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STATISTICAL AND PHYSICOCHEMICAL ASSESSMENT OF SOFT DRINKS AND THEIR IMPACT ON HUMAN HEALTH

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ABSTRACT
The objective of the present investigation is to evaluate soft drinks on various parameters *viz.* physicochemical and microbial parameter in order to assess the safety of these drinks. Four drinks namely Pepsi, sprite, limca and Gatorade were used in the study. The total soluble solids were present in the range of 7.34 to 12.35˚Brix. The concentration of citric acid in the samples was between 0.136 to 0.38%. Reducing sugars was also estimated in the soft drink samples. The pH of the samples was towards the acidic side (2.62 to 3.31). Benzoic acid was only found in sprite and limca. The concentration of benzoic acid was lying as per the standard limit fixed by the European Union. Samples were investigated for the presence of the microbial load. The antimicrobial assay was also performed against probiotic species to study the effect of soft drinks on gut microflora.

KEYWORDS: Benzoic acid; TSS; pH; reducing sugar and Antimicrobial assay.

1. INTRODUCTION

Soft drink is the most popular drink to be consumed on a regular basis to scavenge the thrust. Soft drinks comprise a diverse group of products. They can be categorized in multiple ways on the basis of flavour, carbonation and fruit juice content. Most commonly available forms are: ready to drink and ready to drink after dilution (concentrated syrup)[1]. Soft drinks proposed for human consumption are covered by national regulations based on codes and standards [2].Soft drinks are made up of potable water carbonated under high pressure, acids, colouring agents, sugar and preservatives. It contain around 8-12 %(w/v) of sweetener, 0.05-0.3 %(w/v) of acidulant and 0.1-0.5 %(w/v) of flavouring agent [3].

Water used in the manufacturing of soft drinks should fulfil the physical, chemical and microbiological criteria for drinking water according to European Directive EC 98/1983, US Environmental Protection Agency (EPA), and World Health Organisation (WHO) Standards [4]. The problem arises due to overconsumption of sugary drinks which led to various health hazards (obesity, diabetes mellitus, or non-alcoholic fatty liver disease) [5].

The shelf life of soft drinks varies but there are fewer chances of deterioration due to low pH, carbonation, acid regulators and presence of preservatives. All the ingredients found in the soft drinks should lie under national regulations for example in the United States; soft drinks are regulated by the Food and Drug Administration (FDA). Sections 401 for food standards and 409 for food additives which regulate additives listed on the Food Additives Status List, which includes those additives specified under the Federal Food, Drug, and Cosmetic Act [6]. Benzoic acid is a preservative which works suitably well under acidic condition and widely used in cosmetic and beverages at the pH between 2.2 to 4.0[7].

Soft drinks have been consumed regularly which may affect the dental health and cause other adverse conditions viz obesity, diabetes. In this study, acidity, total sugar content was estimated with the aim of determining its overall effect on human health. The concentration of preservative commonly benzoic acid was evaluated to monitor whether it was lying under the standard limit fixed by the European Union. The microbial load and anti-microbial assay were also conducted to check its safety towards the gut flora.

2. MATERIALS AND METHODS

a. Collection of sample and storage
Soft drinks were collected from local market of New Delhi. Three soft drink samples namely Pepsi, limca and sprite were carbonated and one was non-carbonated orange flavoured energy drink (Gatorade). All the samples were purchased in their PET bottles and stored at 4˚C until used.

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b. Physico-chemical analysis

Degassing of soft drinks

Soft drinks were degassed using sonicator. In sonicator, sound waves propagate from the radiating surface into the liquid media result in alternating high-pressure and low-pressure cycles, with rates depending on the frequency. During the low-pressure cycle, the ultrasonic waves can create small vacuum bubbles in the liquid. These bubbles are migrated to the surface of the liquid. Degassing was performed to minimize the measurement errors. The soft drink was poured into the sterilized container.

