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Physicochemical Properties and Phytochemical Components of Spiced Cucumber-Pineapple Fruit Drink

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ABSTRACT

A blend of equal portions of cucumber (50%) and pineapple (50%) juices was mixed with clove and ginger powder spices at 0.25% (CPCLG1), 0.5 (CPCLG2), 0.75% (CPCLG3) and 1% (CPCLG4) (w/v) respectively to develop a new fruit drink with health benefits. The juice blend without spice extract (CP) was used as reference sample. Samples were analysed for oBrix, pH, acidity, specific gravity, total solids and ascorbic acid (vitamin C). The phytochemicals present in the products were also determined using chemical method and Gas Chromatography-Mass Spectrometry (GC-MS). There was significant reduction in the oBrix from 8.08 to 7.60%, pH (4.41 to 4.36) and total solid (7.96 to 7.71%). Specific gravity was 1.020 while the acidity increased from 0.20 to 0.22% as the levels of spices increased. The phytochemical compounds identified in the products include alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and phlobatannins. The quantitative phytochemical contents showed 0.1 μg/ml Isoquinoline in CP, 0.03271 μg/ml Isoquinoline in CPCLG1; 0.00199 μg/ml Octanoid acid, 0.00224 μg/ml Metroprolol, 0.00231 μg/ml Fumaric acid, 0.00263 μg/ml Benzoquinone, 0.00264 μg/ml Betaxolol, and 0.002471 μg/ml 1-nonene in CPCLG2; 0.00070 μg/ml Limonene and 0.00146 μg/ml Caryophyllene in CPCLG3 and 0.00048 μg/ml P-Benzoquinone, 0.00074 μg/ml 4H-Quinolizine, 0.00017 μg/ml 3-pyridinepropanol, 0.00559 μg/ml Chlorogenic acid, 0.00559 μg/ml Camphene and 0.00089 μg/ml Benzofuranone in CPCLG4. The types and quantities of phytochemicals present in the products were influenced by the formulation of the products.

Keywords: Fruit juice blend, clove, ginger, physicochemical, phytochemicals.

Introduction

Non-alcoholic beverages, especially fruit drinks play a very important role on the diets of people in both developed and developing countries. These beverages are regarded as after meal drinks or refreshing drinks during the dry season in rural and urban centres. Fruits such as oranges and pineapples have been used as the main raw materials in these beverages (Osuntogun and Aboaba, 2004). Recently, consumers' demands have changed considerably with the realisation that foods and drinks (especially spiced fruit drink) contribute directly to good health. The increasing demand for such drinks can be explained by the increasing cost of healthcare, the steady increase in the life expectancy and the desire of older people to improve their health (Mollet and Rowland, 2002).

Nowadays, people are more proactive in taking an initiative to find a food or drink for preventing an illness rather than waiting to cure diseases (Wiki, 2009). Today, drinks are not intended to only satisfy thirst and to provide necessary nutrients for humans but also to prevent nutrition-related disease and improve physical and mental well-being of the consumers thus its function as functional drinks (e.g. spiced fruit drinks) play an outstanding role (Roberfroid, 2000 and Menrad, 2003).

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A fruit product that is non-alcoholic, ready to drink and which contains herbs, spices, vitamins, minerals, amino acids or additional raw fruit or vegetable ingredients in its formulation can be referred to as a functional beverage (Wiki, 2009; 2011a). These drinks are promoted with benefits such as good heart health, improved immunity, digestion, healthy joints, satiety and energy-boosting (Dionex, 2011). The concept of functional food was promoted in 1984 by Japanese scientists who studied the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems. In 1991, the Ministry of Health introduced rules for approval of a specific health-related food category called FOSHU (Food for Specific Health Uses) which included the establishment of specific health claim for this type of food (Roberfroid, 2000; Kwak and Jukes, 2001; Menrad, 2003; Burdock *et al.,* 2006).

In Nigeria, fruits like cucumber and spices (ginger and cloves) are being underutilised as there is no ready-to-drink juice produced from these ingredients (as at the time of this study) which contain some phytochemicals of health benefits. These phytochemicals have been considered to be of crucial nutritional importance in preventing chronic diseases such as cancer, cardiovascular diseases and diabetes (Aruoma, 2003). It has been discovered that regular consumption of fruits, vegetables, herbs and spices has always been associated with health benefits (Liu, 2004). Cucumber, clove and ginger contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals such as alkanoids, flavonoids, tannins, phlabotannins, steroids, saponins among others (Sheetal and Jamuna, 2009). These phytochemicals impart health benefits beyond basic nutrition (Oomah and Mazza, 2000) with the antioxidants having potential to reduce the risk of several deadly diseases in man (Agte *et al.*, 2000). Therefore, there is a great need to discover the presence and the potentials of these chemicals, in order to provide vital information for consumers.

