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Evaluation of the Cariogenic and Erosive Potential of Some Beverages Consumed in Enugu Metropolis, South East Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author CUM conducted the study and drafted the manuscript. Author FNO designed and supervised the study. Author NIO wrote the protocol and assisted in laboratory investigations. Author CNA and managed the analyses of the study. Authors OCO and IPJC managed the literature search and author NSM critically reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Dental caries is a global infectious disease and the most common oral cavity disease. Among the theories which explain caries is the activity of acids produced by bacteria resulting from dietary fermentation of carbohydrates (sugars). Modernization has led to change in our diet and life style and the beverage industries are having a field day. Though people are aware of the harm caused by these beverages and fruit juices on the dental hard tissues, they still choose to guzzle them. The study was an *in vitro* analysis of cariogenic and erosive potential of different beverages mostly consumed in Enugu metropolis, South Nigeria. Seventeen samples were analyzed physically and chemically using the following parameters, pH, titratable acidity (TA), total soluble solid content

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(TSSC), reducing sugars (RS), non-reducing sugars (NRS) and total sugars (TS). Standard analytical techniques were used including potentiometry for endogenous pH, Association of Official Analytical Chemists approved method for titratable acidity, Brix refractometry using Abbe refractometer for TSSC and Fehling's method for sugars. Coke pressented the highest pH (2.83) followed by La casara 2.92 and the least was Bobo strawberry 4.6. California and Holladia recorded the least and highest TA values of 0.04% and 0.26% respectively. Bobo strawberry had the least TSSC (6.81^{o}Brix) and the highest was Hollandia yogurt (16.34^{o}Brix). Seven Up presented the highest reducing sugar 20.45g/100ml. Mirinda and Bobo strawberry had the highest total sugars 68.90g/100ml each. For non-reducing sugars, the values ranged from 0g/100ml (Chivita apple) to 65.45g/100ml (Bobo strawberry). There were significant differences among the samples for reducing sugars (P<0.05). These beverages showed low endogenous pH that were below the critical value for enamel demineralization (pH<5.5). They are potentially hazardous to the teeth and should not be consumed regularly because of their low pH and high sugar content.

Keywords: Dental caries; erosion; pH; sugar; Enugu; Nigeria.

1. INTRODUCTION

Dental caries is a global infectious disease and the commonest disease in the oral cavity [1,2]. Amidst the theories that describe genesis of caries is the activity of acids formed by bacteria as a result of fermentation of carbohydrates (sugars) from diet [2]. There is therefore strong relationship between oral health and sugars. Diet has effect on the soundness, the composition, quantity and pH of the saliva, and the pH of the plaque. Fermentable carbohydrates including sugars after being hydrolyzed by salivary amylase, furnish substrates for the action of oral microflora, which in turn lowers salivary and plaque pH. The corollary is the genesis of tooth demineralization [1].

Physicians, dentists, and parents are facing a serious challenge because of consumption of junk and unwholesome food and drinks leading to co morbidity, including type 2 diabetes, obesity, caries, and dental erosion [2]. Recently, dental erosion has been reported to be a general serious health challenge [3,4,5] and this may have been due to increase in the consumption of soft drinks, fruit juice and sport drinks [5]. Dental erosion is the chemical dissolution of the surface of the teeth due to acid attack without the participation of microorganism. Development has resulted in rapid change in diet and lifestyles. Refined food with increased access to ready-made drink has become the order of the day [6, 7]. Consumption of carbonated drink and fruit juices has been on increase. Majority of the populace see soft drink consumption as safe and harmless, however, there have been a significant health issues linked with frequent consumption of soft drinks [5]. Parents influence, peer pressure, diet fallacies pleasure and tastes have been responsible for the increase in the consumption of these drinks [5,7]. Report has shown that there is a correlation between the quantity and regular consumption of sugar and prevalence and seriousness of caries and erosion [2]. All these carbonated drinks have a pH of 2 to 3 and may cause a pronounced loss of dental hard tissues through erosion and it an increasing challenge to teenagers [2]. Majority of these beverages contain single to double food acidulants namely phosphoric acid and citric acid [5]. Sometimes malic and or tartaric acid may be used. Citric, malic and tartaric acids are erosive because of their inherent tendency to chelate calcium at higher pH [5,6]. The titratable acidity and a low pH are the main cause of erosive lesions, and all these factors including sugar content contribute to the formation of cavity [8].

