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Levels of Benzoic Acid in Soft Drinks and Fruit Juices in Ghana

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Abstract: *Benzoic acid is one of the most commonly used food preservatives in the food and beverage industry in Ghana. The maximum accepted level of benzoic acid in beverages stipulated by national and European legislation is 150 ppm. Thirty four (34) different brands of soft drinks and 16 brands of fruit juices available on the markets and shops in Ghana were analysed for benzoic acid by high performance liquid chromatography with a UV detector. Chromatographic separation was achieved with phenomenex synergi 4µ polar – RP 80A 150×2mm 4 micron column with ammonium acetate buffer (pH = 4.4) and acetonitrile (90:10) as the mobile phase with a flow rate of 0.4 mL/min. The objective of this work was to determine the levels of benzoic acid in soft drinks and fruit juices on the markets of Ghana. The concentration of benzoic acid in the samples was calculated by external standard method and the level of benzoic acid ranged from not detected to 564.00 mg/L for the soft drinks and from not detected to 148 mg/L for the fruit juice respectively. The mean recoveries for both soft and fruit drinks ranged from 107 to 110%. Six (17.60%) of the soft drink samples contained levels of benzoic acid above the 150 mg/L which is the limit set by World Health Organization (WHO). The levels of benzoic acid in the fruit juice samples were within stipulated limit. From the results, the Ghana Food and Drugs Authority needs to monitor and regulate levels of preservatives used by some of the soft drinks manufacturers in Ghana especially those whose products exceeded the set limit.*

Key Words: *Benzoic acid, Chromatography, HPLC, Preservatives, Soft drinks,*

I. Introduction

The use of chemical preservatives has become important in food industries in Ghana and elsewhere [1,2]. Benzoic acid is one of the commonly used chemical preservatives in the food and beverage industry. Ghana has become a middle income country and as a result the production of fast, processed and convenient products especially in food and beverage industry by indigenous manufacturers has increased over the past decade due to lifestyle changes and improved standard of living. This has led to a lot of proliferation of soft and fruit drink industries.

A survey conducted showed that most manufacturers prepared the products with low quality assurance and few accredited laboratories. The use of benzoic acid as an antimicrobial agent has been observed to have adverse effects such as metabolic acidosis, convulsion, hyperactive and hyperpnoea in experimental animals and humans given very high doses of benzoic acid [3]. The development of allergic reactions to benzoates in humans, such as urticaria, non-immunological contact urticaria and asthma, has also been reported in some studies [4,5]. Benzene which is carcinogenic can be formed at very low level (ppb level) in soft and fruit drinks containing both benzoates and ascorbic acid. Exposure to heat and light further stimulate the reaction [6]. The use of food additives is limited by specific regulations. Ghana, as many countries, follows the recommendations by the joint FAO/WHO Expert Committee on Food Additives on the safe use of food additives and limit of 150 mg/L benzoic acid has been established.

Although there are several analytical methods for the determination of levels of benzoic acid, such as spectrophotometric and gas chromatographic (GC) method [7], in this study we used high performance liquid chromatography (HPLC), which has a high specificity and minimum sample preparation and does not need derivatization [8,9,2,10].

In this study, the concentration of benzoic acid was measured in both soft and fruit drinks. The results from this study will provide information for consumers' safety and health protection and also give the baseline data for use by Ghana Standards Authority and the Food and Drug Authority as there is no local literature available for policy work and regulatory activities in Ghana. The information obtained combined with consumption rate can also help to estimate the acceptable daily intake (ADI) of benzoic acid by consumers.

II. Materials And Methods

2.1 Standards and Chemicals

Benzoic acid, extra pure from Gatt Koller (Germany), Acetonitrile, (HPLC grade), Ammonium acetate, 97%, Acetic acid (AR) and Water (HPLC grade) were all obtained from Sigma Aldrich (Germany).

2.2 Chromatographic Conditions

The chromatographic analysis was carried out in a Varian Proster high-performance liquid chromatography equipped with Varian Proster 210-218 SD-1 pumps. The analytical column was Phenomenex Synergi 4µ polar – RP 80A 150×2mm 4 micron column with Proster 325 UV detector.

