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Determination of the sugar content in fruit flavoured drinks by HPLC

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Abstract
Ten common fruit flavored drinks were randomly selected and purchased from the Jos metropolis, Plateau State, Nigeria. Fruit flavoured drinks classified as soft drinks form part of a modern diet in many communities especially for children. These drinks are available as concentrates or in processed form and they are generally considered as a decent swap for whole fruit. However, the fruit flavoured drinks only bear a passing relationship to the real fruit and have high sugar content. Fehling's and Barfoed's tests were conducted to determine the presence of the principal sugars, fructose, glucose and sucrose in the drinks qualitatively. High performance liquid chromatographic analysis was used to quantitate the fructose, glucose and sucrose content using hyperclone amino-bonded carbohydrate column (BDS C18 130 Å, 150 x 4.6 mm) and acetonitrile: water (70:30, pH 6.8) mobile phase system at a flow rate of 0.4 ml/min. 10 µl volumes of each sample were injected and detection was by UV at 190 nm. The results of the analysis showed that the three sugars; fructose, glucose and sucrose were present in 7 out of 10 fruit flavoured drinks at variable concentrations ranging from 0.2 mg/l to 2046.99 mg/l. The remaining 3 fruit flavoured drinks indicated the presence of glucose and sucrose only. The sugar content of some of the drinks calls for caution in children's diet especially with the rise in obesity and dental erosion in tooth enamel associated with sugar sweetened beverages or drinks.

Keywords: HPLC; Fruit drinks, Carbohydrate; Sugars

INTRODUCTION

Fruit flavoured drinks which are classified under soft drinks serve as beverages because they offer good taste and reportedly contain a variety of nutrients naturally found in fruits (Ogden *et al.*, 2007). The emphasis on healthy lifestyles has led to the consumption of pure fruit juices rather than sugary soft drinks in children and adult alike (Benvenuti and Burgess, 2012). Fruit juices are generally considered beneficial or healthy because the juicing process is supposed to make the nutrients more easily accessible to the body. Fruit juice has been reported to provide other health benefits like being a good source of natural vitamins and anti-oxidants. Historically, fruit juices were recommended by pediatricians as a source of vitamin C and as an extra source of water for infants and young children in their diet. Fruit juices command premium prices compared to other soft drinks or liquid refreshments (Benvenuti and Burgess, 2012).

However, drinks commonly classified as fruit juice in our locality is actually fruit flavoured drinks which mainly consists of

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artificial flavours, colours and sugars like fructose, glucose and sucrose. Preservatives like benzoic acid, sorbic acid or sulphur dioxide and natural colours such as anthocyanins and betanin have also been used in their preparations. Other natural sugars used are lactose, maple sugar and molasses (Johnson *et al.*, 2009). Reviews and studies on sugar sweetened beverages have linked drinking of these beverages to greater caries risk in adults and children (Bernabé *et al.*, 2014; Marshal *et al.*, 2003).

Various chromatographic techniques have been used for the analyses of sugars utilizing UV-visible, refractive index and evaporating light scattering detection (Karkacier *et al.*, 2003; Aubin, 2006; Benvenuti and Burgess, 2012). Due to the lack of chromophores in sugars, refractive index or evaporative light scattering would serve as better detection methods however the utilization of amino -bonded column chemistry allows for detection with UV-visible detection.

The study aims to determine the sugar content in ten commonly consumed fruit flavoured drinks sourced from markets in Jos metropolis.

EXPERIMENTAL

Chemicals and reagents. HPLC grade acetonitrile (Riedel-de Haen), Glucose monohydrate (Oxoid), Sucrose (Frost England), D-fructose GPR (Hopkin & Williams Ltd, England) Molisch reagent, Fehling's reagent, Barfoed's reagent. Ten fruit flavoured drinks were obtained from Jos metropolis.

Chromatographic conditions. HPLC system: Cecil 1100 series HPLC system. Column: Hyperclone amino-bond carbohydrate column, C18 5 µ 130 Å, 150 x 4.6 mm. Mobile phase: Acetonitrile-water (70:30 adjusted to pH 6.8). Flow rate: 0.4 ml/min (isocratic). Injection volume: 10 µl. Detection: UV at 190 nm (Karkacier *et al*., 2003).