It costs a lot of money and time to restore extensive tooth wear [5]. Enamel dissolution has a critical pH of 5.5 and any food or drink lower than 5.5 may contribute to or stimulate erosion [1]. The commonest cause of dental erosion in young people is the consumption of acidic beverage such as carbonated sport drinks and fruit juice. These have been reported to be associated with loss of dental enamel, particularly when consumed during periods when there is low salivary flow such as immediately after serious sport activities [3,8,9].

According to recent global soft drink market analysis, Nigeria ranked fourth in the world, coming after United States of America, China and Mexico which ranked first, second and third respectively [10]. Attention has been focused on economic values without considering the health implications or impact of the consumption of soda and other sweetened beverages in sub

Saharan Africa [11]. Recently interest has been kindled to determine some chemical and physical properties of beverages such as endogenous pH, titratable acidity (TA), total soluble solid content (TSSC) or degree Brix (^{0}Bx), and their impact on dental bio film [12,13,14].

In the view of high consumption of soft drinks and other beverages among Nigerians and lack of federal government concern in streamlining this habitude, the purpose of this study was to analyze *in vitro*, the erosive and cariogenic potential of some beverages consumed in Enugu metropolis by assessing some of their physical and chemical parameters.

2. MATERIALS AND METHODS

2.1 Study Design

The evaluation of endogenous pH, titratable acidity, total soluble solids content (TSSC-% Brix) and sugar levels were done on 17 randomly selected soft drinks and fruit juices commonly consumed in Enugu Metropolis South East Nigeria. Samples were bought from Ogbete main market and Shoprite Enugu. Each analysis was done in triplicate. The carbonated drinks were degassed by pouring them into beakers and placed inside the water bath containing warm water. For several minutes, they were vigorously stirred and allowed to stand 15 minutes and stirred once more. The drinks were brought out and allowed to come back to room temperature.

2.2 pH Measurement

The endogenous pH of each fruit juice and soft drink was evaluated at room temperature (28.0°C) with pH meter (Hanna Instruments, USA) immersed into each solution. Buffer standards of pH 7 and 4 were used to calibrate the pH. Into a beaker was placed 20mls of pure beverage/ fruit juice and pH meter was placed into the sample and the value recorded was [13].

2.3 Total Soluble Solids Content (TSSC)

Abbe Refractometer (New Delhi, Delhi) was used to do TSSC analysis. Distilled water was used calibrated the equipment (refractive index 1.3325 and 0° Brix at 28.0°C) [13] and readings of the samples were taken (°Brix or g/100ml).

2.4 Titratable Acidity (TA)

Method adopted by the Association of Official Analytical Chemists (2000) was used to measure the titratable acidity (TA) [15]. The quantity of 0.1 N KOH solutions required for the product to attain a neutral pH or a pH value above it. A 10 ml fraction of the diluted sample was titrated (10% solution of the sample) with the 0.1 N KOH solution until the substance attained a pH value between 8.2- 8.4, equivalent to the end point of the phenophthalein. PH meter (Hanna instruments USA) was used to take the readings [13]. On reaching this value, the volume of KOH was recorded and the percentage of the acid was calculated using the following equation below, and the result was reported as percentage of citric acid.

$$\text{Acidity (\% citric acid)} = \frac{\text{V} \times \text{Nap} \times \text{F} \times \text{meq} - \text{g (Citric acid)} \times 100}{\text{Sample}}$$

Where V=KOH Volume, Nap=Normal concentration of the KOH base; F=Normal correction factor; meq-g=miliequivalent per gram of citric acid; Sample=volume of the beverage.