Determination of acid percentage

Titration was performed to analyse the presence of citric acid in the samples. Five ml of soft drink samples were added into the conical flask and diluted with 50 ml of boiled water. The solution was then titrated against 0.1 N NaOH by adding 0.3 ml phenolphthalein as an indicator. The formula used to calculate a percentage of acidity is as follow:

$$Percentage\ of\ acidity = Respective\ factor \times Titre\ vol. \times Normality\ of\ NaOH \times 100 \div Volume\ of\ sample \times 1000$$

Where, Respective factor of citric acid monohydrate- 0.070, Titre volume-volume of NaOH used (final volume-initial volume)

Total soluble solids

Total soluble solids were determined for all the soft drink samples using refractometer (SER. No.89037 Made in India). It is used to measure the index of refraction of transparent liquids, solids and solutions. It is used to measure 0 to 95% of sugar. TSS was expressed as (%) sucrose or degree Brix. The prism was cleaned with distilled water and calibrated using distilled water. Using dropper one to two drops of soft drink sample was placed on to prism and glass plate was closed. The value of Brix was recorded through the eyepiece.

Estimation of hydrogen ions (pH)

In pH meter, pH Mode was selected and the temperature was set to 25°C. pH electrode was rinsed with deionized water and dried using tissue paper. pH electrode was calibrated using pH 4 buffer and pH 7 buffer. After calibration, ph of soft drinks was recorded in triplicate sets.

Determination of reducing sugar

Reducing sugar was studied using 3, 5-di-nitrosalicylic acid (DNS) method; the standard was prepared using D-glucose. The calibration curve was plotted against the known concentration of standards. 10 µl of soft drink samples were taken in different test tubes and make up the volume to 1 ml with distilled water, 3 ml of DNS reagent was added. The test tubes were incubated at 90 ˚ C for 15 minutes. The absorbance was recorded at 540 nm using U-V spectrophotometer.

Benzoic acid detection

Benzoic acid was analysed by high-pressure liquid chromatography (HPLC, Agilent, USA) equipped with a 4.6 × 150 mm, 5-µm column and diode array detector (DAD) The mobile phase was prepared by mixing 0.05M ammonium acetate buffer with HPLC grade methanol in a ratio of 65 to 35 (1 ml/min). The temperature of the column was 40˚C and the injection volume was 20 µl. The peak of benzoic acid was detected at a wavelength of 254 nm. The calibration curve was obtained using benzoic acid standards (10-40 ppm) with R^2 of 0.998. The samples were prepared in methanol in a ratio of 1:1 and methanol was used as a blank.

c. Microbial analysis

The soft drinks were also investigated for the presence of the microbial load in the previous study [9]. The samples were diluted in maximum recovery diluent and serially diluted to 10^{-3} and 0.1 ml was spread on to plate count agar. The plates were incubated at 30±1℃ for 72±3 hours and evaluated for CFU.

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Antimicrobial assay on probiotics

Probiotic strain Lactobacillus casei Shirota was recovered from the health drink Yakult. This strain was spread on to MRS agar (typically used for Lactobacillus species). The sterile paper disk was dipped into soft drink samples and placed on to agar and incubated at 37˚C for 24 hours. The plates were checked for a zone of inhibition.

d. Statistical Analysis of Data

An analysis of variance (ANOVA) was performed using MS Excel (2007) on the mean result of each group. The t-test was also analysed with the significance of 95% confidence level (P>0.05).

3. RESULTS AND DISCUSSION

a. Physiochemical Characteristics

All the soft drinks were physio-chemically analysed, the summary of data was shown in Fig. 1. pH measure the acid strength by detecting hydrogen ions in the solution, Pepsi and limca were more acidic as compared to Gatorade and sprite. The pH found in Pepsi was 2.62, 2.88 in limca, 3.07 in Gatorade and 3.31 in a sprite. Low pH and high titrable acidity may lead to erosion of enamel surface. Pepsi has pH of 2.62 which cause an extreme change in the surface of the tooth which is also true in the case of energy drink. [10]