Raw pineapple (*Ananas comosus*) juice of 200 – 250 ml is suggested for daily intake (Heun, 2007), the level of consumption of cucumber (*Cucumis sativus*) can be determined by the individual consumer's satisfaction for it is mostly water (about 90% of water), while clove oil has been listed as a "Generally Regarded As Safe" substances by the United States Food and Drug Administration when administered at levels not exceeding 1,500 ppm in all food categories (Jay and Rivers, 1984; Fischer *et al.*, 1990; Anderson *et al.*, 1997 and Waterstrat, 1999). Additionally, the Joint Food and Agriculture Organisation/World Health Organisation (WHO) Expert committee of Food Additives has established the acceptable daily human intake of eugenol in clove (*Eugenia caryophyllus*) at 2.5 mg/kg body weight for humans (Standard human = 60 kg), people in weight range of 50 – 100 kg could safely consume 125 – 250 mg eugenol (Fischer and Dengler, 1990; Nagababu and Lakshmaiah, 1992 and Anderson *et al.*, 1997). Ginger intake should not exceed 4 g daily (this includes the ginger obtained through diet such as from ginger ale, ginger snaps, and ginger bread). Usually, food sources contain not more than 0.5% ginger (Steven and Ehrlich, 2008).

Functional foods or drinks are meant to be taken as part of the regular diet. In some cases, one or more additional ingredients are added that impart health benefits above and beyond those of regular food (Stauffer, 1998). Up to date, in Nigeria, little attention is given to research on the development of functional spiced food/drink products and there is dearth of information on chemical composition of such product. As health awareness grows, more people are turning away from sweet carbonated drinks and are looking for healthier, lower calorie drinks as well as drinks that offer the functionality to meet the specific lifestyle. Thus, there is a need for the development of some spiced fruit drinks (functional drink). The ingredients such as ginger and cloves which are not normally present to any significant extent in pineapple and cucumber juice were introduced to make a spiced fruit drink.

Thus, the thrust of this study is to develop a new spiced fruit drink from the blend of pineapple, cucumber, clove and ginger as well as evaluate the physicochemical properties and the phytochemical constituents of the drink. It is hoped that such product could serve as a functional fruit drink product.

Materials and Methods

Materials

Ripe pineapple *(Ananas comosus)* fruits, cucumber (Cucumis sativus) fruits, cloves *(Eugenia carryophyllata)* and ginger *(Zingiber officinale)* were obtained from Ketu market, Lagos, Nigeria.

Production of spiced fruit drink

Spiced fruit drink was processed from ripe pineapple, green cucumber and spices (ginger and cloves). The pineapple fruits and cucumber were washed, peeled and sliced separately. The juice (single strength) was extracted from each sliced pineapple and cucumber separately using juice extractor. Ginger was dry cleaned, diced and milled into powder while clove was cleaned and also milled into powder. The fruit juices and spices were blended together using juice blender in proportions to get CP (50% cucumber and 50% pineapple), CPCLG1 (50% cucumber, 50% pineapple, 0.25% w/w clove and 0.25% w/w ginger), CPCLG2 (50% cucumber, 50% pineapple, 0.5% w/w clove and 0.5% w/w ginger), CPCLG3 (50% cucumber, 50% pineapple, 0.75% w/w clove and 0.75% w/w ginger), and CPCLG4 (50% cucumber, 50% pineapple, 1% w/w clove and 1% w/w ginger) respectively (Table 1). Each juice was filled into cleaned and sterile plastic bottles and capped properly. The bottled drinks were pasteurised at the temperature of 72°C for 15 mins in a water-bath (Clifton, England).

Total soluble solid content

The total soluble solid content (obrix) in the juice was determined using refractometric method, measured with an Abbe refractometer (Atago, Japan). The refractometer was standardised with distilled water at 20°C. Two drops of juice at 20°C was dropped on the lens (sensitive surface) of the refractometer and measured (AOAC, 2004).

pH and titratable acidity

pH and titratable acidity were determined using AOAC, (2004) procedure. Each drink (25 ml) was pipette into a beaker and inserted pH probe of the pH meter into it after the pH meter has been standardised in buffer 4 and 7 solution at 25°C , it was titrated against 0.1N NaOH solution until the pH meter displayed 8.10 as the end point. The total titratable acidity was then calculated as percentage citric acid as:

$$\% \text{ Acidity} = \frac{0.064 \times \text{Normality of NaOH} \times \text{Titre value} \times 100}{\text{mass of sample (g)}}$$

Titre value (MC) x 0.06404 = % citric acid
0.06404 = ml equivalent weight of citric acid.

Total solids

The total solids (TS) of the samples were determined using digital moisture analyser at 105°C (AOAC, 2004).

Specific gravity

The specific gravity of the sample was determined at 20°C using a density bottle (AOAC, 2004). The specific gravity was calculated as follows:

$$\text{Specific gravity} = \frac{B - C}{A - C}$$

A - Weight of distilled water at 20°C and density bottle
B - Weight of juice at 20°C and density bottle
C - Weight of empty density bottle.

Ascorbic acid content

Ascorbic acid was estimated by 2, 6 - Dichlorophenolindophenol visual titration method according to AOAC, (2004). Standard indophenol solution was prepared by dissolving 0.05 g of 2, 6 - dichloroindophenol in distilled water, made up to 100 ml and filtered. Standard solution of pure ascorbic acid was prepared by dissolving 0.05 g pure ascorbic acid in 60 ml of 20% acetic acid and dilute with distilled water to exactly 250 ml. The dye solution was then standardized against the ascorbic acid solution by titration with 10 ml of the ascorbic

acid solution until a faint pink colour persists for 15 sec. The concentration was then expressed as mg ascorbic acid equivalent to ml of the dye solution.

Qualitative analysis of phytochemicals

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals in the spiced fruit drink using standard procedure (Trease and Evans, 1989; Sofowora, 1993).