2.5 Reducing Sugars, Non-reducing Sugars and Total Sugars

The method adopted by the Association of Official Analytical Chemist (2000) was used for reducing sugars, non-reducing sugars and total sugars and the results were expressed in g/dl [15].

2.6 Reducing Sugars

For the evaluation of reducing sugar, a portion equivalent to 5 g of sample was measured into a 250 volumetric flask and 10ml (20%) neutral lead acetate solution was added to it, the solution was diluted to mark with distilled water and filtered. An aliquot of 25 ml of clarified filtrate was transferred into 500 ml volumetric flask containing 100 ml of distilled water. Potassium oxalate (10%) was then added in small amount until there is no further precipitation. The solution was made to mark and filtered through a Whatman No 1 filter paper. The filtrate was transferred to a 50 ml burette.

2.7 Titration

Into 250 ml conical flask 5mls of Fehling's A and B were pipette and mixed, then 10 mls of distilled water and few glass beads were added and

Table 1. Brand name, ingredients and manufacturers of the tested beverages

S/N	Commercial brand/name	Active principal ingredients	Manufacturer
1	Sprite	Carbonated water, sugar, citric acid, lime and lemon flavours sodium citrate sodium Benzoate	Coca cola
2	Fanta	Carbonated water, sugar, citric acid, Ascorbic acid, stabilizers (E414, E415), orange flavor, sodium benzoate, colourants, sunset yellow and flavor Tartrazine.	Coca-cola
3	Coke	Carbonated water, sugar, caramel colour, phosphoric acid, cola flavor, caffeine	Coca-cola
4	5 Alive Apply fruit Nectar	Water, concentrated apple juice 25% minimum sugar, pectins, citric acid, Apple flavor caramel colour, Vit. C and E	Coca-cola
5	7up	Sweetened carbonated beverage: carbonated water, sugar, citric acid malic acid, sodium citrate lemon and lime flavours	Seven up bottling company
6	Pepsi	Carbonated water sugar, caramel, phosphoric acid, caffeine, Gum Arabic, cola flavor	Seven up bottling company
7	Mirinda orange	Sweetened carbonated beverage: sugar, citric acid Arabic gum, sodium benzoate; Ester gum, orange flavor, sunset yellow, Tartrazine	Seven up bottling company
8	La casera (Apple drink)	Carbonated water, Apple juice 4%, sugar, Apple flavor, Acidulants: Malic acid and citric acid preservative: sodium benzoate, colour: caramel	Classic beverage Nigeria Ltd (CB)
9	Ribena (Blackcurrant)	Water, sugar, black currant juice concentrate, Glucose syrup, citric acid, vitamin C, Preservative: (Potassium sobate), Grape colour (E163)	GlaxoSmithKline
10	California sun (straw berry drink juice)	Water, sugar, Xanthan Gum, Strawberry, concentrate, sodium benzoate potassium sorbate, Pectin, Ascorbic acid (35mg/100l) Citric acid, strawberry flavours, Red carmozine, Aspartame, Acesulfame-k	Frutta Juice and SERVICES Nig Ltd
11	Bobo (Straw berry milk drink)	Water, full cream milk, sugar, strawberry juice, citric acid, carboxy methyl cellulose, sodium citrate, strawberry flavor, milk flavor, ponceau 4 R, Potassium sorbate, Acesulfame-k, sucrose, fatty acid esters, vitamin C	Bobo food and beverage limited
12	Bobo (orange milk drink)	Water, full cream milk, sugar, orange juice, citric acid, carboxymethyl cellulose, sodium citrate, orange flavor, milk flavou, Poceau 4R, Yellow tartrazine, potassium sorbate, Acesulfame-k, Vitamin C	Bobo food and beverage limited
13	Twist (Apple fruit milk drink)	Milk powder, apple fruit juice, butter oil, sugar, stabilizers, water, vitamins	Condia Nutrition

