12.2 and then to 53 mmol H^+/L. In this study, the evaporation of palm sap to a tenth of its volume yielded syrup with a sugar content of 66.22%, corresponding to a sap sugar content of about 6.62%, indicating that it had undergone some lactic fermentation in the process of tapping and handling, resulting in a decrease in sugar content and a corresponding increase in titrable acidity to a value of 0.77, and decrease in pH to 3.73 (similar to the value of 3.97 recorded for oil palm sap and a sugar content of 6.18% previously found [50]). Also, sucrose was the dominant sugar and glucose and fructose were minor constituents; sugar content was relatively high, in agreement with the fact that the sap was tapped in the morning and preserved in ice shortly after collection, in order to minimize fermentation until its evaporation to produce the syrup.

The syrup had a considerable titrable acidity and vitamin C content. Titrable acids, as well as vitamin C, provide a desirable tart flavour in foods, and their presence gives the distinct impression of fresh fruit. Karamoko et al. [49] detected several organic acids in freshly collected *Elaeis guineensis* palm sap; these included oxalic, citric, tartaric, malic, ascorbic, formic, lactic, propionic, and acetic acids. However, according to the authors, three of these—oxalic, acetic, and propionic—were not native to the exudates, but were produced by adventitious organisms during the tapping process. The concentration of these organic acids in the syrup contributes to its flavour; according to Eze and Ogan [50], titrable acidity is an important determinant factor for the sour taste in wines. Vitamin C

is an antioxidant, which acts by donating hydrogen atoms to molecular oxygen. In doing so, it is preferentially oxidized. This ability to scavenge oxygen protects the flavour as well as the colour of an array of beverages [1]. Vitamin C plays an important role in the synthesis of collagen, by preventing the oxidation of ferrous iron cofactor of prolyl hydroxylase, thus offering protection against scurvy. Increased intake of this vitamin is associated with a reduced risk of chronic diseases such as cancer, cardiovascular disease, and cataract, probably through antioxidant mechanisms [51].

Hydroxymethyl furfural (5-HMF) is a decomposition product of fructose. It is formed during the heat treatment of foods (such as in the evaporation of palm sap to produce sugar) and is an indicator of the extent of this; it also contributes to the flavour of foods and may function as an antioxidant [52].

The phytochemicals present in the oil palm sugar and the aqueous extract of *A. melegueta* pepper are shown in Table 4.

Table 4. Phytochemical groups present in oil palm sugar and alligator pepper aqueous extract.

Phytochemical Group	Oil Palm Sugar	*A. melegueta* Pepper Aqueous Extract
Alkaloids	- [a]	+ [b]
Tannins	-	+
Saponins	+	+
Glycosides	+	+
Terpenoids	-	+
Flavonoids	+	+

[a] Absent; [b] Present.

The alligator pepper extract contained alkaloids, tannins, saponins, glycosides, terpenoids, and flavonoids; the palm sugar only contained glycosides, flavonoids, and saponins. These phytochemical groups contain dietary phytochemicals known to exhibit biological activity resulting in potential health benefits [27–29].

Antioxidant capacity values for the beverage ingredients—oil palm sugar solution and *A. melegueta* pepper extract—are given in Table 5. Included for comparison are values for butylated hydroxyquinone (BHT), a highly potent synthetic antioxidant used in foods.

Table 5. Antioxidant capacity values for oil palm sugar solution, *A. melegueta* pepper extract, and BHT.

Characteristic	Oil Palm Sugar [a]	*A. melegueta* Pepper Aqueous Extract	BHT [b]
Total phenolic content (mg GAE [c]/mL)	0.216 ± 0.010	0.130 ± 0.005	-
DPPH radical scavenging activity (%)	28.31 ± 0.99	12.40 ± 0.20	84.07 ± 2.55
β-carotene bleaching antioxidant activity (%)	26.58 ± 2.42	51.27 ± 0.57	72.00 ± 3.62
Reducing power (mg % AAE [d])	39.69 ± 0.77	-	5.12 ± 0.02

Values are expressed as mean ± SEM (n = 3). [a] 20% syrup in distilled water; [b] 0.02% in 70% ethanol; [c] GAE: Gallic Acid Equivalent. [d] AAE: Ascorbic Acid Equivalent.