The mobile phase contained a mixture of acetonitrile-ammonium acetate buffer adjusted to pH 4.4. The chromatographic separation was achieved with isocratic elution condition at a flow rate of 0.4 mL/min and 20 µL of sample were injected. The UV-spectrophotometer was used to determine the wavelength of maximum absorption of the benzoic acid prior to the analysis

2.3 Calibration Curve of Standard of Benzoic acid Solutions

Standard solutions containing 1.0, 5.0, 15.0, 25.0, and 50.0 mg/L of pure benzoic acid were prepared. 20 µl of each standard was injected. The peak areas were measured and those of the analytes were plotted against the concentration (mg/L) of the benzoic acid. Least square linear regression analysis was used to determine the slope, y-intercept and the correlation coefficients of the standards plots. The quantification of the samples was based on comparison of the peaks of the standards with those of the samples.

2.4 Sample Preparation

Thirty four (34) different brands of soft drinks and 16 fruit juices were purchased from the open markets in Kumasi, Ghana in three batches. No extraction was done, but the samples were filtered and centrifuged at 3000 rpm for 30 minutes and the supernatant taken. Samples were diluted prior to the analysis.

2.5 Limit of detection (LOD) and Limit of quantification (LOQ), Recovery study and Reproducibility Analysis
The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC analysis were obtained. The recovery studies and the reproducibility of the method were also obtained and shown in table 3 and table 4 respectively.

2.6 Statistical analysis

A descriptive statistical analysis for means, median, standard errors and coefficient of variation were computed. A one way analysis of variance (ANOVA) was carried out using SPSS version 17, at a significance level of 5%.

2.7 Estimated daily intake of Benzoic Acid

The estimated daily intake of benzoic acid from the soft drinks and fruit juices were calculated. Since there is no data on the consumption per capita of soft drinks and fruit juices in the country, the codex (2012) report on the consumption of soft drinks per day and a report by the Business and Financial Times, a News Paper in the country was used in the estimations.

III. Results And Discussion

The external standard method was used to calibrate the chromatographic system for benzoic acid quantification. Peak identification of the benzoic acid in various soft drinks and fruit juices were based on the comparison between the retention times of standard compounds and was confirmed by spiking known standard compounds to the sample. For that purpose standard solution prepared with pure benzoic acid ranging from 1.0 to 50.0 mg/L were used. The Linearity concentration of benzoic acid and the UV absorbance at 238 nm was obtained and correlation coefficient for the standard curve was 0.998. The HPLC system used presented optimum separation with minimal bandwidth with elution time of 7.5 ± 0.27 min for benzoic acid and 10 min was set for the run of each analysis. A total of 34 (n = 34) soft drinks and 16 (n = 16) fruit juices were analysed.

For the soft drink analysis, the concentration of benzoic acid detected in the samples ranged from not detected (nd) to 564.00 mg/L. Out of the 34 soft drink samples analysed only one sample had no levels of benzoic acid, 6 samples showed levels above the set standard for benzoic acid or benzoates in soft drinks (150 mg/L) with the rest within the range of zero to 150 mg/L. The mean concentration for all the samples was 70.20 mg/L which was below the mean concentration recorded by [11,8], (Tfouni *et al*, 2002 in Brazil, 259.2 mg/L and Khosrokhavar *et al*, 2010 in Iran, 163.8 mg/L). The standard deviation, the standard error of mean and the coefficient of variation were 119.00, 11.78 and 169.52% respectively. The high value of the coefficient of variation may be due to fact that the samples used for the analysis were produced from different companies with different production methods and quality assurance. The calculated coefficient of variation from three samples produced from the same company was 1.52%. The total estimate of intake of benzoic acid for soft and fruit drink for adults and children was 0.19 and 1.14 mg/L respectively (Table 5). Soft drinks contributed substantially to the intake of benzoic acid or benzoates with 0.19 mg/L for adults and 1.14 mg/L for children. The intake of benzoic acid for fruit juice was 0.00072 and 0.0043 mg/L for adults and children respectively.

