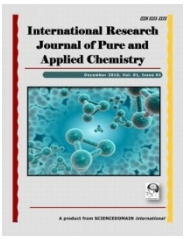

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RP-HPLC Determination of Benzoic Acid in Samples of Children Food Distributed in Aden-Yemen

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMS and SKB managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Reverse phase high-performance liquid chromatographic method that allows the determination of the preservative benzoic acid is described. Separations were affected by using an initial mobile phase of methanol–acetate buffer (pH 4.4), (35:65). Separation of benzoic acid is achieved in 7.10 min. Analytical performance of separation such as the limit of detection, limit of quantification, linear range and reproducibility were evaluated. The method was applied to determine the quantities of benzoic acid in 32 local and imported children food products distributed in Aden-Yemen, comprising, dry milk and chesses, food for infants and young children; cakes, juices and jam. The results show that the ranges of benzoic acid in the studied samples vary greatly from 1.8 mg/L to 849.3 mg/L.

Keywords: RP-HPLC; children food analysis; benzoic acid; Aden –Yemen.

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1. INTRODUCTION

Preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods during its shelf life. In addition, they prevent consumer hazards owing to the presence of microbial toxins or pathogenic microorganisms and economic losses due to spoilage. The role of preservatives has become more prominent with the increase in production of treated and convenience foods. These fields have been interested in consumers, health professionals, commercial and industrial agencies, because they are widely consumed in the diet by most segments of the population, also it can exert adverse health effects, especially for children and pregnant women. In fact, such food additives are subject to regulation, since an excessive or inappropriate use of them may present food-safety problems and can introduce a risk factor [1].

The European scientific committee on food has recommended that children should pay particular attention to intake, since there is evidence suggesting that the intake of some additives express on a body weight basis may be markedly higher than that of adults. Utilizing benzoic acid (E210) as well their salt sodium benzoate (E211); potassium benzoate (E212); calcium benzoate (E213) is allowed by European legislation, and its presence must be declared on the label [2]. WHO's International Program on Chemical Safety (IPCS) suggests that a provisional tolerable intake would be five mg/kg body weight per day [3]. For this reason, using food additives in different countries is limited by definite regulations. These preservatives are permitted by legislation, but their use demands special care. Efficient analytical techniques able to detect the content of this preservative are available such as spectrophotometric methods [4-5], chromatographic methods [6-7] and capillary electrophoresis methods [8]. The spectrophotometric methods in the analysis of food preservative presents major inconvenient, since the interferences required almost an additional extractive step. HPLC methods are more attractive for this purpose, offering the possibility to analyze food additives without a prior step, with high accuracy and precision, and a very good detection limit, as well. The objectives of present work were to determine the quantities of benzoic acid in 32 regional and imported children food products distributed in Aden-Yemen, comprising, dry milk and cheese, food for infants and young children, cakes, juices and jam.

1.1 Experimental Part

1.1.1 Sampling

Samples were collected in August 2010, from different supermarkets in the Aden-governorate The analyzed food products comprised of cakes (14 samples), juices and drinks (eight samples), jams (three samples), dry milk and cheese (three samples), and food for infants and young children, mainly manufacturers of grain (four samples).

2. PREPARATION OF SAMPLES

2.1 Liquid Samples

10mL of liquid sample was diluted with 25 mL of methanol into 50mL volumetric flask and mixed well at 50ºC for about 10 min. on sonicator (vibrating heating plate) (BANDELIN RK 100H, Germany). The volumetric flask was next subjected to vortex mixing (Model ALC, Germany) for five min. The contents were filtered through a 0.45 µm syringe filters (Sartorius

AG 37070 Goettingen Germany). The content then transferred into another 50mL volumetric flask and diluted with methanol up to the mark. The clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done.

2.2 Solid Samples

Solid samples were finely ground in an electric mixer prior to the extraction. About 1.0g of sample is accurately weighed in a screw capped test tube. 25 mL of methanol was added, and placed in a sonicator that was maintained at 50ºC for 30 min. The test tube was next subjected to vortex mixing for 5 min. The contents were filtered through a 0.45 µm syringe filters. The content then transferred into a 50 mL volumetric flask and diluted with methanol up to the mark. The clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done.

3. CHEMICALS AND REAGENTS

Chemicals and reagents used were obtained from the (BDH), VWR, UK, acetic acid (99.8%), benzoic acid extra pure, ammonium acetate (Analar); methanol (HPLC grade).