Test for tannins: $FeCl_3$ (0.1%) was added to 5 ml of the spiced fruit drink and observed for brownish green or a blue black colouration, which shows the presence of tannins.

Test for phlobatannins: A-10 ml of aqueous spiced fruit drink sample was boiled with 2 ml 1% HCl acid in a test tube. A deposition of a red precipitate that occurred indicated the presence of phlobatannins.

Test for saponins: A-20 ml of the spiced fruit drink sample was measured into test tube and boiled in a water bath, then filtered. Ten (10) ml of the filtered sample in a test tube was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

Test for flavonoids: Few drops of 1% NH_3 solution was added to the aqueous solution of spiced fruit drink in a test tube. A yellow coloration was observed which indicated the presence of flavonoid compounds.

Test for terpenoids: Five (5) ml of aqueous solution of spiced fruit drink sample was mixed with 2 ml of $CHCl_3$ in a test tube. Three (3) ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown colouration was formed which showed the presence of terpenoids.

Quantitative analysis using gas

Chromatography – Mass Spectrometer (GC-MS)

Quantitative analysis of phytochemicals of spiced fruit drinks was carried out using Gas Chromatography – Mass Spectrometer (GC-MS), Agilent Technologies Inc. Germany. The instrument employed the following conditions: Column HP5 column (30 mm× 1D 35 μM film 0.25 μM, composed of 100% Dimethyl poly siloxane), initial temperature 80°C to hold for 4 mins, ramp at 8°C/min to final temperature 310°C to hold for 12 mins; Helium (99.999%) was used as carrier gas at a constant flow of 3 ml/min and an injection volume of 1 μl was employed. Total GC running time was 37 mins while database NIST 08 was used.

Statistical analysis

Data obtained from the physicochemical study were subjected to Analysis of Variance (ANOVA) and means were separated by Duncan's New Multiple Range Test (DNMRT) using SPSS 17.0 software (SPSS, Inc, Chicago, IL).

Results and Discussion

Physicochemical properties of spiced fruit drink

Table 2 shows the °Brix, pH, acidity, total solid, specific gravity and ascorbic acid contents of freshly prepared spiced fruit drinks from the blends of CP, CPCLG1, CPCLG2, CPCLG3, and CPCLG4 respectively. Sample CP with the °Brix (8.08) was significantly different ($p < 0.05$) from other samples. The °Brix of CPCLG1 (7.97), CPCLG2 (7.77), CPCLG3 (7.67) and CPCLG4 (7.60) reduced as the amount of spices increased. Spices (especially clove, ginger, cinnamon, mace, anise, cardamom, and nutmeg) could reduce or eliminate sugar as recommended by Hertzler (2011). The pH values of CP (4.41) and CPCLG1 (4.39) were significantly different ($p < 0.05$) from CPCLG2 (4.37), CPCLG3 (4.36) and CPCLG4 (4.36) though the pH values were relatively similar which can be attributed to the proximity of the total titratable acidity (citric acid) of all the samples (0.21 to 0.22%) with no significant different ($p < 0.05$). According to Kaanane, *et al.*, (1988), the minimal change in pH can be explained by relationship existing between pH and free acid content. The high pH of a food is used as an indicator of bacterial spoilage (i.e. the food with high pH is more susceptible to microbial spoilage). The spiced fruit drinks were acidic in

nature therefore, becoming more resistant to bacterial spoilage (Richard and St-Piere, 2006).

There was no significant difference ($p > 0.05$) in the acidity of all the samples CP (0.21%) and CPCLG1 (0.21%), CPCLG2 (0.21%), CPCLG3 (0.21%) and CPCLG4 (0.22%). There was a significant difference ($p < 0.05$) in the total solid content of the spiced fruit drinks CP (7.96%) and CPCLG1 (7.80%) while no significant difference ($p > 0.05$) was noticed between samples CPCLG2 (7.70%), CPCLG3 (7.70%) and CPCLG4 (7.70%). Total solid is the amount of dry material remaining after all the water is evaporated (Richard and St-Pierrie, 2006).

Table 1: Percentage composition of fruit juice and spices in each sample

Sample code	Cucumber (%)	Pineapple (%)	Clove (%)	Ginger (%)
CP	50	50	0	0
CPCLG1	50	50	0.25	0.25
CPCLG2	50	50	0.50	0.50
CPCLG3	50	50	0.75	0.75
CPCLG4	50	50	1	1

CP, cucumber-pineapple juice, CPCLG, cucumber-pineapple-clove-ginger juice

The specific gravity of all the samples CP (1.021), CPCLG1 (1.021), CPCLG2 (1.021), CPCLG3 (1.022) and CPCLG4 (1.022) showed no significant difference ($p > 0.05$). This was in line with the amount stated for all beverages, non alcoholic (including soft drinks and juices) and fruit drinks (low calories and undiluted) as 1.01 – 1.03 (FAO, 2011). The specific gravity of a juice is often determined by the quantity of sugar or fruit present in that juice – the more sugar or fruit present in a juice, the denser the juice becomes (Science Bob, 2010). Fruit is one of the best low dense foods because of its high water content, which provides high volume and weight. To stay within low density guidelines, it is important to either consume fruit juice or whole fruit that has not been dehydrated than to eat whole fresh and processed fruits that do not contain added sugar (Ashley, 2011).

