Electronic Journal of University of Aden for Basic and Applied Sciences

EJUA-BA Vol. 5 No. 3 (2024)

https://doi.org/10.47372/ejua-ba.2024.3.376

ISSN: 2708-0684

RESEARCH ARTICLE

MONITORING OF BENZOIC ACID LEVELS IN SOFT DRINKS CONSUMED IN ADEN, YEMEN

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Received: 25 August 2024 / Accepted: 29 August 2024 / Published online: 30 September 2024

Abstract

Benzoic acid is widely used as a food preservative due to its effectiveness in inhibiting the growth of yeast and mold. The undissociated form of benzoic acid is the most potent. In acidic beverages like soft drinks, benzoic acid can react with additives such as ascorbic acid to release benzene, which can pose health concerns. This study aimed to determine the levels of benzoic acid in soft drink samples commonly consumed in Aden, Yemen. Eighteen soft drink samples were analyzed using high-performance liquid chromatography with a UV detector. The highest benzoic acid concentration was found in a FIFA orange sample at 173.87 ppm, while the lowest was 8.40 ppm in a DREAM red sample. Some samples, including MIRINDA orange, FANTA apple, and DILSI red, had no detectable levels of benzoic acid, despite their labels indicating its presence. Overall, the benzoic acid concentrations in all tested samples were within the permissible limits set by local, regional, and international standards.

Keywords: Benzoic acid, Food additives, Soft drinks, High-performance liquid chromatography.

1. Introduction

Food additives are chemical substances that are intentionally added to foods to achieve specific functional purposes, such as preserving food quality, improving appearance, taste, or texture, and facilitating processing [1, 2]. Based on their specific functions, food additives can be classified into various categories, including antimicrobials, antioxidants, emulsifiers, and flavoring agents [3-5].

Benzoic acid is a widely used food preservative due to its effectiveness in inhibiting the growth of yeast and mold. It is typically more effective in its undissociated form, which predominates in acidic environments [6, 7]. In acidic beverages like soft drinks, benzoic acid can potentially react with other additives, such as ascorbic acid, to form benzene, a compound that can raise health concerns [8, 9].

The acceptable dietary intake levels of benzoic acid span a range of food categories, with the following permitted concentrations: various foods 200-1000 ppm, prepared salads and confectionery up to 1500 ppm, food supplements and preserved vegetables at 2000 ppm, liquid egg at 5000 ppm, cooked seafood between 2000-6000 ppm, soft drinks at 150 ppm, alcohol-free beer at 200 ppm, sacramental grape juice at 2000 ppm, and liquid tea concentrates at 600 ppm. The FDA allows benzoic acid usage in food items in quantities up to 1000 ppm (0.1%) [10, 11]. In terms of aquatic toxicity, benzoic acid has been found to have low to moderate toxicity to different aquatic species based on reliable tests. For instance, previous reports have shown that the cyanobacterium Anabaena inaequalis exhibited the lowest EC50 value of 9 mg/liter (cell multiplication inhibition) [12].

Monitoring the levels of food additives, including benzoic acid, is essential to ensure compliance with safety regulations and to assess consumer exposure. Rigorous quantitative methods of analysis, such as high-performance liquid chromatography (HPLC), must be used to assess additive levels in a wide range of food matrices, as multiple additives or groups of additives with comparable functionalities may coexist within a single food product [13-16]. It is necessary to compare the actual amounts of additive use and consumption to safety standards (acceptable daily intakes) and permissible limits set by local, regional, and global regulatory bodies [10, 11].

The primary objective of this study was to determine the levels of benzoic acid in soft drink samples commonly consumed in Aden, Yemen, using HPLC with UV detection. The results were then compared to the local, regional, and international standards to assess the safety and compliance of the analyzed soft drinks.

2. Materials and Methods

2.1. Chemicals and Instrumentation

Analytical grade benzoic acid (reference standard), HPLC-grade acetonitrile and methanol, ammonium acetate, deionized water, orthophosphoric acid, and 0.1 M sodium hydroxide were used in this study. The HPLC system was a JASCO LC-NET model equipped with a UV detector. Other equipment included an ultrasonic bath, vacuum pump, electronic balance, pH meter, and magnetic stirrer.

2.2. Sample Collection

A total of 18 commercially available soft drink samples, including popular brands such as PEPSI, SHANI, MIRINDA, FANTA, DREAM, DILSI, and FIFA, were randomly purchased from local markets in Aden, Yemen for analysis.

2.3. Sample Preparation

The soft drink samples were homogenized and filtered through filter paper to remove any particulates [17]. To degas the carbonated beverages, the samples were sonicated for approximately 15 minutes until the carbon dioxide bubbles were completely removed.

2.4. Standard Solution Preparation

A 1000 ppm stock solution of benzoic acid was prepared by dissolving 1 g of the reference standard in 1000 mL of the mobile phase. Working standard solutions at concentrations of 40, 120, and 160 ppm were prepared by diluting the stock solution with the mobile phase.

2.5. Mobile Phase Preparation

The mobile phase was prepared by mixing acetonitrile (40%) and ammonium acetate (0.04%) in deionized water. The pH of the mobile phase was adjusted to 4.2.