The palm sugar and solution and *A. melegueta* pepper extract had a considerable total phenolic content. The total phenolic content assay by the Folin-Ciocalteu reagent is also a measure of the reducing capacity of a substance [53], and would include, in addition to phenolic compounds, non-phenolic compounds, including vitamin C, 5-HMF, and Maillard reaction products. Both solutions therefore exhibited a considerable reducing capacity. Although there are numerous phytochemicals consumed in the diet, polyphenols constitute the largest group, and have attracted much attention due to their antioxidant properties. The antioxidant activity of regularly consumed fruits and vegetables reflects their phenolic and vitamin C composition [54,55].

Compared with BHT, which had a high value, the palm sugar solution exhibited modest DPPH radical scavenging activity, while the aqueous *A. melegueta* extract had a lower level of activity, but a high β-carotene bleaching activity. The palm sugar solution exhibited moderate β-carotene

bleaching activity; both the sugar solution and the pepper extract had lower values than that of BHT. The palm sugar exhibited a high reducing power, but BHT had a relatively low value.

Some nutritional characteristics of beverage formulations containing the palm sugar, alligator pepper, and citric acid are shown in Table 6. All the preparations had an equal carbohydrate content.

Table 6. Chemical characteristics of beverage formulations.

Characteristic	Palm Sugar + Distilled Water	Palm Sugar + Citric Acid + Water	Palm Sugar + *A. melegueta* Pepper Extract + Water	Palm Sugar + Citric Acid + *A. melegueta* Pepper Extract + Water
Total carbohydrate (%) [a]	13.28 ± 0.89	13.28 ± 0.89	13.28 ± 0.89	13.28 ± 0.89
Titrable acidity (%)	0.72 ± 0.00	0.79 ± 0.00	0.19 ± 0.01	0.80 ± 0.02
pH	3.48 ± 0.01	3.12 ± 0.01	3.51 ± 0.01	3.14 ± 0.01
Vitamin C (mg/100 mL)	34.00 ± 0.10	34.00 ± 0.10	36.00 ± 0.00	35.00 ± 0.00
5-Hydroxymethyl furfural (5-HMF), µmol/L	201.70 ± 0.45	202.15 ± 0.53	201.87 ± 0.27	202.81 ± 0.95

[a] Calculated from the total carbohydrate content value in Table 3.

The addition of citric acid to the palm sugar solution resulted in a slight increase in titrable acidity and a corresponding decrease in pH. Addition of the *A. melegueta* extract to the palm sugar solution decreased its titrable acidity, with a slight increase in pH to 3.51, indicating the alkalinity of, and some neutralisation by, the extract. The combination of citric acid, palm sugar, and *A. melegueta* pepper extract had a higher titrable acidity, and lower pH, with these values being similar to the values for the sugar and citric acid formulation. The addition of citric acid did not cause large shifts in the pH values, indicating the ability of the palm sugar to buffer the effect of this acid, thereby preventing a sharp increase in acidity and the production of a very sour taste. Eze and Ogan [50] observed a similar phenomenon in (the increasing organic acid content of) fermenting oil palm sap, which they attributed to the efficient buffering of the protons by the weak organic acids produced. Formulations containing the alligator pepper extract had a vitamin C content (36.00 and 35.00 mg/100 mL) similar to those that did not (34 mg/100 mL); thus, each contained 57 to 60% of the recommended dietary allowance (RDA) of 60 mg/100 mL for the vitamin [51], with content comparable to those of mandarin, grapefruit, and lemon citrus varieties [56].

Vitamin C stability is inversely proportional to the pH of the medium in which it is present [57] and this compound, when present in juices of a lower pH, tends to be less susceptible to degradation. The optimum stability of ascorbic acid in the presence of oxidizing agents has been exhibited at a pH of about 3.0–4.5 [58], a range that includes the values for all the formulations in this study. The presence of heavy metals such as iron and copper in a beverage system can greatly accelerate vitamin C degradation, with a resulting decrease in beverage quality. The use of citric acid can promote vitamin C stability by rendering various heavy metals unavailable for the catalysis of its oxidation [1]. 5-HMF values were similar for all the formulations, indicating that this compound was mainly contributed by the palm sugar.