Table 2: Physicochemical composition of freshly prepared spiced fruit drink

Sample	°Brix (%)	pH	Acidity (%)	Total Solids (%)	Density(g/ml)	Vitamin C (mg/100 ml)
CP	8.08^{a}	4.41^{a}	0.21	7.96^{a}	1.02	36.67^{a}
CPCLG1	7.97^{b}	4.39^{b}	0.21	7.80^{b}	1.02	34.67^{b}
PCLG2	7.77^{c}	4.37^{c}	0.21	7.70^{c}	1.02	34.33^{bc}
CPCLG3	7.67^{d}	4.36^{c}	0.21	7.70^{c}	1.02	33.00^{c}
CPCLG4	7.60^{d}	4.36^{c}	0.22	7.70^{c}	1.02	31.00^{d}
			(NS)		(NS)	

Mean with the same superscript letter along the same column are not significantly different ($p < 0.05$); NS = Not significant. **CP,** cucumber-pineapple juice, **CPCLG**, cucumber-pineapple-clove-ginger juice

There were significant difference ($p < 0.05$) in the ascorbic acid (vitamin C) contents of the freshly prepared CP, CPCLG1, CPCLG3 and CPCLG4. Sample CP showed the highest value (36.67 g/100 ml) of ascorbic acid followed by CPCLG1 (34.67 g/100 ml), CPCLG2 (34.33 g/100 ml), CPCLG3 (33.00 g/100 ml) and CPCLG4 (31.00 g/100 ml) respectively. The amount of ascorbic acid in fresh spiced fruit drinks slightly decreased with increasing amount of spices; however, these were significantly lower than from the value for guava juice (80.1 mg/100 g), almost similar to passion fruit juice (39.1 mg/100 g) and higher than the value for lemon juice (10.5 mg/100 g) (Suntornsuk, *et al.,* 2002). Since ascorbic acid is soluble in water, it is readily lost via leaching from cut or bruised surfaces of raw material; however in processed foods the most significant losses result from chemical degradation (Tannenbaum, 1985). Transformation of ascorbic acid to diketoglutaric acid due to reaction with air and metal ions may also contribute to the losses encountered (Harris, 1975 and Addo, 1981).

Phytochemical constituents of spiced fruit drinks

The phytochemical constituents of the spiced fruit drink are shown in Table 3. The result of qualitative analysis on each spiced fruit drink showed the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and phlobatannins. CP contained alkaloids, flavonoids, steroids and terpenoids while CPCLG1 contained alkaloids, flavonoids, steroids, tannins and terpenoids. Alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and phlobatannins were found to be present in CPCLG2, CPCLG3 and CPCLG4. Saponins, tannins and phlobatannins were absent in CP while saponins and phlobatannins were absent in CPCLG1 and this may be due to the absence or very small percentage of spices (clove and ginger). These compounds are known to complex with protein in the body (Osuntogun, 1984 and Afolabi *et al.*, 1988).

Table 3: Qualitative analysis of phytochemical constituents of spiced fruit drink

Samples	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Terpenoid	Phlobatannins
CP	+	+	-	+	-	+	-
CPCLG1	+	+	-	+	+	+	-
CPCLG2	+	+	+	+	+	+	+
CPCLG3	+	+	+	+	+	+	+
CPCLG4	+	+	+	+	+	+	+

+ = present, – = absent

CP, cucumber-pineapple juice, **CPCLG**, cucumber-pineapple-clove-ginger juice

The curative properties of fruits and spices are perhaps due to the presence of various secondary metabolites which are the non-nutritive plant compounds. These classes (such as alkaloid, tannin, anthraquinone and flavonoid) of compounds are known to have curative activity against several pathogens and therefore could suggest the use traditionally/locally and scientifically for the treatment of various illnesses (Hassan *et al.*, 2004, Usman and Osuji, 2007). The phytochemicals are known to have antimicrobial activity (Ebana *et al.*, 1995). Tannin has been found to possess a stringent properties that hasten the healing of wounds and inflamed mucous membranes (Okwu, 2004, Kozioc, and Marcia, 1998). Tannin and flavonoid are thought to be responsible for anti-

diarrheal activity (Enzo, 2007). Usman and Osuji, (2007) reported that tannin has been widely applied topically to sprains, bruises and superficial wounds. Similarly, Elmarie and Johan, (2001) reported tannin to have antibacterial effect.