2.6. HPLC Analysis

The HPLC analysis was performed using a C18 column at 25°C. The mobile phase was pumped at a flow rate of 1.5 mL/min with a 40:60 (v/v) ratio of acetonitrile to ammonium acetate solution. The injection volume was 20 µL, and the UV detection wavelength was set at 228 nm. Prior to sample injection, the column was washed with deionized water and methanol to remove any residual salts and organic materials [18].

3. Results and Discussion

3.1. Identification of Benzoic Acid

This study aimed to analyze real samples of commonly consumed soft drinks from the Aden markets to determine the concentration of benzoic acid. A sensitive and reliable HPLC-UV method was developed to detect the presence of benzoic acid in the soft drink samples. An initial test was conducted to ensure that the measurement of benzoic acid was not affected by the elimination of the CO_2 content in the samples.

The HPLC instrument is a highly accurate and valuable analytical tool for the determination of benzoic acid. The concentration of benzoic acid in the samples was calculated based on the peak areas and retention times. In this work, the limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were determined through a calibration graph (**Figure1**) using standard benzoic acid solutions at concentrations of 40, 120, and 160 ppm.

Once the presence of benzoic acid in the samples was confirmed, the quantification was performed using the calibration curve equation y = a + bx, where a is the slope, b is the intercept, x is the concentration, and y is the peak area. The correlation coefficient (R^2) of the calibration curve was 0.9998, indicating excellent linearity. The peak areas for the experimental runs used in calculating the corresponding benzoic acid concentrations are presented in **Table 1**.

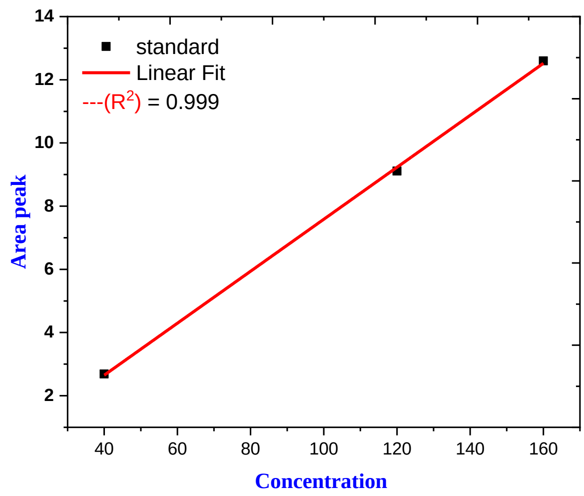

Fig. 1. Calibration curve of standard benzoic acid

Table 1. Calibration table for benzoic acid standards

No.	Concentrations (ppm)	Area (µv.s)	Retention time (min.)
1	40	2686831.5	3.413
2	120	9114036.0	3.403
3	160	12603503.0	3.461

The LOD was calculated as 3 times the residual standard deviation of the y-intercept divided by the slope of the calibration curve [1]. This resulted in LOD and LOQ values of 1.5 and 5 ppm, respectively, which are far below the reference points for benzoic acid in soft drinks, as suggested by the Yemen Standard Organization [19].

3.2. Levels of the benzoic acid in samples

The levels of benzoic acid in the selected brands of soft drinks (PEPSI, SHANI, MIRINDA, FANTA, DREAM, DILSI, and FIFA) were examined chromatographically. The chromatographic analysis results of the samples under study are presented in **Figures 2-6** and **Table 2**. The results obtained for these soft drink samples are summarized in **Table 2**. In 3 samples (16%), benzoic acid could not be detected, either because the compound was not present or the developed method was not suitable for its quantification. The concentrations of benzoic acid in 3 samples (16%) were identified at levels near the estimated limit of quantification (LOQ) of the method, which was determined to be 5 ppm. These samples included MIRINDA orange, FANTA apple, and DILSI red. The chromatograms of a 160 ppm standard of benzoic acid are shown in **Figure 1,** and an actual soft drink sample containing 10.4 ppm of benzoic acid is presented in **Figure 2**, demonstrating that the estimated LOQ is a reasonable value.

The current study indicated that the highest concentration of benzoic acid was found in the FIFA orange sample, reaching a level of 173.87 ppm, while the lowest concentration was detected in a DREAM red sample at 8.40 ppm. Interestingly, the concentrations of benzoic acid were at the lower end of the detection limit in the samples of MIRINDA orange, FANTA apple, and DILSI red soft drinks.

The HPLC analysis of the three samples, PEPSI, SHANI, and MIRINDA, revealed that the concentrations of benzoic acid were 10.52 ppm, 12.72 ppm, and not detected (ND), respectively, as presented in **Table 2**. The highest concentration of benzoic acid, 12.72 ppm, was detected in the SHANI sample, while the lowest concentration of 10.52 ppm was found in the PEPSI sample. When compared to the findings reported by other researchers, the overall concentration of benzoic acid in the current study was lower than the mean value of 336 ± 190 ppm observed by Onwordi et al. [20] in carbonated drinks from Nigeria. This is a significantly higher level than the concentrations detected in the present investigation. Furthermore, our results were also lower than the mean concentration of 163.8 ppm recorded by Khosrokhavar et al. [21] in Iran and the 70.20 ppm reported by Kusi and Acquaah [22] in Ghana.