The antioxidant capacities of the palm sugar solution and formulations are shown in Table 7.

Table 7. Antioxidant capacity of beverage formulations.

Characteristic	Palm Sugar + Distilled Water	Palm Sugar + Citric Acid + Water	Palm Sugar + *A. melegueta* Pepper Extract + Water	Palm Sugar + *A. melegueta* Pepper Extract + Citric Acid + Water
DPPH radical scavenging activity (%)	28.31 ± 0.99 [a]	27.58 ± 0.79 [a]	54.10 ± 2.09 [b]	79.83 ± 0.60 [c]
Reducing power mg % ascorbic acid equivalent (AAE)	39.69 ± 0.77 [a]	39.91 ± 1.10 [a]	41.35 ± 2.56 [a]	45.19 ± 0.19 [b]

Data are reported as mean ± SD (n = 3); [abc] means with different superscripts on the same row differ significantly ($p < 0.05$).

Beverages containing palm sugar alone, and with added citric acid, exhibited a similar DPPH radical scavenging activity and reducing power; that which contained *A. melegueta* pepper and palm sugar had a higher DPPH radical scavenging activity, but similar reducing power. The higher DPPH radical scavenging activity was due to the additional phenolic content provided by the alligator pepper extract (0.013 mg GAE/mL, from a 10-fold dilution in the formulation), and lower acidity than for the beverage containing palm sugar alone. Higher DPPH radical quenching in less acidic media has also been observed in tea infusions [59], and it was postulated that the change in hydrogen ion concentration caused the change of the mechanism of the scavenging process of DPPH radicals by phenolic compounds, with the decrease in pH leading to the domination of the Proton Coupled Electron Transfer (PC-ET) mechanism [60].

The combination of citric acid, palm sugar, and alligator pepper exhibited the highest DPPH radical scavenging activity and reducing power. Citric acid has several important functions in beverages, including flavour modification, pH control, the chelation of metals, and catalysis of the conversion of sucrose to invert sugar [1]. Since citric acid had no significant effect on the antioxidant capacity when added to the palm sugar alone, and the synergistic effect observed when it was added to the combination of the palm sugar and *A. melegueta* extract probably resulted from its chelation of pro-oxidant metal(s) in the sugar and pepper extract, thereby making them unavailable for the catalysis of oxidative reactions. Indeed, Okwu [18] reported the presence of the pro-oxidant metals iron (1.80 ± 0.22 mg/100 g) and copper (0.63 ± 0.22 mg/100 g), and Odebunmi et al. [13] found an iron content of 37.80 ± 0.16 mg/kg in alligator pepper seeds. Oboh et al. [10] reported an iron content of 2.90 ± 0.14 mg/100 g in oil palm syrup. A higher DPPH scavenging activity, resulting from the removal of proxidant metal ions from tea infusions, has been observed by previous workers [59].

The DPPH [61] and pH [62,63] values of a number of commercial ready-to-drink beverages, which base their marketing strategies on antioxidant potency and are regarded as functional drinks [61], and values for the palm sugar-*A. melegueta* pepper-citric acid beverage formulations, are shown in Table 8.

Table 8. Comparison of DPPH and pH of formulations with reported values for beverages with reported health benefits.

Beverage	Average DPPH Radical Scavenging Activity (% Inhibition)	pH
Palm sugar + *A melegueta* pepper + citric acid + water	79.83 ± 0.60 [a]	3.14 ± 0.01 [a]
Palm sugar + *A. melegueta* pepper + water	54.10 ± 2.09 [a]	3.51 ± 0.01 [a]
Pomegranate	50.10 ± 1.90 [b]	2.93–3.20 [c]
Red wine	35.2 ± 2.2 [b]	3.30–3.50 [d]
Palm sugar + water	28.30 ± 0.99 [a]	3.48 ± 0.01 [a]
Concord grape juice	28.20 ± 6.10 [b]	2.80–3.00 [c]
Palm sugar + citric acid + water	27.58 ± 0.79 [a]	3.12 ± 0.01 [a]
Iced green tea	22.30 ± 2.60 [b]	3.72 ± 0.01 [c]
Blueberry juice	20.60 ± 1.40 [b]	3.11–3.33 [c]
Cranberry juice	19.20 ± 0.60 [b]	2.30–2.50 [c]
Acai juice	18.3 ± 1.2 [b]	2.05–3.50 [c]
Orange juice	12.70 ± 1.0 [b]	3.30–4.19 [d]