The presence of phlobatannin suggests the diuretic property of the plant (Awoyinka *et al.*, 2007). The terpenoids have also been shown to decrease blood sugar level in animal studies (Luo *et al.*, 1999). Steroids showed the analgesic properties (Sayyah *et al.*, 2004 and Malairajan *et al.*, 2006). Singh, (2006) reported the role of steroid as anti-inflammatory and analgesic agents. Steroid and Saponin are responsible for central nervous system activities (Argal and Pathak, 2006). Phytochemicals such as terpenoid, flavonoid, tannin, steroid and alkaloid have anti inflammatory effects (Manach *et al.*, 1996; Latha *et al.*, 1998; Liu, 2003; Akindele and Adeyemi, 2007 and Iikay *et al.*, 2007); Glycoside, flavonoid, tannin and alkaloid have hypoglycemic activities (Cherian and Augusti, 1995). Alkaloids are heterocyclic indole compounds which have been proved to have pharmacological properties such as hypotensive activity (Lata and Veenapani, 2010), anticonvulsant activity (Atefeh *et al.*, 2010), antiprotozoal, antimicrobial and antimalarial activities (Frederich, *et al.*, 2002). Mensah *et al.*, (2009) concluded that the plants which possessed tannin; cardiac glycoside and alkaloid, are the most effective for managing hypertension and gives protection for the heart. Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cell against all stages of carcinogenesis (Okwu, 2004). Flavonoid, in intestinal tract lowers the risk of heart disease (Okwu, 2005). Phenolic compounds are extensively used in disinfections and remain the standard with which other bactericides are compared (Gill, 1992). According to Lewis and Elvin-Lewis (1977), alkaloid and glycoside act with defense mechanism as phyto-protective agent against invading microorganism. According to Gonzalez *et al.*, (1994), the optimal effectiveness of a medicinal plant may not be due to one main active constituent, but to the combined action of different compounds originally in the plant.

The results of phytochemical quantitative analysis of spiced fruit drinks are shown in Table 4. The spiced drink CP had 100% Isoquinoline at 21.414 min retention time as shown in Figure 1. Figure 2 showed CPCLG1 with 32.71% Isoquinoline at 6.686 min retention time. Figure 3 showed CPCLG2 with 1.99% octanoid acid at 22.570 min retention time, 2.24% Metroprolol at 18.725 min retention time, 2.31% Fumaric acid at 17.277 min retention time, 2.63% Benzoquinone at 16.551 min retention time. 2.64% 2.64% Betaxolol (2.64%) at 20.081 min retention time, 2.74% Methallyl cyanide at 16.551 min retention time and 4.71% 1-nonene at 21.409 min retention time. CPCLG3 had 0.70% Limonene at 22.702 min retention time and 1.46% Caryophyllene (bicyclo) at 9.833 min retention time as shown in Figure 4. CPCLG4 had P-Benzoquinone (0.48%) at 25.986 min retention time, 0.74% 4H-Quinolizine at 16.745 min retention time, 0.17% Sesquiterpene (3-pyridinepropanol) at 8.111 min retention time, 5.59% Chlorogenic acid (cyclohexane) at 16.619 min retention time, 5.59% Camphene (methylidene) at 16.619 min retention time, 0.89% Benzofuranone (coumesterol) at 22.107 min retention time, 3.98% Sesquiterpenes (3-pyridineacetonitrile) at 8.111 min retention time of as shown in Figure 5.

In the phytochemicals quantitative analysis of spiced fruit drinks, CP and CPCLG1 contained 0.1 and 0.03271 μg/ml Isoquinoline respectively. The Isoquinoline derivatives are used as anti-hypertension agents (such as quinapril, quinapirilat, and debrisoquine). CPCLG2 contained 0.00199 μg/ml octanoid acid, 0.00224 μg/ml Metoprolol, 0.00231 μg/ml Fumaric acid, 0.00263 μg/ml Benzoquinone, 0.00264 μg/ml Betaxolol, 0.002471 μg/ml Methallyl cyanide and 0.002471 μg/ml 1-nonene. Octanoid acid has antifungal properties and could be used against yeast infection (Isabel, 2011). Metroprolol is a beta-adrenergic blocking agent that is used for treating high blood pressure, heart pain, abnormal rhythms of the heart and

some neurologic condition. It also reduces the force of contraction of heart muscles thereby lower blood pressure, and for treating migraine headache (Ferlex, 2011a). Fumaric acid serves as a food additive, it is used as an acidity regulator and is denoted by the E number E297. Fumaric acid which is non-toxic is a food acidulant used since 1946. It is generally used in beverages and baking powders for which requirements are placed on purity. It is generally used as a substitute for tartaric acid and occasionally in place of citric acid, at a rate of 1.36 g of citric acid to every 0.91 g of fumaric acid to add sourness, similar to the way malic acid is used. It is also used as a coagulant in stove top pudding mixes (Wiki, 2011b). Benzoquinone is an oxidizing agent and hydrogenation reagent. It also has antitumor (inhibition) activity and antibacterial effect. Betaxolol is a beta blocking agent and used for the management of hypertension, reduction of exercise heart rate and glaucoma (Lipworth *et al.*, 1991 and Farlex, 2011b), Methallyl cyanide and 1-nonene (C9H18 colourless clear liquid, naturally occurring also in cowpea) are used in food application as flavouring agent in snack food and condiments (Anonymous, 2011). CPCLG3 contained 0.00070 µg/ml d-Limonene, 0.00146 µg/ml Caryophyllene. D-Limonene is a phytonutrient in a class of chemicals called terpenes, d-limonene smells like oranges, it has been shown to induce apoptosis, which is the "death signal" of cancer cell, or prevents it from dividing. It also increases the levels of enzymes in the liver that can detoxify carcinogens. In animals, limonene has been shown to slow the growth of cancers of the pancreas, stomach, colon, skin and liver. It has been shown experimentally to have protective effects against certain types of cancer and was evaluated in phase I clinical trials with advanced cancer patients (Gould, 1997). Caryophyllene is a natural bicyclic sesquiterpene with a strong spicy woody odour and it is anaesthetic, antifungal, antiseptic and antibacterial agent (Beneforce, 2011). CPCLG4 contained 0.00048 µg/ml Benzoquinone, 0.00074 µg/ml 4H-Quinolizine, 0.00017 µg/ml 3-pyridinepropanol, 0.00559 µg/ml Chlorogenic acid, 0.00559 µg/ml Camphene (methylidene), – 0.00089 µg/ml Benzofuranone (coumesterol), 0.00398 µg/ml Sesquiterpenes (3-pyridineacetonitrile). Benzoquinone is an oxidizing, tannin agent and hydrogenation reagent. It also has antitumor (inhibition) activity and antimicrobacterial effect. 4H-Quinolizine is for the treatment of glaucoma while 3-pyridinepropanol is used to combat nausea, vomiting or dizziness associated with motion sickness and has been studied for its gentle, stimulating effects. Chlorogenic acid (cyclohexane) has therapeutic effect, it is a naturally occurring phenolic acid which is a carcinogenic inhibitor and has antibiotic and anti-inflammatory effect, it is also a potent antioxidant, regular ingestion helps the flow of bile and thus reduces the adverse effects of bile stagnation (Subhuti, 2006), camphene (methylidene) which contain anti-