Table 2. Levels of the benzoic acid (ppm) in samples

No.	Sample	Concentration of Benzoic Acid (ppm)				Retention time (min)			
		Recurrence			Mean ± SD	Recurrence			Mean
		1	2	3		1	2	3	
1	PEPSI	10.38	10.26	10.92	10.52 ± 0.35	3.942	3.967	3.933	3.950
2	SHANI	13.42	12.35	12.40	12.72 ± 0.60	3.242	3.233	3.350	3.275
3	MIRINDA orange	ND	ND	ND	ND	ND	ND	ND	ND
4	FANTA red	11.12	10.86	10.79	10.92 ± 0.17	3.525	3.025	3.592	3.380
5	FANTA orange	29.40	28.98	30.46	29.61 ± 0.76	3.558	3.492	3.367	3.473
6	FANTA apple	ND	ND	ND	ND	ND	ND	ND	ND
7	FANTA lemon	42.11	40.64	41.52	41.42 ± 0.74	3.467	3.467	3.700	3.546
8	DREAM black	13.60	14.30	8.58	12.16 ± 3.12	3.350	3.383	3.425	3.386
9	DREAM RED	8.38	8.40	8.41	8.40 ± 0.02	3.575	3.542	3.525	3.547
10	DREAM orange	8.66	8.71	8.79	8.72 ± 0.07	3.417	3.458	3.433	3.436
11	DREAM apple	58.47	61.70	64.44	61.54 ± 2.99	4.117	4.117	4.083	4.106
12	DILSI red	ND	ND	ND	ND	ND	ND	ND	ND
13	DILSI orange	9.22	9.79	9.65	9.55 ± 0.30	4.025	4.142	3.908	4.025
14	DILSI apple	36.82	38.28	32.81	35.97 ± 2.83	4.200	4.150	4.183	4.177
15	FIFA red	171.28	172.03	_	171.66 ± 0.53	3.492	3.517	_	3.504
16	FIFA orange	172.55	175.19	_	173.87 ± 1.87	3.450	3.475	_	3.4625
17	FIFA apple	145.21	142.16	_	143.67 ± 2.18	3.467	3.458	_	3.4625
18	FIFA lemon	158.06	157.44	_	157.75 ± 0.44	3.608	3.592	_	3.600

ND = not detection limit

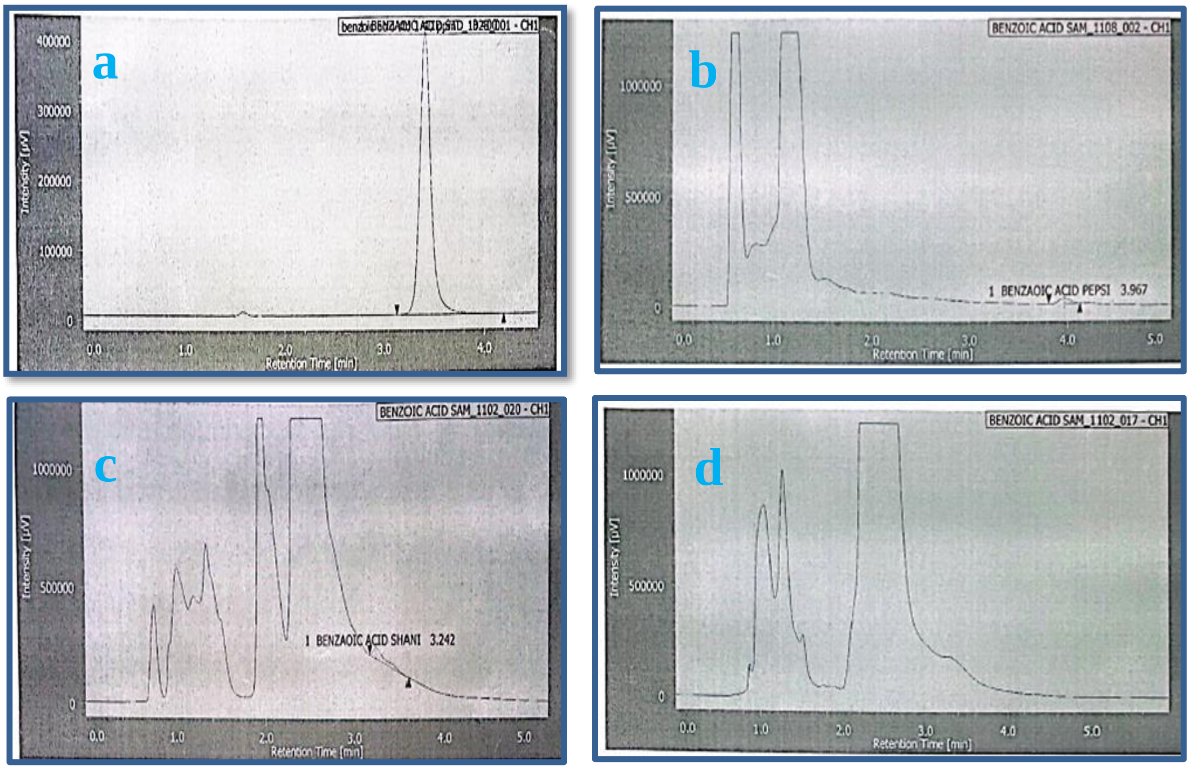

Fig. 2. Benzoic acid content in (a) standard solution (b) Pepsi (pH=2.39), (c) Shani (pH=2.8) and (d) Merinda orange (pH=2.2)