Standard preparation. The standard sugars (glucose, fructose and sucrose) were blended with acetonitrile - water (70:30) to make a 5 mg/10 ml stock solution. Four further dilutions of the standards were made to construct a four-point calibration curve using the standard values. Each standard was injected in duplicate.

Sample preparation. Samples of various fruit drinks purchased from the local market in Jos. A 1g sample of each drink was directly mixed with 4 ml of water. The mixtures were homogenized using a magnetic mixer/stirrer for 20 minutes at 3000 rpm and then centrifuged at 4000 rpm for 30 min at room temperature. The supernatants were then filtered using a Whatman No. 42 filter paper. The filtrate (30 ml) of each sample was blended with 70 ml of acetonitrile and 10 µl volumes injected on to the column (Karkacier *et al*., 2003).

Molisch's test. A 2ml volume of carbohydrate solution was poured into a test tube into which a drop of Molisch's reagent (10% α-naphthol in ethanol) was added. A 1ml portion of conc. H_2SO_4 was poured down the side of the test tube to form a layer at the bottom. The presence of a reddish-violet colour at the interface indicates a positive test for carbohydrates.

Fehling's test. Fehling's solution A (aqueous solution of $CuSO_4$) was mixed in equal proportion with Fehling's solution B (Sodium potassium tartrate). The sugar solution (2 ml) was added to it after mixing and boiled. The presence of a red precipitate indicates a positive test for reducing sugars and aldehydes.

Barfoed's test. To 2ml of Barfoed's reagent (cupric acetate in acetic acid or lactic acid) was added an equal volume of the sugar solution. The mixture was boiled for 5

minutes in a water bath and allowed to stand. The presence of a brick-red cuprous oxide precipitate indicates the presence of reducing sugars (Pasto *et al*., 1992; Hendrickson *et al*., 2001; Pavia *et al*., 2005).

RESULTS AND DISCUSSION

The labeling on the fruit juice boxes showed that out of 10 drinks sampled, 4 contained between 4 -10 % of different fruit juice concentrates while some of the other drinks did not specify the amounts present (Table 1). Four of the drinks contained vitamins like ascorbic acid with no stated quantities. The qualitative analysis using basic tests such as Molisch reagent for sugars gave positive results for all the sugars while Fehling's and Barfoed's reagents tested negative for sucrose (Table 2). The amino bonded column used in this assay is useful for separating sugars and oligosaccharides.

Underivatised monosaccharides are reported to be quickly resolved on amino columns through normal phase partitioning based on the interaction of sugar hydroxyl groups with the amino phase. Carbohydrate detection is most sensitive at UV wavelength of 190 nm, although solvent impurities can create noise and limit sensitivity at this wavelength. However, acetonitrile: water mobile phase resolves the most common monosaccharides well since water and the sugars compete to form hydrogen bonds with the amino phase.

The retention time decreases as the water content in the mobile phase is increased (Binder et al., 1980). The retention time of fructose, glucose and sucrose were determined from the chromatograms of the standards as 2.54, 3.45 and 4.32 mins (Figure 1). HPLC analyses of the fruit flavoured drinks showed that the drinks contained the three main sugars; fructose, glucose and sucrose in 7 out of 10 samples while fructose was not detected in three of the samples (Table 3).

In fruit juice analysis, the variation in sugar content among different cultivars is used to assess the quality by consideration of the ratios of fructose, glucose and sucrose (Benvenuti & Burgess, 2012). As shown in Figure 2, Drink 3 contained the highest amount of glucose (2046.99 mg/l) and sucrose (1380.27 mg/l) while Drink 6 contained the highest amount of fructose (88.15 mg/l).

The World Health Organization in 2003 recommended that people consume less than 10 % of their total calories from free sugars which includes added sugars and the sugars in fruit juices, fruit concentrates, honey and syrups. Under the new draft guideline, a person consuming below 5% of total energy intake per day would have additional benefits. Five percent of total energy intake is considered to be equivalent to around 25 g (ca. 6 teaspoons) of sugar per day for an adult of normal Body Mass Index (BMI) (WHO, 2003; 2014). From reports by other investigators, the prevalence of dental decay has been linked to continuous leaching of Ca^{2+} ions, phosphorus, oxygen and hydrogen from the tooth enamel due to exposure to the acids in soft drinks and fruit juice consumption (Low and Alhuthali, 2008; Barbour *et al.,* 2006; van Egyen *et al.,* 2005, Tahmassebi *et al.,* 2006).