14	Capri-sonne orange drink	Water, sugar, orange juice concentrate, natural fruit drink, no artificial flavours, colours, sweeteners or preservatives added	CH1 limited
15	Chivita (Apple juice)	100% fruit juice, 0% added sugar, no preservative No artificial colours, no artificial flavor	CHI Limited
16	Chivita (Orange)	10% fruit juice, 0% added sugar, no preservative No artificial colours, no artificial flavor	CHI Limited
17	Hollandia yoghurt	Yoghurt base, sugar, stabilizer, water	CHI Limited

heated to boiling. Three drops of 1% methylene blue were added and the mixture was titrated with the already made sample solution until the reducing sugar present in the sample was completely reduced by the Fehling`s solution showing blue to brick-red colour.

The percentage of reducing sugars was calculated using the following equation.

$$\text{Reducing sugars \%} = \frac{\text{Dilutions} \times \text{Factor of Fehling (gm)} \times 100}{\text{Weight of sample} \times \text{Titre}}$$

2.8 Total Sugars

Method used by Calvalcanti as per protocol where 5ml of concentrated hydrochloric acid was added to 25 ml of each beverage and put in double boiler for 10 minutes. The mixture was cooled and 5ml diluted saturated lead acetate and distilled water was added. Ten milliliter of mixture of Fehlings was added followed by 40ml of distilled water. These were heated to boiling for 4min. Titration was done with 25 ml of diluted filtrate against the Fehlings solution in the presence of methylene blue until brick red precipitate was obtained [13]. The spent volume was recorded and the total sugar content was calculated using the equation below;

$$\text{Total sugar content (\%)} = \text{FEQ} \times \text{dilution} \times 100/\text{VTITRATION}$$

Where FEQ=Equivalence Factor; VTITRATION= volume spent

2.9 Non-reducing Sugars

Subtracting the reducing sugars from the total sugars and multiplying the value by the conversion factor of glucose in sucrose which is 0.95 gives the estimated value of non-reducing sugar. Grams of sucrose per dl of sample were used to express the result.

2.10 Determination of Fehling Factor

Into 500 ml volume flask containing 50 mls of distilled water, 4.75 g of analar grade sucrose was added followed by 5mls of conc. HCL (1+1). This was boiled in a water bath at 70^{o} C for 1hour cooled and neutralized with NaOH solution and then made up to volume, mixed and then 50 mls was transferred to 100 ml flask and finally made up to volume. It was then transferred to a burette and titrated using 5 mls each of Fehling solution.

$$\text{Fehling factor} = \frac{\text{Titre} \times \text{weight of sucrose in g}}{500}$$

2.11 Statistical Analysis

Data generated were analyzed with GraphPad Prism version 5.0. The student's t-test was used to calculate differences between means while analysis of variance (ANOVA) was used to estimate difference between group mean. Categorical variables were presented as frequencies (percentages) while continuous variables were presented as mean ± standard deviation. $P< 0.05$ was considered statistically significant.

3. RESULTS

The results of the physical and chemical parameters differed among the evaluated beverages. Table 2 shows the distribution of pH and TA mean values for the tested beverages. All the beverages tested showed pH below the critical value of 5.5. The pH values ranged from 2.83±0.01 (Coke) to 4.56±0.01 (Bobo straw berry). The least TA value was recorded for California sun 0.04±0.002 while the highest was Hollandia yoghurt 0.26±0.001.

Table 3 shows the distribution of TSSC, reducing sugars, non-reducing sugars and, total sugars of the tested samples. The least TSSC was shown by Bobo straw berry 6.81±0.01 while the highest TSSC was presented by Hollandia yoghurt (16.32)±0.01.

The highest reducing sugars value reported for 7 up (20.45± and the least was caprisonne-orange

2.76±0.01. Bobo straw berry milk drink and chivita (apple juice) had no reducing sugar.

Non-reducing sugars values ranged from 1.42±0.01 for california sun to 64.77±0.02 for Hollandia yoghurt.