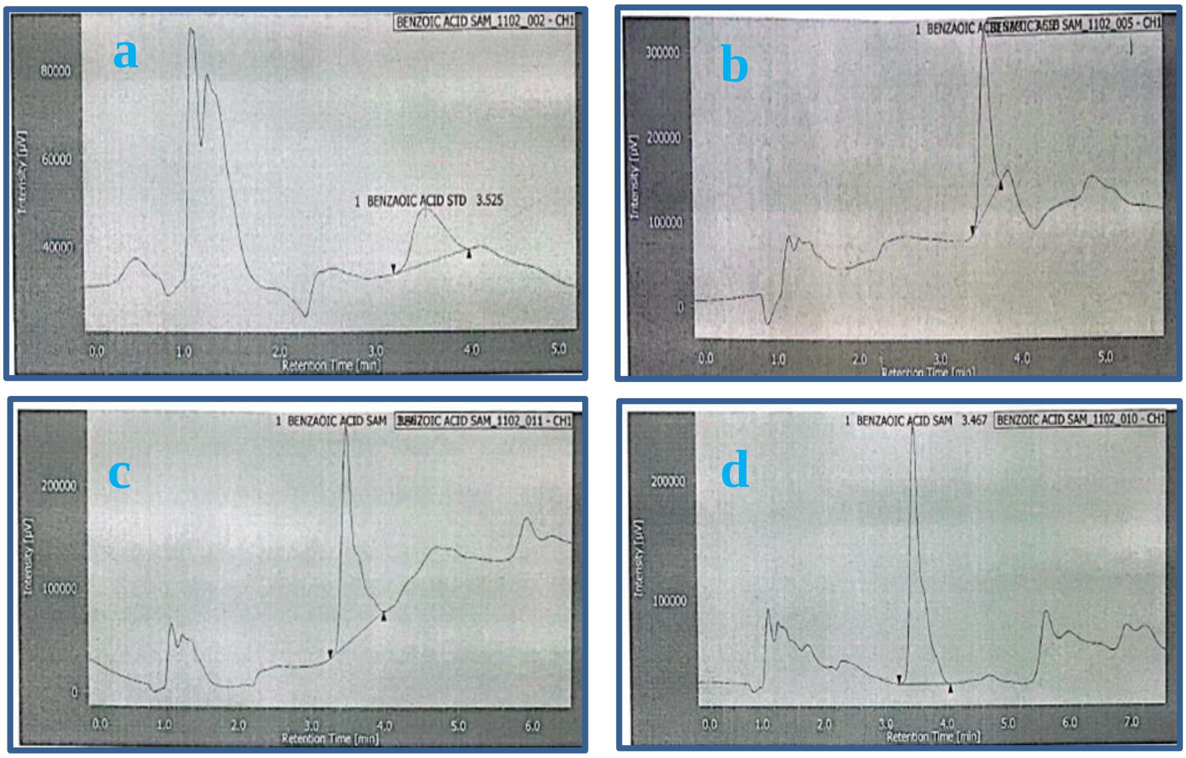

Fig. 3. Benzoic acid content in (a) FANTA Red (pH=3.37), (b) FANTA Orange (pH=3.18), (c) FANTA Lemon (pH=3.25), and (d) FANTA Apple (pH=3.25)