[a] This study, [b] [60], [c] [61], [d] [62].

The palm sugar and the palm sugar and citric acid solutions had higher DPPH assay values than iced green tea, blueberry juice, cranberry juice, acai palm juice, and orange juice. The palm sugar and alligator pepper formulation had a higher value than pomegranate juice and red wine. They all had acid pH values ranging from 2.05 to 4.19, which lie within the range for natural fruit juices. Thus, based on this favourable comparison, coupled with the high phenolic (and other phytochemicals) content and demonstrated health benefits of palm sugar and aqueous extracts of *A. melegueta* seeds, these beverages incorporating them may be regarded as functional.

The results of the sensory evaluation of the different formulations are presented in Table 9.

Table 9. Descriptive sensory characteristics of formulations.

Organoleptic Properties	Syrup + H_2O	Syrup + Citric Acid	Syrup + *A. melegueta* Pepper	Syrup + Citric Acid + *A. melegueta* Pepper
Colour	Light brown	Light brown	Light brown	Light brown
Smell	Fruity palm wine smell	Smells like orange juice. Has a slight palm wine smell.	Spicy, palm wine smell	Spicy, smells like orange juice. Has a slight palm wine smell
Taste	Sweet	Sweet and sour	Sweet and peppery; slightly astringent	Sweet, sour and peppery; slightly astringent
Mouth feel (between tongue and palate)	Light like water	Light like water	Light like water	Light like water,
Like/dislike	Like	Like much	Like much	Like very much
Refreshing (pleasantly new, different and interesting): Yes/ no	Yes. New, pleasant, but just average.	Yes. Pleasantly new, different and interesting.	Yes. Pleasantly new, different and interesting.	Yes. Pleasantly new, different and interesting.

All the formulations had a light brown colour. The palm sugar solution had a fruity palm wine smell. Citric acid is the preferred acidulant in the beverage industry because its pleasant sour taste and thirst quenching effect are characteristic of citrus fruits [1]. The addition of citric acid altered the taste and smell of the palm sugar solution. It introduced an orange juice smell, which was stronger than the fruity palm wine smell, as well as a pleasant sour taste and (possibly) protection for vitamin C. The formulation containing the alligator pepper extract and palm sugar had a spicy palm sap smell, and that containing palm sugar, alligator pepper, and citric acid had a spicy, orange juice, and a slight palm sap smell. The palm sugar solution alone had a sweet taste, while the combination of sugar with citric acid had a sour-sweet taste. The palm sugar and *A. melegueta* pepper formulation was sweet and slightly peppery, and the sugar solution containing citric acid and alligator pepper extract was sweet, sour, and slightly peppery. The formulations had a light and smooth mouth feel; in addition, formulations containing alligator pepper extract were mildly astringent. All the sensory characteristics observed were pleasant. The beverage formulation containing palm sugar, alligator pepper, and citric acid (which had the highest DPPH radical scavenging activity and reducing power) was the preferred product based on the sensory evaluation. Thus, the beverage formulations present pleasant ways for the delivery of the medicinal constituents of oil palm syrup and *A. melegueta* pepper, and the associated health benefits.

4. Conclusions

Non-alcoholic, non-carbonated drinks were formulated from sugar derived from the sap of the African oil palm (*Elaeis guineensis* Jacq) tree, the aqueous extract of *Aframomum melegueta* seeds, and citric acid. Their antioxidant capacity, and carbohydrate, vitamin C, and phytochemicals content, coupled with their pleasant organoleptic characteristics, indicate that they may be suitable for use as refreshing functional beverages.

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