4. DISCUSSION

Beverage consumption has been on increase and this is dangerous to oral health. Strong relationship exists between sugars and oral health. In addition to sugar, other factors that influence the cariogenic potential of drinks or food include frequency of intake, consistency and form, time span of exposure, composition of food, eating order, flow of saliva, buffer presence, and oral hygiene [1]. Regular intake of sugary drinks has become a major cause of caries and dental erosions. They are known to cause harm to the teeth because of two properties namely the low pH and titratable acidity of the drinks which can cause erosion on the dental hard tissues and secondly, the fermentable sugars in the drinks which are acted upon by plague micro-organisms to produce acid in the dental plaque, resulting in demineralization thereby leading to caries [16]. Sugars that we consume may occur naturally or may be added [1]. The contents of these drinks include aspartame, phosphoric acid, maleic acid, citric acid, phosphates, sugar, caffeine, water and fluoride depending on the type. Some of these beverage are classified as carbonated drinks, energy drinks or fruits juices.

The practical method to assess erosive potential of beverages is to evaluate its pH.

Our study showed that all the seventeen tested beverages had pH lower than 5.5 which is the critical value for dental demineralization.

The pH ranged from 2.83 for coke to 4.56 for Bobo strawberry. Some researchers reported a range of 3.32-4.44 [8]. The TA which is the amount of base required to bring a solution to neutral pH were low in our work. It ranged from 0.04-0.26. The type of acid contained in the beverage also enhances the demineralization power of the drink; citric acid has a greater erosive potential than maleic, phosphoric acid and hydrochloric acid [5,17,18]. The high erosive potential of the citric acid is associated with its power to form complexes with the calcium ions present in the hyroxiapatite [19]. Beverages with citric acid are known to be potentially erosive [20]. The risk of erosion has a strong relationship to the quantity and frequency of consumption. Whenever there is an erosive challenge, behavioral factors act to play a role in modifying the level of erosive tooth wear. The method of taking in these beverages (gulping, sipping, use of a straw) affects the duration of contact between the teeth and challenge, consequently the rate and length of exposure to an erosive

Table 2. Distribution of the beverages based on their mean values and standard deviation for endogenous pH and titratable acidity

	Name of beverage	Endogenous pH Mean ± SD	Titratable acidity Mean ± SD
Coca-cola	Sprite	3.75 0.01	0.07 0.001
	Fanta	3.27 0.01	0.11 0.001
	Coke	2.83 0.01	0.08 0.001
	5 Alive	3.17 0.01	0.18 0.001
7-up bottling company	7 up	3.71 0.01	0.12 0.001
	Pepsi	2.84 0.01	0.05 0.002
	Mirinda	3.17 0.01	0.08 0.002
Classic beverage limited	La casara apple drink	2.92 0.01	0.12 0.090
GlaxoSmithKline	Ribena	3.28 0.01	0.08 0.001
Frutta Juice and services Nig Ltd	California sun	3.75 0.01	0.04 0.002
Bobo food and beverage limited	Bobo strawberry milk drink	4.56 0.01	0.16 0.001
	Bobo Orange	4.48 0.01	0.15 0.002
Condia nutrition	Twist apple fruit milk drink	4.26 0.02	0.13 0.002
Chi- Limited	Capri-sonne orange drink	4.00 0.01	0.08 0.001
	Chivita (apple juice)	4.04 0.02	0.09 0.06
	Chivita (orange)	4.41 0.01	0.20 0.001
	Hollandia yoghurt	4.30 0.01	0.26 0.001

Table 3. Reducing Sugars (RS), Non-reducing Sugars (NRS), Total Sugars (TS) and Total Soluble Solute Content (TSSC) all in g/dl of various beverages