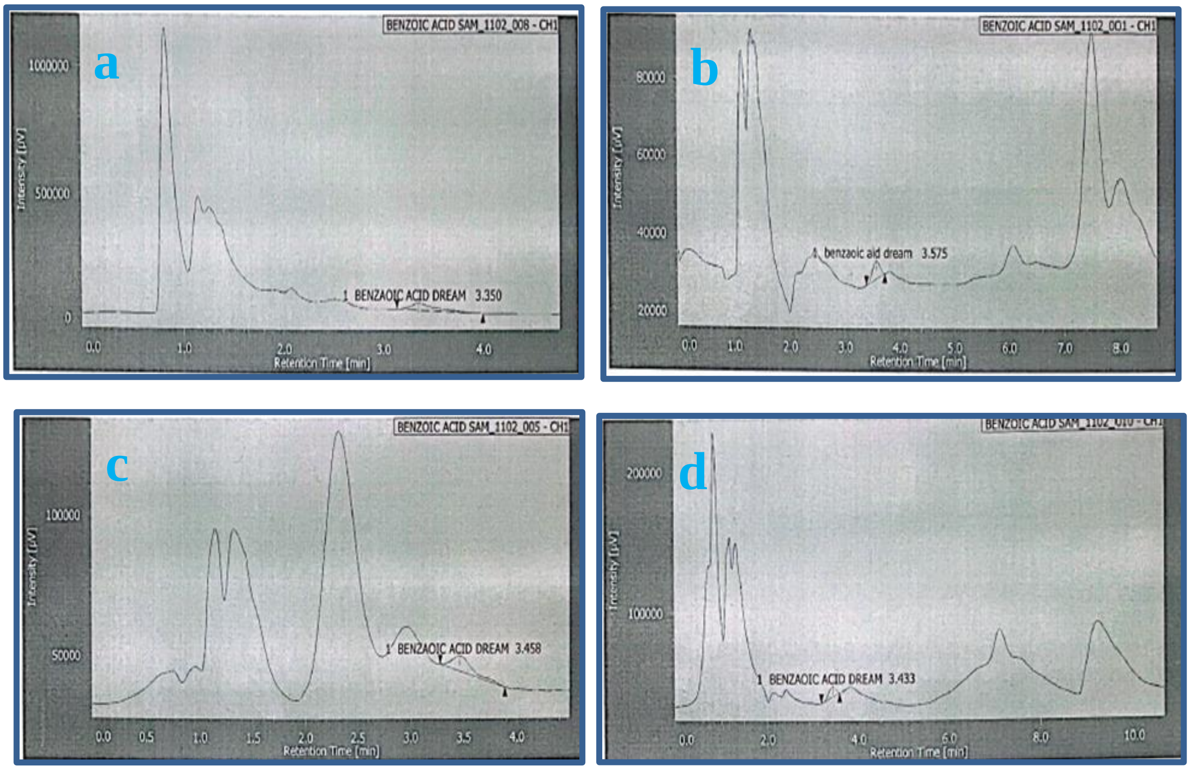

Fig. 4. Benzoic acid content in (a) Dream black (pH=3.08), (b) Dream Red (pH= 3.34), (c) Dram orange (pH=3.23) and (d) Dream Apple (pH=2.84)

The results represented in **Table 2** show that the mean concentration of benzoic acid in the FANTA soft drink samples varied significantly. The FANTA red, FANTA orange, FANTA apple, and FANTA lemon samples contained 10.92 ppm, 29.60 ppm, not detected (ND), and 41.42 ppm of benzoic acid, respectively. The highest mean concentration was found in the FANTA lemon sample at 41.42 ppm, while the lowest mean concentration was observed in the FANTA red sample at 10.92 ppm. Interestingly, no detectable levels of benzoic acid were found in the FANTA apple sample. The obtained results in this study were lower than those reported by Tfouni and Toledo [23], who determined the levels of benzoic acid in per capita daily intake in Brazil to be 259.2 ppm.

Regarding the DREAM soft drink samples, **Table 2** shows that the concentrations of benzoic acid were 12.16 ppm, 8.40 ppm, 8.72 ppm, and 61.56 ppm for the DREAM black, DREAM red, DREAM orange, and DREAM apple, respectively. The highest mean concentration of benzoic acid in the DREAM soft drink samples was found in the DREAM apple at 61.54 ppm, while the lowest was in the DREAM red sample at 8.40 ppm.

The concentration of benzoic acid was also measured in both soft and fruit drinks by Kusi and Acquaah [22], who used an external standard method. The level of benzoic acid ranged from not detected to 564.00 ppm for the soft drinks and from not detected to 148 ppm for the fruit drinks. The results obtained in the current study were generally lower than those reported [22].

For the DILSI soft drink samples, the concentrations of benzoic acid were ND, 9.55 ppm, and 35.97 ppm for the DILSI red, DILSI orange, and DILSI apple, respectively. The data in Table 2 indicate that the highest mean concentration of benzoic acid in the DILSI soft drink samples was found in the DILSI apple at 35.97 ppm, while the lowest mean concentration was observed in the DILSI orange sample at 9.55 ppm. No detectable levels of benzoic acid were found in the DILSI red sample.