4. CHROMATOGRAPHIC CONDITIONS

Analytical separation was carried out on HPLC from Perkin-Elmer, Germany, model (Series – 200). Using (EXCGL 120 ODS-A) C18 column. (15cm x 4.6mm, 6µm). At room temperature (22±2ºC). The detector used was a Perkin-Elmer, Germany UV-VIS model (Series – 200), set at 233 nm. The volume of sample injected was 20 µL. The aqueous phase was prepared by adding 3.8 grams of ammonium acetate in one liter of water. pH was adjusted to 4.4 using acetic acid. The mobile phase used was methanol–ammonium acetate buffer (pH 4.4) (35:65 v/v) for 10 minutes. The flow rate was 1mL /min. . External calibration were done using standard benzoic acid solution.

5. STATISTICAL ANALYSIS

The results of the RP-HPLC analysis of standard solutions of benzoic acid as well as the studied samples were expressed as mean. Data were analyzed using a proprietary computer program for interactive scientific graphing and data analysis, Origin-8.1, from Origin lab Corporation.

6. RESULTS AND DISCUSSION

6.1 Optimization and Validation

The typical chromatogram resulting from injected standard benzoic acid solution in our work is shown in Fig. 1, resulting chromatogram indicates that retention time of benzoic acid was 7.10 minutes.

Linearity between the concentration of benzoic acid and the UV absorbance at 233 nanometers was maintained over the concentration range of 5-100 mg/L. The calibration curve was obtained by plotting the concentration vs. peak area as shown in Fig. 2.