Table 4: Quantitative analysis of phytochemicals of Spiced fruit drinks

Sample	Phytochemicals	Quantity (µg/ml)
CP	Isoquinoline	0.1
CPCLG1	Isoquinoline	0.03271
CPCLG2	Octanoid acid	0.00199
	Metroprolol	0.00224
	Fumaric acid	0.00231
	Benzoquinone	0.00263
	Betaxolol	0.00264
	1-nonene	0.00247
CPCLG3	Limonene	0.00070
	Caryophyllene	0.00146
CPCLG4	P-Benzoquinone	0.00048
	4H-Quinolizine	0.00074
	3-pyridinepropanol	0.00017
	Chlorogenic acid	0.00559
	Camphene	0.00559
	Benzofuranone	0.00089

CP, cucumber-pineapple juice, CPCLG, cucumber-pineapple- clove-ginger juice

fungal properties, as well as anti-bacterial qualities thus often used to treat bacterial infections in the intestines. It could also be used in the treatment of wounds on the skin (DHC, 2011). Benzofuranone (coumesterol) is a phytochemical that belongs to the group called coumestans and is a natural organic compound that is thought to decrease risk of stomach cancer, coumestrol could help reduce risk of prostate and breast cancer by preventing estradiol binding to estrogen receptors (Farlex, 2011c). Sesquiterpenes (3-pyridineaceto

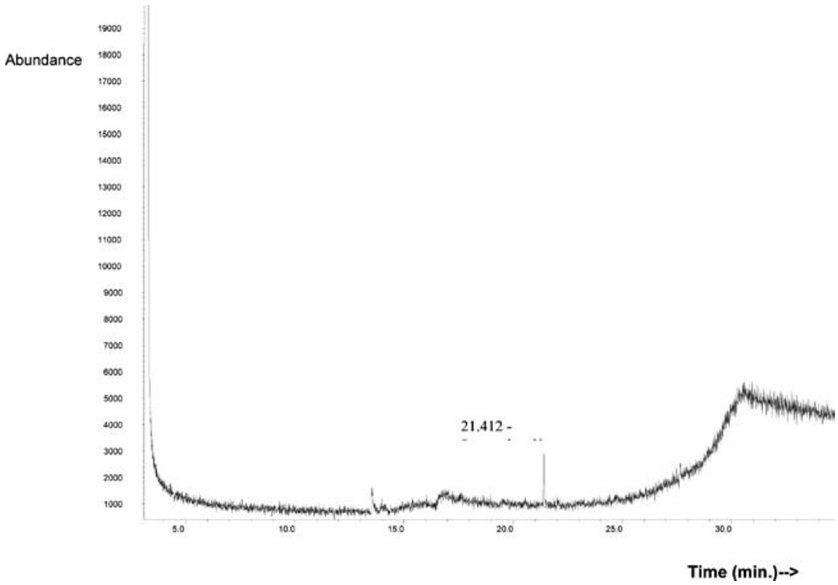

Fig. 1: Chromatogram of phytochemical constituents of freshly prepared spiced fruit drink (CP)