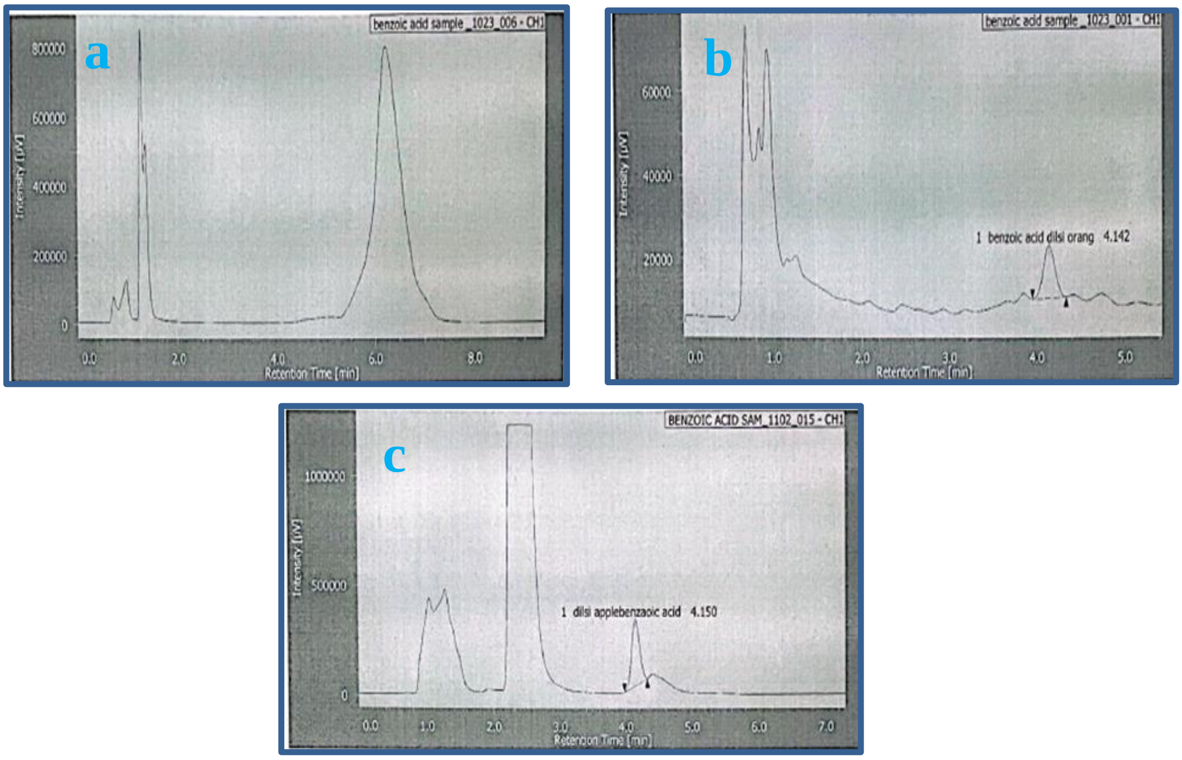

Fig. 5. Benzoic acid content in (a) DILSI Red (pH= 2.85), (b) DILSI Orange (pH=2.85), and (c) DILSI Apple (pH=2.65)

The AOAC liquid chromatographic method [24] was collaboratively tested for the determination of benzoic acid in orange juice. This method is applicable for the determination of 0.5–10 ppm benzoic acid in orange juice, where benzoic acid in solid-phase extracted orange juice is separated by liquid chromatography on a C18 column.

The analysis of the FIFA soft drink samples revealed significant levels of benzoic acid. As shown in **Table 2**, the FIFA Red, FIFA Orange, FIFA Apple, and FIFA Lemon samples contained 171.66 ppm, 173.87 ppm, 143.67 ppm, and 157.75 ppm of benzoic acid, respectively. The highest mean concentration of benzoic acid was observed in the FIFA Orange sample at 173.87 ppm, while the lowest was found in the FIFA Apple sample at 143.67 ppm. The benzoic acid concentration in this group of FIFA soft drinks ranged from 143.67 ppm to 173.87 ppm.

In a study by Javanmardi et al. [25], the researchers analyzed 54 different food samples, including soft drinks, UHT milk, ketchup, and bread. The results indicated a high prevalence of benzoic acid, with 92.5% of the samples testing positive. The detected levels ranged from 3.5 to 1520 ppm, which aligns with the concentration range reported in the current study.

Furthermore, a suitable HPLC method for determining benzoic acid in foodstuffs was collaboratively tested on various products such as orange squash, cola drinks, beetroot, and pie filling [26]. This method is applicable for benzoic acid concentrations ranging from 50 to 2000 ppm. The performance characteristics for benzoic acid in orange squash and cola drinks were reported as 471.4 ppm and 1234.4 ppm, respectively [26].

When comparing the concentration of benzoic acid in all soft drink samples in the current study, it was observed that the levels were within the allowed limits set by local, regional, and international standards, as shown in **Table 3** [19].