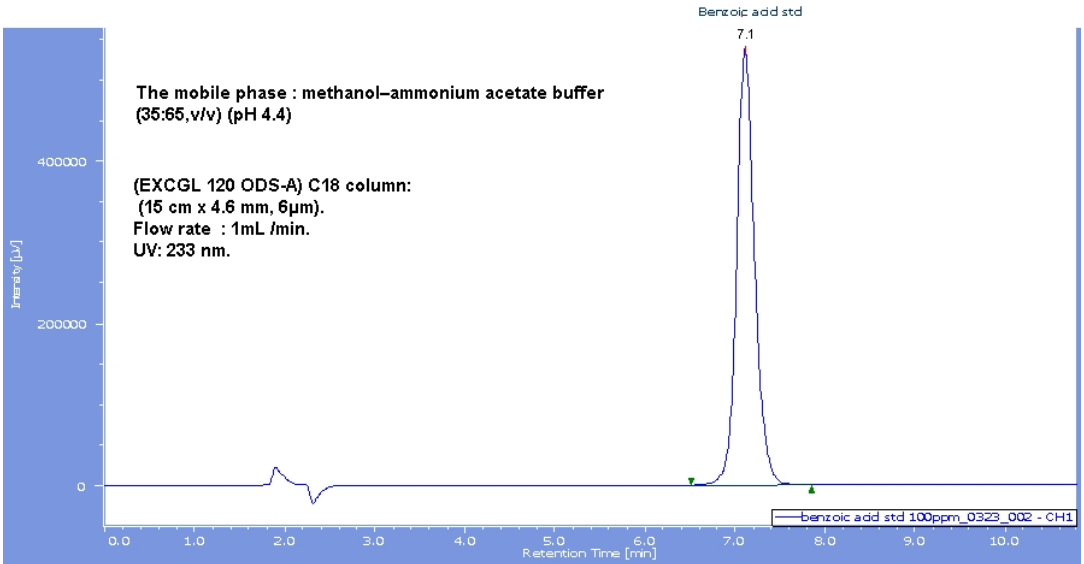

Fig.1 chromatogram of 100mg/L standard benzoic acid solution

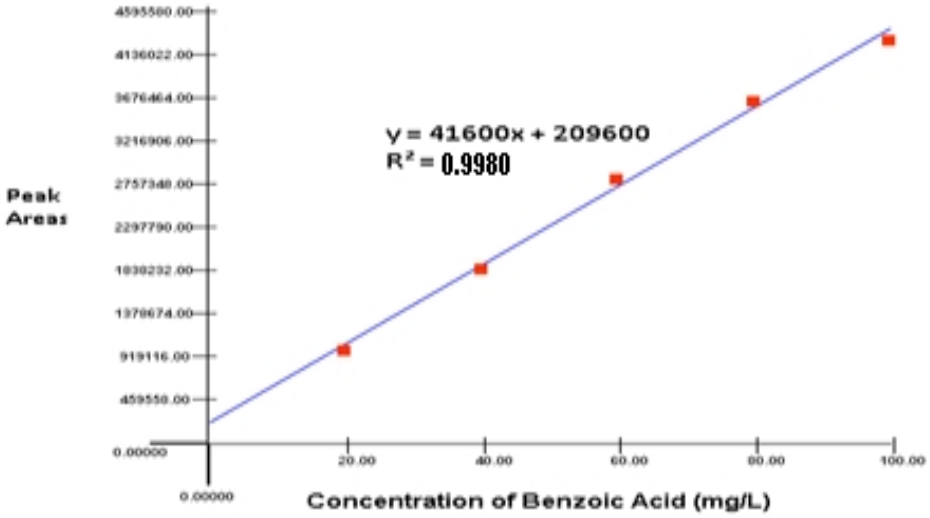

Fig.2. Calibration curve for standard Benzoic Acid

The average percentage of deviation or variation is equal to 1.654%, which is small when compared with the reference deviation ratio (±15%). This small variation indicates the stability of measurement. The limit of detection (LOD) is defined as the smallest peak detected with a signal height three times that of the base line while the limit of quantitation (LOQ) refers to the lowest level of analyte which can be determined with an acceptable degree of confidence. (LOQ) value is often calculated as 10 times the signal height to the baseline. In our work, detection and quantitation limits were estimated by successively decreasing the concentration of the prepared standards, down to the smallest detectable

peak. This concentration was multiplied by 3 and 10 to obtain the detection and quantitation limits, respectively. Table 1 show the analytical performance of the proposed method.

Table 1. Analytical performances of RP-HPLC method

Parameter	Benzoic acid
limit of detection (LOD) (mg/L)	0.473
limit of quantitation (LOQ) (mg/L)	1.435
Linear range (mg/L)	5-100
The correlation coefficient (R^2)	0.9980

6.2 Application to Commercial Samples

The selected children foods in this study were chosen to be fall under five categories. The mean concentration of benzoic acid in each sample in the different categories was shown in Tables 2, 3, 4, 5 and 6.

Category (1) consists of three samples of dry milk and chesses. Benzoic acid means concentrations in these samples' ranges from 2.749 to 94.943 mg/kg. Sample1and 2 had a small mean concentration of benzoic acid 2.749 mg/kg, and 11.207 mg/kg respectively, while sample 3 had a mean concentration of 94.943mg/kg, Table 2.

Table 2. Concentrations of benzoic acid (mg/kg) in category (1), dry milk and cheese samples

Samples no.	Mean concentration (mg/kg) ± SD	Mean concentration range of benzoic acid in the category
1	2.749±0.318	2.749 - 94.943
2	11.207±2.398	
3	94.943±2.826	

n=3

The content of benzoic acid in these studied samples may be attributed to the nature of the sample. The presence of different amounts of (hippuric acid) in samples of dry milk and cheese, and the culture (thermophilic) in milk and (mesophilic) in cheese, converts (hippuric acid) in milk and cheese to benzoic acid Furthermore, other factors, such as feeding habits and conditions of storage and pollution may be expected to result in the presence of benzoic acid. This explanation is supported by a number of studies, such as the European Commission which stated that, most of the benzoic acid were derived from (hydroxycinnamic acids) as components of consumer feed ingested by animals [9]. Wibbertmann and others in 2005 [10] said that, benzoic acid can be seen naturally in many animals and plants. It is naturally found in many foods, including milk products and did not exceed the average values of 40 mg/L of food, and the maximum benzoic acid added to food for purpose of conservation were 1000 mg/L. Campell in 2010 [11], found that benzoic acid occurs naturally in milk at low concentration, a few milligrams per kilogram in fermented dairy,a high concentration lies in the range 20-50 mg/L. During the process of fermentation lactic acid bacteria turning (hippuric acid) which occurs naturally in milk to benzoic acid.