A systematic review conducted by Malik *et al.,* 2014 on the impact of sugar sweetened beverages on blood pressure, showed that the consumption of these drinks was associated with higher BP leading to increased incidence of hypertension. They suggest that interventions to reduce the intake of sugar sweetened beverages should be an integral part of public health strategy to reduce the incidence of hypertension.

Conclusion. The sugar content of the fruit flavored drinks ranged between 0.2 and 2046.99 mg/L. The amount of fruit juice concentrate was shown on the label of 4 drinks with a range of 4-10% while that of the vitamins were not stated.

Further studies may help to correlate the amount of sugar to the recommended daily requirements.

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Table 1: Description of the drinks analyzed

s/n	Juice concentrate	Some other ingredients	Batch No Manufacturing/ Expiry dates	Manufacturer	NAFDAC Reg. No.
1	4% apple juice	Water, sugar, malic acid, citric acid, colour, caramel, Na benzoate	5210227171DL4 27/02/13-28/08/13	La casera PLC Nig. Ltd.	01-4278
2	10% pineapple juice	Water, sugar, pineapple flavour, xanthan gum,β-carotene, ascorbic acid, pectin, pineapple flavour	02AP020 11/04/13- 11/01/14	Baniaz H.C. Ltd, Nigeria	B1-2981
3	Mixed fruit (min. 5%)	E 330 (citric acid) ascorbic acid (E300), stabilizer (E 415)	Y250413016 28/03/13- 27/12/13	Chi Nig. Ltd.	A1-7979
4	Apple juice-N.S	Water, sugar, citric acid, vitamin	Y08213B14 08/02/13- 07/11/13	Chi Nig. Ltd.	
5	Milk drink	Water, sugar, citric acid, CMC, Na citrate, lactic acid, apple flavour, fast green, yellow tartrazine, Na tripolyphosphate	A3-22 01/03/13- 31/08/13	VIJU Industries Nig. Ltd.	A1-3482
6	10% whole soybeans	Water, sugar, vegetable oil, whole milk powder (1%)	23/11/12/R 23/11/13	Jugo- express Int. Nigeria	01-5100
7	Apple concentrate – N.S	Natural flavour, water	Y081012B1B 08/10/12 – 07/07/13	Chi Ltd, Nig.	01-3343
8	Strawberry Concentrate- N. S	Yoghurt base, sugar, stabilizer (E440), colour (E124)	Y060313B19 06/03/13 – 05/09/13	Chi Ltd, Nig.	01-7347
9	Pineapple concentrate, coconut juice-N.S	Water, skimmed milk, pectin, citric acid	Z090213C1 09/02/13 – 08/11/13	Chi Ltd, Nig.	01-7288
10	Blackcurrant juice concentrate	Water, sugar, glucose syrup, citric acid, vitamin C, potassium sorbate, grape colour (E163)	LOT DT13CU 03/2013 – 12/2013	Glaxosmith-Nigeria	01-0902

N.S- Not stated

Table 2: Qualitative tests for fructose, glucose and sucrose

Test	Fructose	Glucose	Sucrose
Molisch	+ve	+ve	+ve
Fehling	+ve	+ve	-ve
Barfoed	+ve	+ve	-ve