Company name	Name of beverage	TSSC Mean + SD	NRS Mean + SD	TS Mean +SD	RS Mean +SD
Coca-cola	Sprite	12.61 0.02	11.35 0.01	15.22 0.01*	3.28 0.01
	Fanta	12.14 0.01	2.71 0.01	9.75 0.01	6.89 0.01
	Coke	10.46 0.01*	8.26 0.01	14.35 0.01^	5.64 0.01
	5 Alive	12.85 0.01	5.64 0.01	15.22 0.01*	9.28 0.01
7-up bottling company	7-up	9.74 0.01	43.25 0.01	55.97 0.02	20.45 0.01
	Pepsi	8.28 0.01	19.47 0.02	36.65 0.02	16.16 0.01
	Mirinda	11.90 0.02	60.3 0.01	68.90 0.01	5.46 0.01
Classic beverage limited	La casara Apple drink	9.98 0.01	1.71 0.01	14.37 0.01^	12.57 0.01
GlaxoSmithKline	Ribena	11.90 0.01	27.32 0.01	36.65 0.02	7.88 0.01
Frutta juice and service Nig Ltd	California sun	12.61^ 0.01	1.43 0.01	13.74 0.02	12.24 0.01
Bobo food and beverage Ltd	Bobo strawberry milk drink	6.81 0.01	65.45 0.01	68.90 0.01	- -
	Bobo orange	7.06 0.02	14.85 0.02	29.76 0.02	14.14 0.01
Condia Nutrition	Twist Apple fruit milk drink	13.31 0.01	8.21 0.01	15.70 0.01	7.06 0.01
Chi-Limited	Capri-sonne orange drink	12.61^ 0.01	8.19 0.01	11.36 0.02	2.76 0.01
	Chivita (apple juice)	11.42 0.01	-	-	-
	Chivita (orange)	10.46* 0.02	7.68 0.01	17.25 0.01	9.28 0.01
	Hollandia yoghurt	16.32 0.01	64.77 0.02	68.20 0.02	-

RS: Statistical significant differences (p<0.0001) were recorded in all the samples for RS
TS: Statistical significant differences (p<0.0001) were recorded in all except those with the same symbols
TSSC: Statistical significant differences (p<0.0001) were recorded in all except those with same symbols
There were variations in physical and chemical parameters evaluated in these beverages

agent is very significant [5,16,20]. A single acidic attack is inconsequential but if repeated, the power of saliva to handle the acid attack may be overwhelmed. If the challenge is regular and there are few or no protective factors, this may be hostile as in caries susceptible people [7].

Brix is another evaluated parameter which is a measure of dissolved solids (proteins, lipids, mineral salts, vitamins, glucides, organic acids, pigments and other substances. It has a correlation with the stickiness of the ingested foods which facilitates its retentions on the dental surfaces [8]. In our study, the TSSC (Brix values) ranged from 6.81 for Bobo straw berry to 16.32 for Hollandia yoghurt. The fruit juices in our study seemed to have high Brix 10.46 for chivita orange to 13.31 for Twist Apple fruit drink. This is in consonance with the work done in Brazil [8]. Sugar content of these beverages showed variation. These include, reducing sugar, Non-reducing sugars and total sugars. The total sugar ranged from 9.75 for Fanta to 68.90 for Bobo straw berry and Mirinda respectively. Chivita (Apple juice) recorded no total sugar. Worthy to note is the high values of these beverages. The values are higher than the ones reported by other researchers [8,21,22]. Frequent consumption of these drinks combined with poor oral hygiene may lead to caries. Reducing (glucose) and non-reducing sugars (Sucrose) can be metabolized by *S. mutans*. Notwithstanding, these microorganisms have greater capacity of forming glucans from sucrose. This sugar is a substrate for glucosyl transferase (GTF) which can be of three types GTF-B, GTF-C and GTF-D. The glucans mediate the adherence of microbial cells to dental surface, favoring bio film formation [8,23].

5. CONCLUSION

The beverages assayed in this study had low pH and elevated total sugar content except chivita apple and so differ in their erosive and cariogenic potentials. Regular consumption of these drinks whether soft drinks or fruit juices should be discouraged. Parents and care givers should be educated on the consequences of these beverages on dental hard tissues when consumed regularly especially by children. Healthy dietary habit and proper oral hygiene produce should be encouraged. Oral education should be incorporated into school curriculum. Habits that aid remineralization should be encouraged.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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