When comparing the results of this category with similar previous studies, we found that, Sieber and others in 1995 [12] found that the amount of benzoic acid in samples of Swiss

cheese lies in the range 12- 40 mg/kg, Urbien, and Leskauskait in 2006 [13] found that, the amount of benzoic acid in samples of fermented milk in Lithuania lies in the range 14-23 mg/L, Ahmet and others in 2008 [14] found that, the amount of sodium benzoate in Turkish samples of dry milk and cheese located in the range 112.1- 280.4 mg/kg, Smith in 2008, [15] found that benzoic acid occurs naturally in milk at low levels, also found that the amount of benzoic acid in samples of pasteurized milk and milk brewed located in ranges 4- 6 and 30-60 mg/L respectively, and that the amount of benzoic acid in samples of white cheese lies in the range 9-18 mg/kg, Ishiwata and others in 1999 [16] found that the amount of benzoic acid in 399 Japanese sample of milk and cheese, located in range 1.0-16 mg/kg, Ping qi and others in 2009 [17] found that the amount of sodium benzoate in 31 samples of Chinese's milk powder located in the range 19-110 mg/kg Fig. 3.

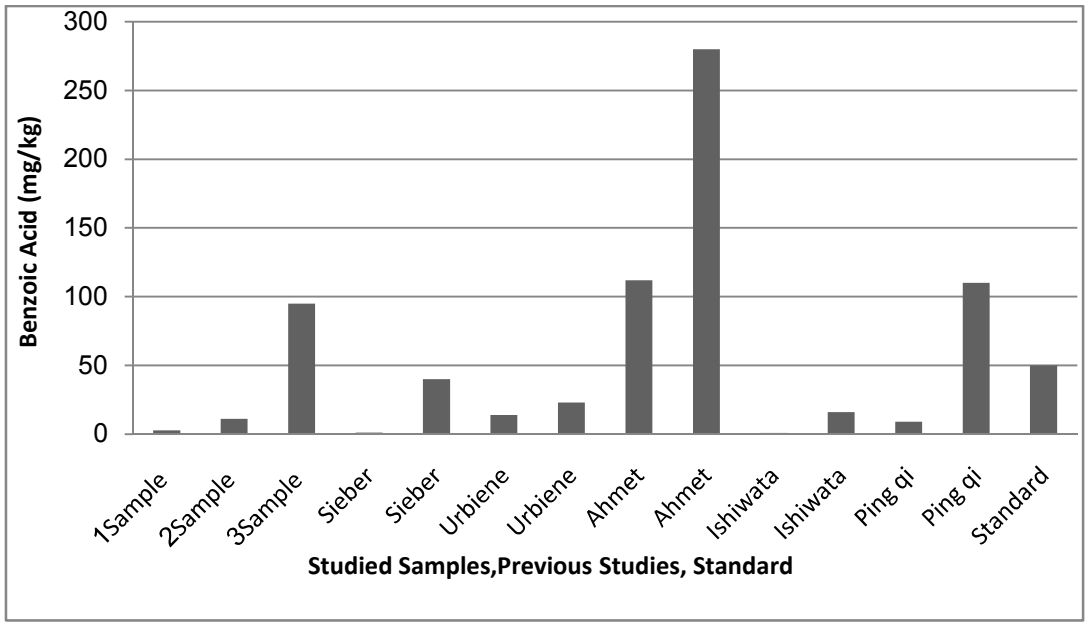

Fig. 3. Catagory (1) Dry milk and Cheeses

Category (2) consists of four food samples for infants, and young children manufactures from grains, the mean concentrations of benzoic acid in these sample's ranges from 84.753 mg/kg to 180.066 mg/kg, Table 3.

Table 3. Concentrations of benzoic acid (mg/kg) in category (2), food for infants and young children, mainly manufacturers of grain samples

Samples No.	Mean concentration (mg/kg) ± SD	Mean concentration range of benzoic acid in the category
4	84.753±0.591	84.753-180.066
5	91.245±5.818	
6	114.842±5.546	
7	180.066±3.126	

n=3

Most standards do not permit to adding benzoic acid to dry milk. From these, Yemeni standard specifications No. 442 issued 2003, No. 586 issued 2003. No. 470 issued 2004. No.1091 issued 2005, No. 373 issued 2008, Codex Alimentarius Commission Specification No. 192 issued 1995, European specifications for food additives' EU No. 95/2/EC [9-15,18-24]. Only Yemeni standard specification No. 860 issued 2004 [16,25] states that the maximum allowable ascorbic acid or benzoic acid or their salt in milk is 50 mg/L (individually or collectively). The presence of milk and grain samples in this group is likely to be one of the reasons for the possibility of a natural occurrence of benzoic acid in this group. Wibbertmann and others in 2005 [18,10], reported that benzoic acid occurred naturally in many plants and animals. Most standards did not allow adding benzoic acid in the canned baby food manufacturer, mainly of grain, such as, Yemeni standard No.1028 issued in 2004, and No. 22 issued in 2009, Gulf Standard No. 677 issued in 2007, [26-28] which specializes in infant and children's food and the Codex Alimentarius Commission specification No. 73, No.74 issued in 1981, [29-30] and Codex Alimentarius Commission specification for canned foods for children mainly manufactures from grain, No. 192 issued 1995, [23]. Therefore, the presence of benzoic acid as a preservative for this category of children food is a violation of the specification.