+ve: positive and -ve: negative

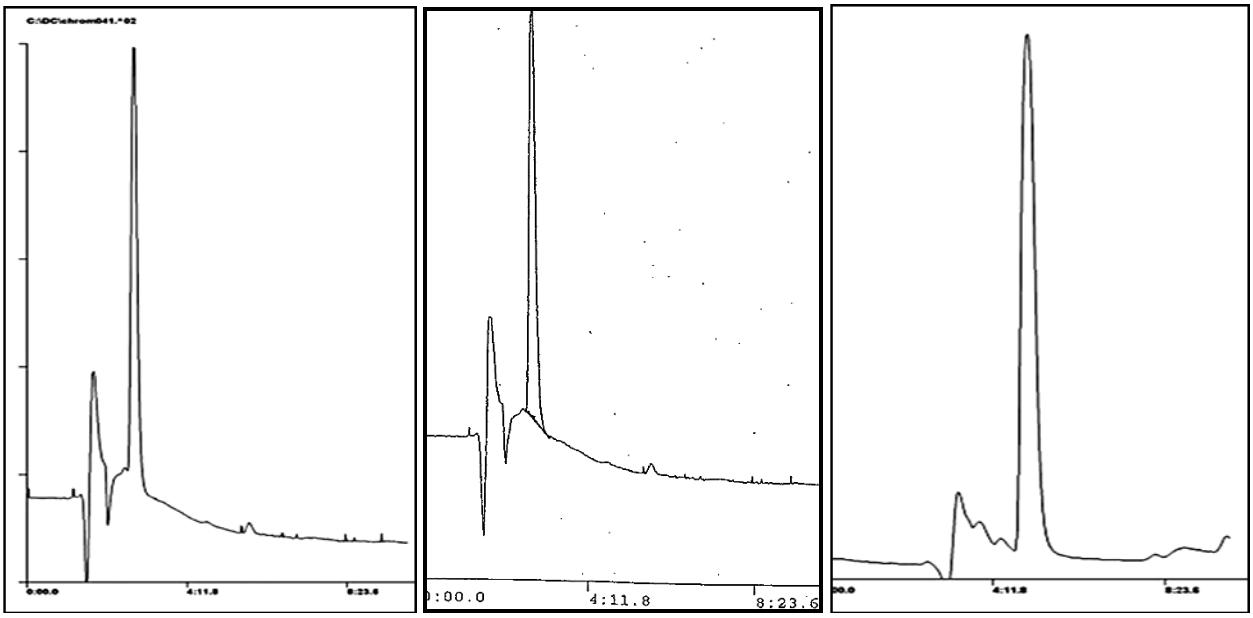

Figure 1: Chromatographic profiles of the standard sugars (fructose, glucose and sucrose)

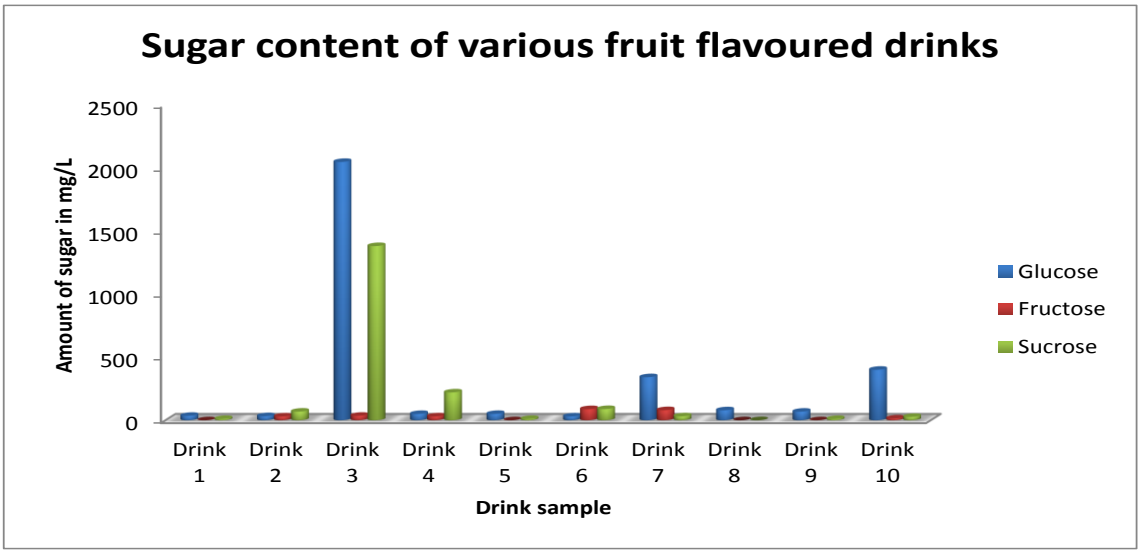

Figure 2: Bar chart showing the levels of glucose, fructose and sucrose in each fruit flavoured drink.

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