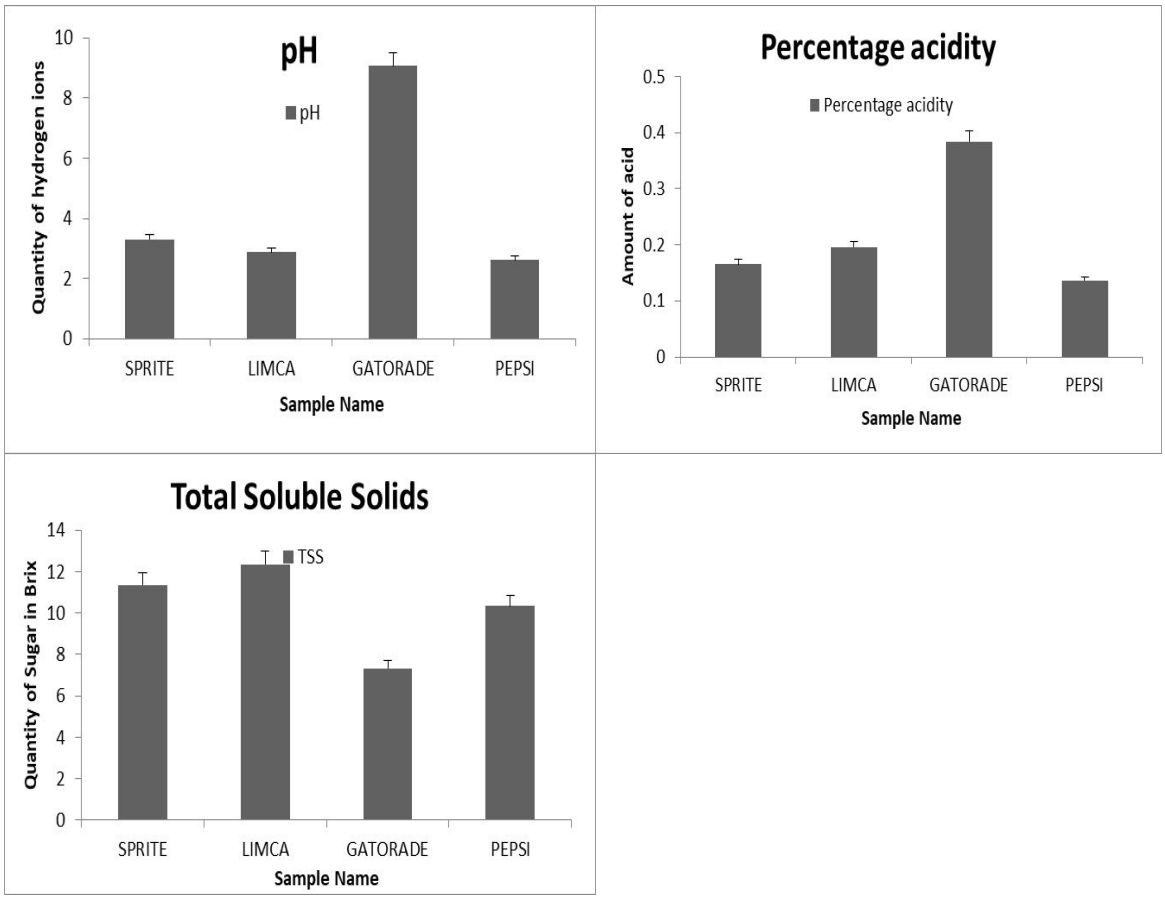

Fig. 1 Indicating Physico- chemical parameters of different soft drinks

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The concentration of citric acid in different samples was determined by titration. The concentration of acid was lying in a range of 0.136 to 0.384%. The presence of phosphoric acid in soft drinks, promote bone resorption and other ingredients in soft drinks excrete calcium in the urine. The high level of phosphoric acid acts as an exogenous source of phosphorus which causes hyperphosphatemia. The inhibition of hydroxylase from hyperphosphatemia causes hypocalcemia. [11] Menopausal women are inherently at higher risk of bone fracture and osteoporosis which may be magnified with increased consumption of soft drinks. [12]

The total soluble solid content of soft drinks was measured using refractometer in ˚Brix. Total soluble solids were lying in the range of 7.34 to 12.35˚Brix, it not only measures carbohydrates but it also measures organic acid, protein, fat and minerals found in the solution. The ratio of total soluble solids to acid was analysed to indicate the taste of the drink either sweet or tart in flavour. The higher value of total soluble solid as compared to acid may impart a sweet flavour to drink whereas a lower value of soluble solid indicates tart flavour shown in Fig. 2. The sugar in drinks are metabolized by plaque causing microbes which produce organic acid, later these acids were added to demineralization process leading to dental caries. [13]