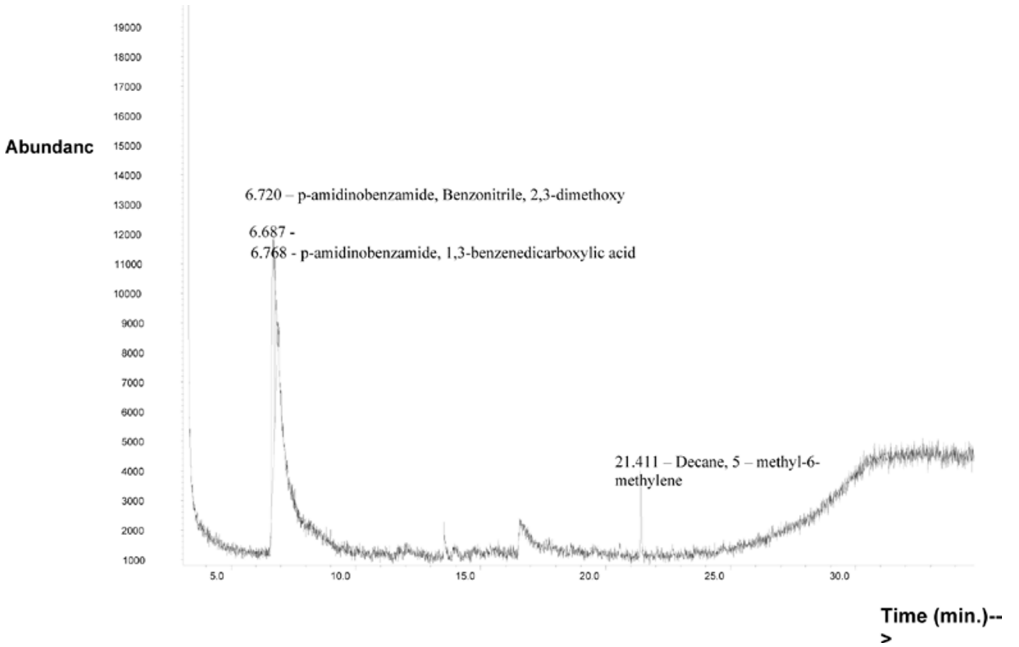

Fig. 2: Chromatogram of phytochemical constituents of freshly prepared spiced fruit drink (CPCLG1)

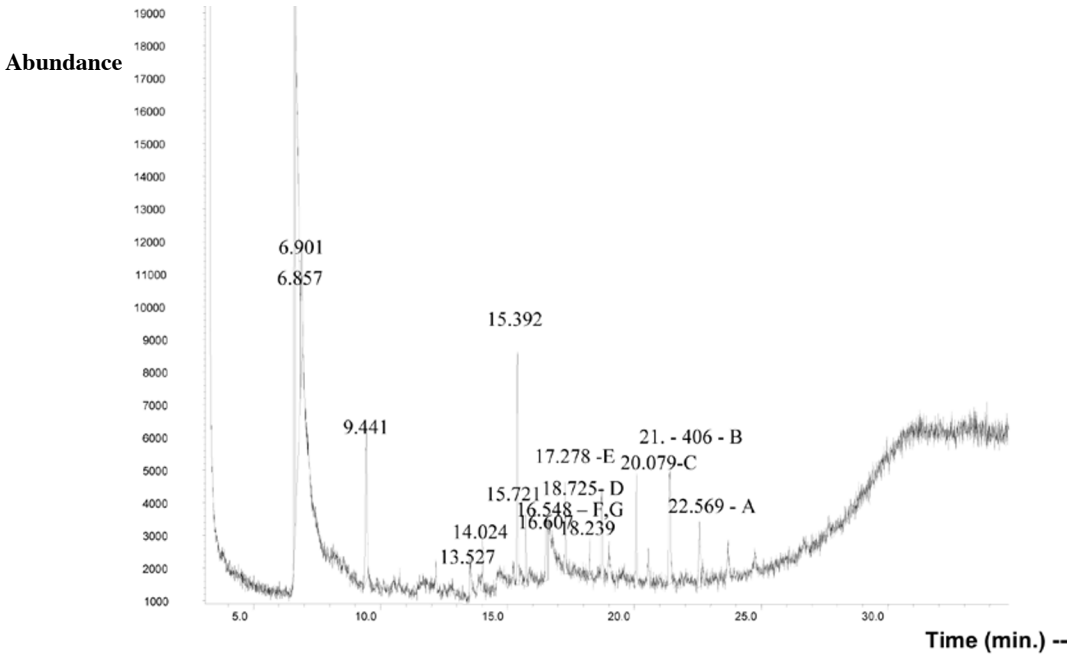

Fig. 3: Chromatogram of phytochemical constituents of freshly prepared spiced fruit drink (CPCLG2)
Key: A = Octanoid acid, B = 1-Nonene, C = Betaxolol D = Metroprolol, E = Fumaric acid, F = Methallyl cyanide, G = Benzoquinone