Category (3) consists of fourteen samples of cake. Mean concentrations of benzoic acid in these sample's range from 1.778 mg/kg to 849.295 mg/kg. It is divided into three groups, first one, which consist of samples 8 to 12 with mean concentration range of benzoic acid from 1.778 mg/kg to 5.492 mg/kg, the second group which consists of samples 13 to 17 with mean concentration of benzoic acid from 27.220 mg/kg to 94.670 mg/kg, and the third group which consists of samples 18 to 21 with mean concentration of benzoic acid from 108.107 mg/kg to 849.295 mg/kg, Table 4.

Table 4. Concentrations of benzoic acid (mg/kg) in category (3), cakes samples

Samples No.	Mean concentration (mg/kg) ± SD	Mean concentration range of benzoic acid in the groups	Mean concentration range of benzoic acid in the category
8	1.778±0.707	1.778 – 5.492	1.778 - 849.295
9	2.206±0.933		
10	2.587±0.429		
11	4.689±0.411		
12	5.492±0.076		
13	27.220±1.924	27.220 - 94.670	
14	46.417±4.677		
15	66.151±2.185		
16	69.103±5.928		
17	94.670±5.129		
18	108.107±8.484	108.107- 849.295	
19	298.949±5.516		
20	632.029±4.065		
21	849.295±5.609		

n=3

Most standards do not permit to adding benzoic acid to cakes. Such as, Yemeni Standard No. 586 issued in 2003[19], and No. 104 issued in 2006 [31], which specializes in all types of cakes not include mixtures of different kinds of cakes or cakes prepared for special

purposes, stated the use of sorbic acid up to 0.4% of the weight of flour and did not mention the use of sodium benzoate or benzoic acid.

Food Additives of the European Union No. 95/2/EC [24], did not allow adding benzoic acid in the cake, and it permits adding sorbic acid, and the maximum allowable is 1000 mg/L. As well as the specification for the Codex Alimentarius Commission specification. No.192 issued in 1995 on food additives [23], has stated that the maximum permitted benzoic acid is 1000 mg/L for products filling fruit pastry and bakery s goods products and did not mention a cake within the food in this specification. Therefore, according to these mentioned specifications, all cake samples studied are a violation of the specification. The presence of unusual amounts of benzoic acid in the studied samples of cake can be attributed to the quality of materials used in its manufacture.

Category (4) consists of eight samples of juices, the mean concentrations of benzoic acid in these sample's ranges from 22.942 mg/L to 175.940 mg/L, Table 5.

Table 5. Concentrations of benzoic acid (mg/L) in category (4), Juices samples

Samples No.	Mean concentration (mg/L) ± SD	Mean concentration range of benzoic acid in the category
22	22.942±3.889	22.942 - 175.940
23	46.053±5.974	
24	66.621±0.818	
25	71.001±6.036	
26	77.508±5.230	
27	115.060±2.218	
28	125.003±2.057	
29	175.940±0.617	

n=3

The concentrations of benzoic acid in samples of juices and drinks were within the permitted national and international limits. Such as Yemeni standard specializes in drinks and juices. No.1859 issued in 2007 [32]. The Codex Alimentarius Commission specification on food additives No.192, issued in 1995 [23]. All these standards reported that the maximum permitted benzoic acid in juices, and beverage is 1000 mg/L. Concern has been expressed that in soft drinks and beverage, benzoic acid and its salts may react with ascorbic acid (vitamin C) in presence of transition elements forming small quantities of poisonous benzene [33].