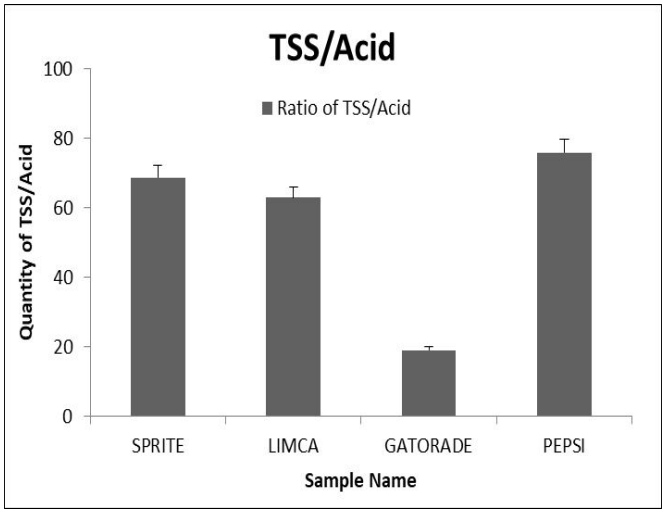

Fig. 2 Represent TSS to Acid ratio of different samples

Reducing sugar was estimated using glucose standard curve, the concentration of reducing sugar was found in all the sets of the sample. Sprite, Pepsi, limca and Gatorade contain 237.8, 271, 115 and 155 mg/l. Reducing sugars have reducing properties and contain free aldehyde and ketone group.

The concentration of benzoic acid was analysed using High-Pressure Liquid Chromatography (HPLC), benzoic acid is used in soft drinks as a preservative. The concentration of benzoic acid was found under the permitted limit of European Union (EU). The maximum acceptable limit was 150 ppm, whereas sprite and limca contain 85.65 and 89.70 ppm of benzoic acid.

b. Evaluation of microbial load

The microbial load was not found in any soft drink samples due to less favourable conditions for the microbial growth. The presence of preservatives, an acid regulator in the drinks was a primary reason to hinder the microbial growth. Even though there are varieties of microbial species which can tolerate lower pH but due to proper plant sanitation and packaging, the occurrence of contaminants was further minimized.

c. Antimicrobial assay

The antimicrobial assay was performed to analyse the effect of soft drinks on the gut microflora. The probiotics are becoming popular these days because it contains good bacteria that line our digestive tract and provide various health benefits (absorb nutrients, fight infection). To determine the inhibitory effect of these soft drinks on gut microflora, an antimicrobial assay was performed. No zone of inhibition appeared around any soft drink

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samples which clearly showing that the components of soft drinks having no antagonistic effect on the gut flora in Fig. 3. It was evident that components of soft drinks are safe for consumption and have no influence on gut microflora.

Fig. 3 Antimicrobial assay on MRS agar plate, C stands for control (sterilized water) whereas p, s, g and l stand for Pepsi, sprite, Gatorade and limca.

d. Statistical study

ANOVA was performed in which p-value was greater than 0.05 which indicates that there was no significant difference in the mean value of data with the confidence level of 95%. The value of F calculated was less than the F crit it clearly proves null hypothesis shown in table 1.

Table 1. Represented ANOVA test values (F<F crit) and P-value>0.05

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.453	3	2.15	0.040	0.98	6.59
Within Groups	212.66	4	53.16			
Total	219.11	7				

Various research groups investigated excessive consumption of sweet carbonated soft drinks induces thiamine deficiency. A frequent elevation in thiamine requirement results in overt beriberi heart disease. [14] Report on excessive aspartame consumption showed the inhibitory effect on appetite. [15]

4. CONCLUSIONS

The soft drinks used in this study were acidic in its nature. The concentration of preservative viz benzoic acid only found in two drinks and was as per the standard limit preset by the European Union. The soft drinks samples were free from any microbial load as well as found compatible with the gut microflora.

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6. CONFLICT OF INTERESTS

The author declares that there is no conflict of interests regarding the publication of this paper.

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