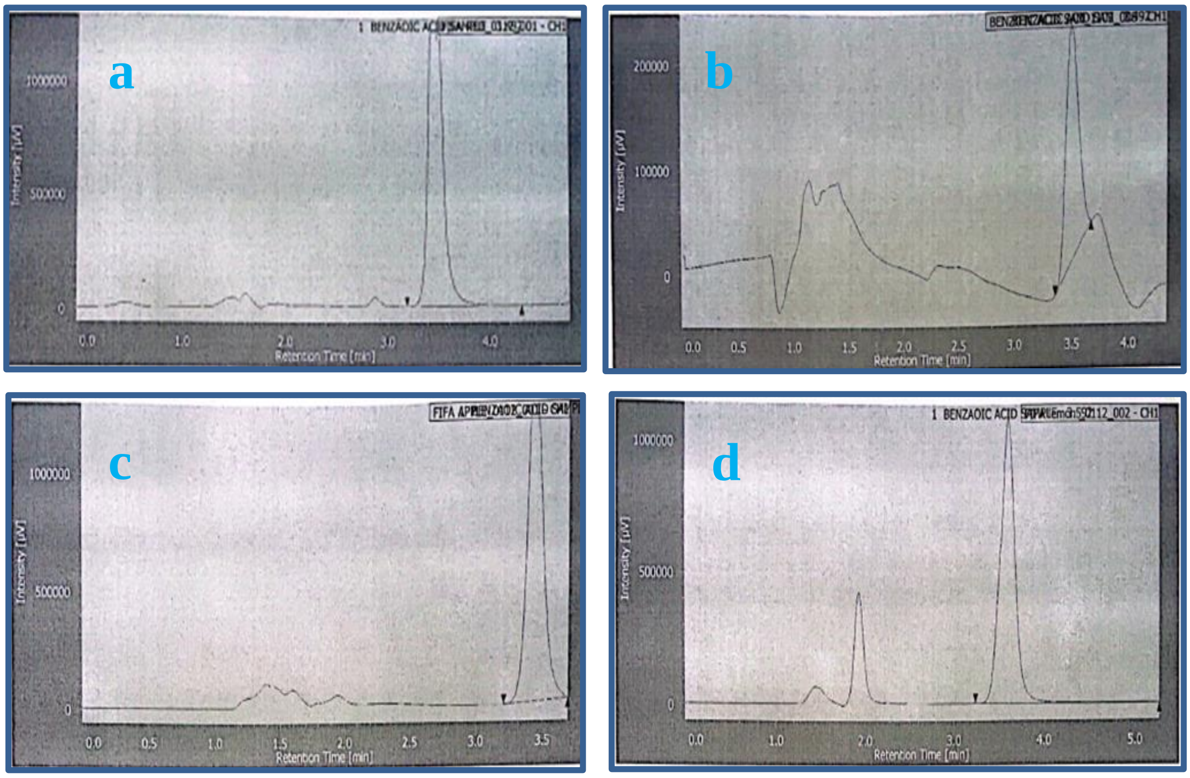

Fig. 6. Benzoic acid content in (a) Fifa Red (pH= 3.37), (b) Fifa Orange (pH=2.85), and (c) Fifa Apple (pH=2.78), and (d) Fifa Lemon (pH=2.78)

Table 3. Permissible limits of benzoic acid in soft drinks, according to specifications

No.	State or organization	Specification name	Benzoic acid concentration mg/L
1	Republic of Yemen The Ministry of Industry, the Yemeni Organization for Standardization, Metrology and Quality Control	Yemeni Standards No. (586/2003) C_6H_5COOH, C_6H_5COONa and C_6H_5COOK in food preservation	1000
2	Gulf standard specification	C_6H_5COOH, C_6H_5COONa and C_6H_5COOK in the preservation of ingredients (1994/172)	1000
3	World Health Organization Food and Agriculture Organization	ALINORM 07/30/12	1000

3.3. pH values of the samples

In food additives, an increase in the carbon chain length of the alkyl group (R) generally leads to a decrease in water solubility but an increase in solubility in non-aqueous solvents. This structural change also tends to decrease the toxicity while enhancing the antimicrobial effect of the additive.

Although benzoic acid exhibits its preservative effect in solutions with pH < 4, certain food additives can be effective at higher pH levels, up to around pH 7 or higher. This extended pH range of activity is not achievable with conventional chemical preservatives. The individual additives or a mixture of additives are often blended with benzoic acid to reinforce the antimicrobial effect on foods across a wider pH range of 4-8.

The data presented in **Table 4** suggests that an increase in temperature leads to a decrease in the pH value of the soft drink samples. This is due to the increased ionization of the molecules within the acidic soft drink matrix. As soft drinks are inherently acidic, the rise in H ions results in a lower pH value.

Table 4. pH values of the samples

Sample	pH in different degrees	
	At 12 °C	At 25 °C
PEPSI	2.74	2.37
SHANI	3.32	3.28
MIRINDA Orange	2.85	2.59
FANTA Lemon	3.60	3.35
FANTA Orange	3.58	3.18
DILSI Red	3.89	3.22
DILSI Orange	3.75	2.96
DILSI Apple	3.10	2.85

The reported pH ranges for different beverage categories are: carbonated drinks (2.46-4.10), fruit juices (2.54-3.86), sports drinks (2.59-2.61), and dairy drinks (3.83-5.21) [27]. Generally, these beverages are acidic in nature, which can help inhibit bacterial growth. However, the acidity of these drinks can also lead to the demineralization of teeth, with sports drinks being the most acidic among the studied samples.

3.4. Health implications of benzoic acid in soft drinks

In this study, we have discussed the environmental impact of benzoic acid and its discharge into water and soil due to its usage as a preservative in various products. Building upon this, it is essential to also consider the health implications of benzoic acid, particularly in soft drinks.

Benzoic acid and its salts are widely used as preservatives in various food and beverage products, including soft drinks, due to their antimicrobial properties. However, the presence of benzoic acid in acidic beverages, such as soft drinks, can pose potential health concerns.

When benzoic acid is mixed with ascorbic acid (vitamin C) in soft drinks, it can undergo a chemical reaction to form benzene, a known carcinogenic compound [12]. Exposure to benzene has been linked to various health issues, including cancer, reproductive problems, and neurological disorders [28-30].