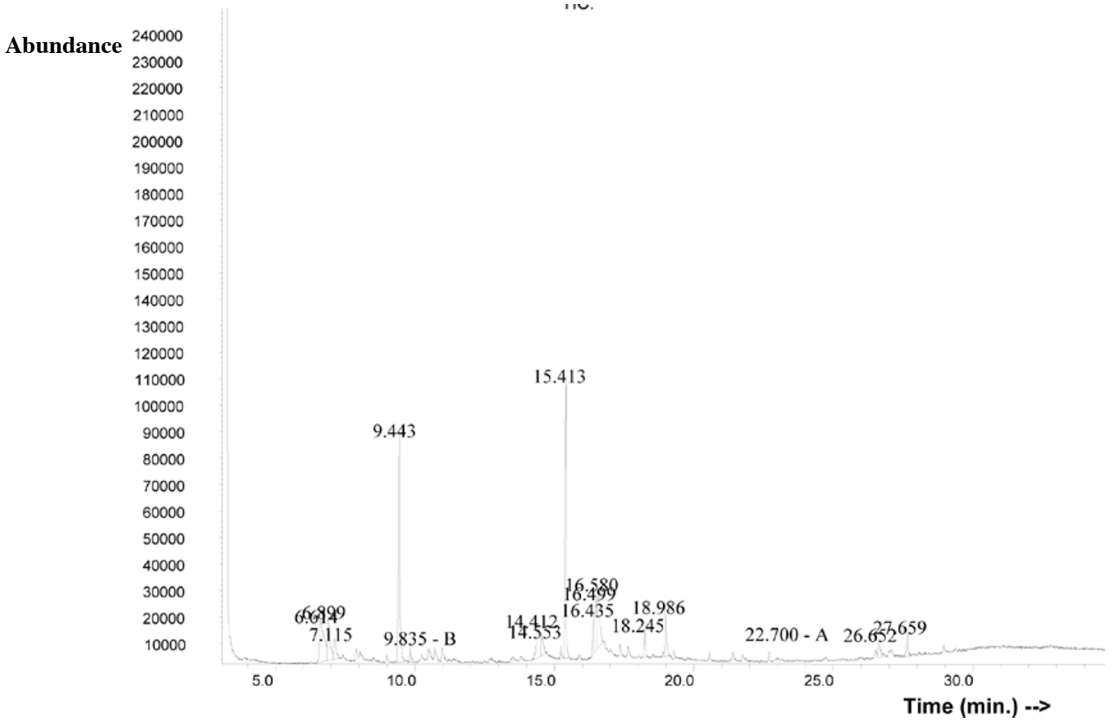

Fig. 4: Chromatogram of phytochemical constituents of freshly prepared spiced fruit drink CPCLG3
Key: A = Limonene, B = Caryophyllene

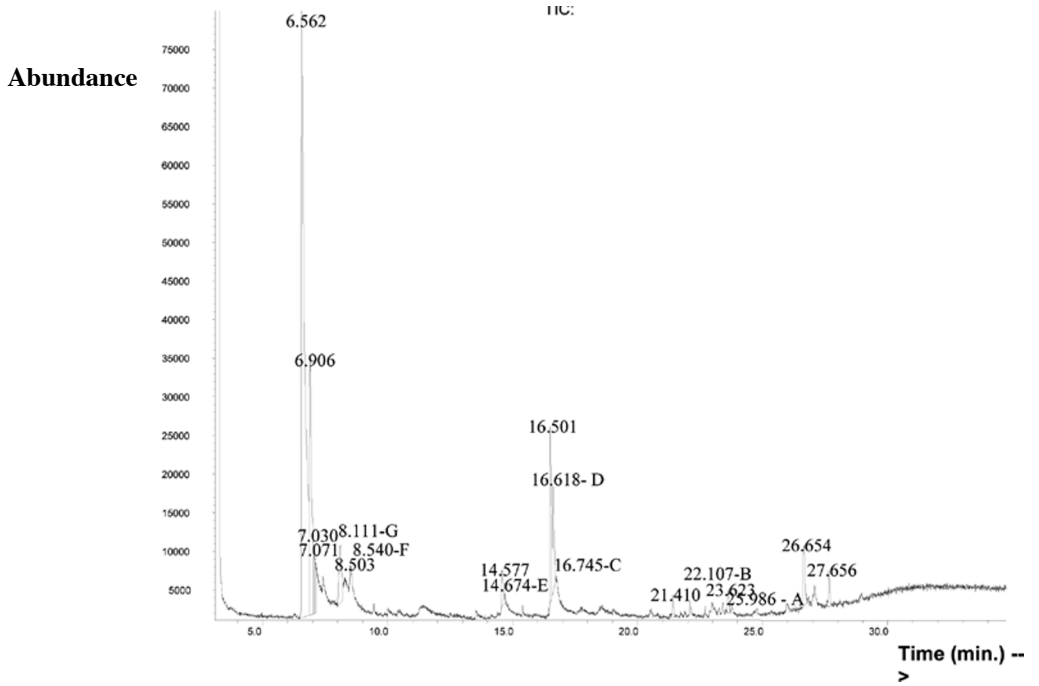

Fig. 5: Phytochemical constituents of freshly prepared spiced fruit drink (CPCLG4)
Key A: = P-Benzoquinone, B = Benzofuranone (coumesterol), C = 4H-Quinolizine, D = Camphene (methylidene), E = Chlorogenic acid, F = Caryophellene (Bicyclo 2.2.1.), G = Sesquiterpene.

nitrile) are anaesthetic, antifungal, antiseptic and antibacterial agent (Beneforce, 2011).

Conclusion

The spiced fruit drink CPCLG2 was the most acceptable drink compared to CP, CPCLG1, CPCLG3 and CPCLG4 in terms of brix, pH, acidity, total solid, ascorbic acid (vitamin C) and phytochemicals. Spiced fruit drink from the blend of pineapple, cucumber; clove and ginger are good source of phytochemicals such as alkaloid, flavonoid, saponin, steroid, tannin, terpenoid and phlobatannins, which were more pronounced in CPCLG2, CPCLG3 and CPCLG4. Active constituents such as fumaric acid, betaxolol, benzoquinone,benzofuranone,camphene,chlorogenic acid, quinolizine, pyridinepropanol, of great health benefits, were obtained quantitatively in the spiced fruit drink, especially in CPCLG2 and CPCLG4. These findings can form the basis of further studies to identify other active compounds, which may be health benefit and subsequently may lead to new functional drink (nutraceutic/ therapeutic drinks) discovery and development. As the phytochemicals are not considered as nutrients; there are no official recommendations of how much of each active constituent should be consumed in order to obtain the most beneficial effects. Therefore, attempts should be made to determine the quantitative requirements of these active constituents of the phytochemicals for consumption.

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