This also shows that the intake of benzoic acid is higher in soft drinks than fruit juices. This is because most of the fruit juices producers use pasteurization as the method in preserving their products. The low level of the per capita consumption of fruit juice shows that Ghanaians do not take in much of fruit juice. This may be due to the abundance of natural fruits in the country. The potential daily intake of benzoic acid calculated are within the range of 0 – 5 mg/L body weight per day by JECFA and WHO, 1987 [12]. The estimated daily intake of benzoic acid calculated for the soft drink and fruit juice was below that of Tfouni and Toledo, 2002 for the same product (soft drinks and fruit juice) who conducted a survey of the daily estimates of benzoic acid in Brazilian foods

Table 1. Descriptive statistics of soft drinks

Statistic	Number screened	Mean conc. (mg/L)	Median conc. (mg/L)	Standard deviation	SEM	CV (%)	Min.	Max.
Soft drink	34	70.20	27.60	119.00	11.78	169.52	nd	564

SEM= standard error of mean CV= coefficient of variation Min= minimum Max= maximum nd= not detected Conc = concentration

Sixteen (16) samples of fruit juices were analysed for the levels of benzoic acid. The concentration of the samples ranged from not detected to 148 mg/L with a mean of 31.00 mg/L. One product had no level of benzoic acid. The standard deviation, the standard error of the mean and the coefficient of variation were 43.62, 6.32 and 140.70 respectively. The high percentage of coefficient variation from the 16 fruit juices was observed because the fruit juices were produced from different companies and therefore each company had its own standard of production. Most of the fruit juices that had labels which displayed as 'no preservatives' actually had some levels of benzoic acid in them.

Table 2. Descriptive statistics of fruit juices

Statistic	Number screened	Mean conc. (mg/L)	Median conc. (mg/L)	Standard deviation	SEM	CV (%)	Min.	Max.
Fruit juice	16	31.00	7.70	43.62	6.32	140.70	nd	148

SEM= standard error of mean CV= coefficient of variation Min= minimum Max= maximum nd= not detected Conc = concentration

The limit of detection for both soft drinks and fruit juices was 0.03 mg/L and the limit of quantification was also 0.10 mg/L. The mean recoveries for soft drinks ranged from 108 to 110% and that of the fruit juices also ranged from 107 to 110% (Table 3). The precision of the method was also evaluated (Table 4). The coefficient of variation of 3.11% was found, which not only indicate the high reproducibility of the method but also indicates that this preservative is stable for a long period of time.

Table 3. Results for recoveries of spiked standards to various samples

Sample	Mean recovery (%)
Kalyppo guava	108.88 ± 4.30
Pina juice	110.98 ± 0.68
Malta guinness	110.20 ± 0.85
Coca cola	107.68 ± 2.60

Table 4. Reproducibility of benzoic acid

Statistic	Number screened	Mean conc (mg/L)	Median conc (mg/L)	Variance	Standard deviation	SEM	CV (%)	Min.	Max.
Standard	6	26.43	26.74	0.68	0.82	0.34	3.11	25.08	27.17

SEM= standard error of mean CV= coefficient of variation Min= minimum Max= maximum Conc = concentration

Table 5. Estimated daily intake (EDI) of benzoic acid

Food category	Analytical concentration (mg/L)	Consumption product (mg/person day^{-1})	EDI[a] (mg/L)	EDI[b] (mg/L)
Soft drink	70.20	0.162	0.19	1.14
Fruit juice	31.00	0.0014	0.00072	0.0043
		Total	0.19072	1.1443

[a] Average body weight (adult) = 60 kg

[b] Average body weight (children) = 10 kg

IV. Conclusion

The analytical determination of benzoic acid as preservative is not only important for quality assurance purposes but also for consumer interest and protection. This work used a simple, fast HPLC method for the determination of benzoic acid in soft and fruit drinks samples in Ghana. The results showed that the benzoic acid concentration varied between different kinds of soft drink samples, with some levels above the maximum values established by national and international legislation and authorities. Most of the products that declared "no preservatives" on some of the fruit juice were not in accordance with their label claims. There was a great significant difference between the levels of benzoic acid in the soft drinks and fruit juice as carried out by the one way analysis of variance at a significant level of 5% with the soft drinks having high levels than the fruit juice. This may be attributed to the fact that most of the fruit juice manufacturers pasteurized their products and therefore tend to add little of the benzoic acid. In conclusion, for producers whose products exceed the legislation limit, it is recommended that whenever possible there should be judicious use of additives and benzoic acid levels should be regulated and only used in the control of yeast and bacteria in food products. A regular monitoring and education should be organized for soft drinks and fruit juice manufacturers in Ghana. This work has given us a firsthand information to the levels of benzoic acid in soft and fruit drinks on the Ghanaian market and also the daily intake of benzoic acid by children and adults.

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