Furthermore, high levels of benzoic acid consumption have been associated with other health concerns. Benzoic acid can irritate the gastrointestinal tract, causing symptoms such as nausea, vomiting, and diarrhea [31]. Additionally, there is evidence that benzoic acid may have adverse effects on the nervous system, potentially leading to headaches, dizziness, and hyperactivity, particularly in children [32,33].

The potential health risks associated with benzoic acid in soft drinks highlight the importance of monitoring and controlling its levels in these products. Continuous surveillance and enforcement of regulations are crucial to ensure the safety of consumers and promote transparency in the food industry.

Overall, our findings underscore the need for further research and regulatory measures to mitigate the environmental and health risks associated with benzoic acid.

4. Conclusion

The study has not only investigated the levels of benzoic acid in commonly consumed soft drinks in Aden, Yemen but has also highlighted the potential health concerns associated with this food preservative.

The findings indicate that the concentrations of benzoic acid in the analyzed samples were within the permissible limits set by local, regional, and international standards. However, the study emphasizes the potential health risks posed by the presence of benzoic acid in acidic beverages, particularly when combined with ascorbic acid. The formation of the carcinogenic compound benzene and the various gastrointestinal and neurological effects linked to high benzoic acid consumption underscore the need for continuous monitoring and control of this food preservative.

The study underscores the importance of transparent labeling and consumer awareness regarding the presence of benzoic acid and other food additives. This information empowers consumers to make informed choices and encourages the food industry to adopt responsible practices in the use of preservatives.

Overall, this comprehensive analysis, including the assessment of the potential health implications, provides a robust framework for ongoing efforts to ensure the safety and quality of soft drinks and other food products. The findings will contribute to the development of more effective regulations and strategies to safeguard public health in the region.

Acknowledgments

The authors extend their heartfelt appreciation to the Supreme Authority for Drugs and Metrology in Aden, Yemen, for granting access to their state-of-the-art laboratories for the analysis of the soft drink samples. The unwavering support and collaboration of the Authority's staff were crucial in the successful execution of this research.

Declarations

Conflict of Interest: The authors state that there are no financial conflicts or personal relationships that could have impacted the research presented in this paper.

Ethical Approval: The authors affirm that the study was conducted in strict accordance with all applicable ethical guidelines and procedures.

Sample Availability: Samples of the compounds examined in this study can be obtained from the corresponding author upon a reasonable request.

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مقالة بحثية

مراقبة مستويات حمض البنزويك في المشروبات الغازية المستهلكة في عدن، اليمن

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استلم في: 25 أغسطس 2024 / قبل في: 29 أغسطس 2024 / نشر في 30 سبتمبر 2024

المُلخّص

يُستخدم حمض البنزويك على نطاق واسع كمادة حافظة للأطعمة نظرًا لفعاليته في منع نمو الخميرة والعفن. يُعتبر الشكل غير المتأين من حمض البنزويك هو الأكثر فعالية. في المشروبات الحمضية مثل المشروبات الغازية، من الممكن أن يتفاعل حمض البنزويك مع المواد المضافة مثل حمض الأسكوربيك لإطلاق البنزين، مما يمكن أن يشكل مخاطر صحية. هدفت هذه الدراسة إلى تحديد مستويات حمض البنزويك في عينات من المشروبات الغازية التي تُستهلك بشكل شائع في عدن، اليمن. تم تحليل ثمانية عشر عينة من المشروبات الغازية باستخدام كروماتوغرافيا السائل عالية الأداء مع كاشف الأشعة فوق البنفسجية. تم العثور على أعلى تركيز لحمض البنزويك في عينة من مشروب برتقال فيفا (FIFA) بتركيز 173.87 جزء في المليون، بينما كان الأدنى 8.40 جزء في المليون في عينة من مشروب دريم الأحمر (DREAM). بعض العينات، بما في ذلك مشروب ميرندا البرتقال (MIRINDA)، ومشروب فانتا (FANTA) بنكهة التفاح، ومشروب ديلسي الأحمر (DILSI)، لم يكن لها مستويات قابلة للكشف من حمض البنزويك، على الرغم من أن ملصقاتها تشير إلى وجوده. بشكل عام، كانت تركيزات حمض البنزويك في جميع العينات المختبرة ضمن الحدود المسموح بها وفقًا للمعايير المحلية والإقليمية والدولية.

الكلمات المفتاحية: حمض البنزويك، المواد المضافة للأطعمة، المشروبات الغازية، كروماتوغرافيا السائل عالية الأداء.

How to cite this article:

N. A. Al-halmi, A. A. M. Saeed, M. S. S. Al-Salimi, M. G. Abobakr, Kh. Y. Ahmed, O. Kh. Mustafa, F. S. Hood, R. S. Hussein, N. M. Saeed, Z. O. Taleb, Y. K. Manea, “MONITORING OF BENZOIC ACID LEVELS IN SOFT DRINKS CONSUMED IN ADEN, YEMEN”, *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 5, no. 3, pp. 288-298, September. 2024. DOI: https://doi.org/10.47372/ejua-ba.2024.3.376