When comparing the content of benzoic in studied juices and beverages with previous studies, we found that, Pylypiw and Grether in 2000 in the United States [34], found that the concentration of sodium benzoate in kinds of juices' ranges between 12-38 mg/L. Mota and others in 2003 [35], found that benzoic acid in samples of juices in Portuguese ranges between 179-198 mg/L. Visti and others in 2003 in Finland [36] found that the concentration of benzoic acid in kinds of juices' ranges between 52-602 mg/L. Oladipo and others in 2009 in Nigeria [37] found that the concentration of benzoic acid in kinds of canned fruit juices ranges between 250 -1000 mg/L. Cornelia and Elena in 2009 [38] found that the concentration of benzoic acid in samples of the juice in the city of Bucharest, Romania ranges between 30.29 - 107.75 mg/L. Khosrokhavar and others in 2010 in Iran [39], found that the concentration of sodium benzoate in types of drinks' ranges between 3.9-164.0 mg/L, Fig. 4.

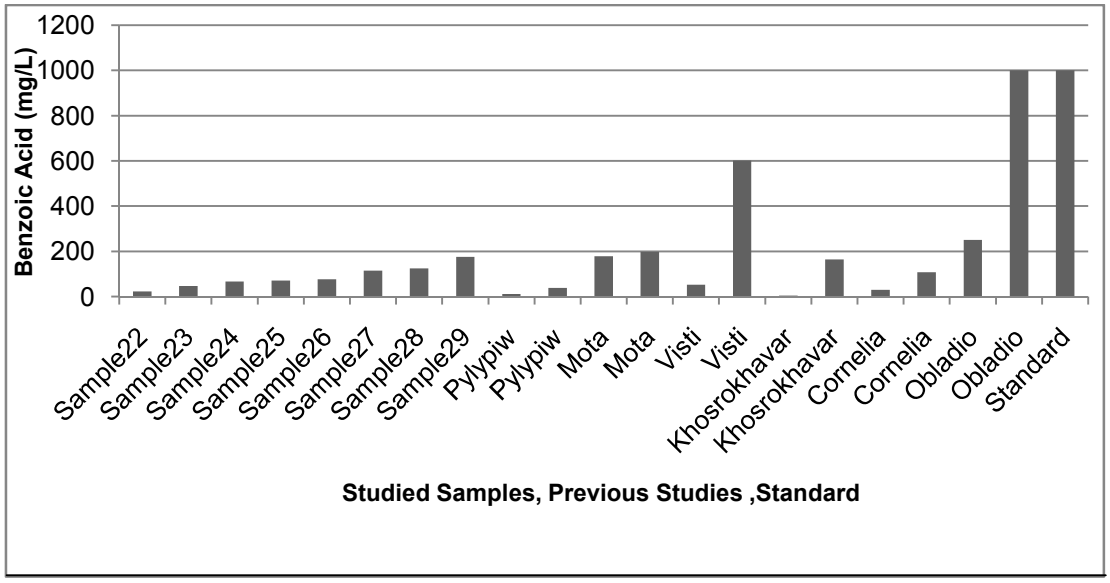

Fig. 4. Category (4) Juices Samples

Category (5) consists of three samples of jam, the mean concentrations of benzoic acid in these sample's ranges from74.016 mg/kg to 519.688 mg/kg, Table 6.

Table 6. Concentrations of benzoic acid (mg/kg) in category (5), Jam samples

Samples No.	Mean concentration (mg/kg) ± SD	Mean concentration range of benzoic acid in the category
30	74.016±0.252	74.016 - 519.688
31	357.019±6.092	
32	519.688±29.368	

n=3

The contents of benzoic acid in studied samples of jam are within the ranges of some previous studies. Ferreira and others in 2000 [40] found the concentration of benzoic acid in samples of Portuguese jam ranges between 413.9 - 1501.0 mg/kg. Mota and others in 2003 [35] found the concentration of benzoic acid in samples of Portuguese jam ranges between 646-798 mg/kg. Wibbertmann and others in 2005 [10] found the concentration of benzoic acid in samples of British jam lies in the range 20-333 mg/kg. Saad and others in 2005 [41] found the concentration of benzoic acid in samples of Malaysian jam lies in the range 1756.3-1872.1 mg/kg.

The concentrations of benzoic acid in studied samples of jam were within the amount of benzoic acid allowed in jam according to the local, regional and international standard, which permits 1000 mg/L of benzoic acid. Such as Yemeni standard No. 586 issued 2003 [19], Codex Alimentarius Commission specification No.192 issued in1995 [23] and Codex Alimentarius Commission specification No.296 issued in 2009 [42] as well as Scientific Committee on Consumer Products (SCCP/0891/05) [43], Fig. 5.

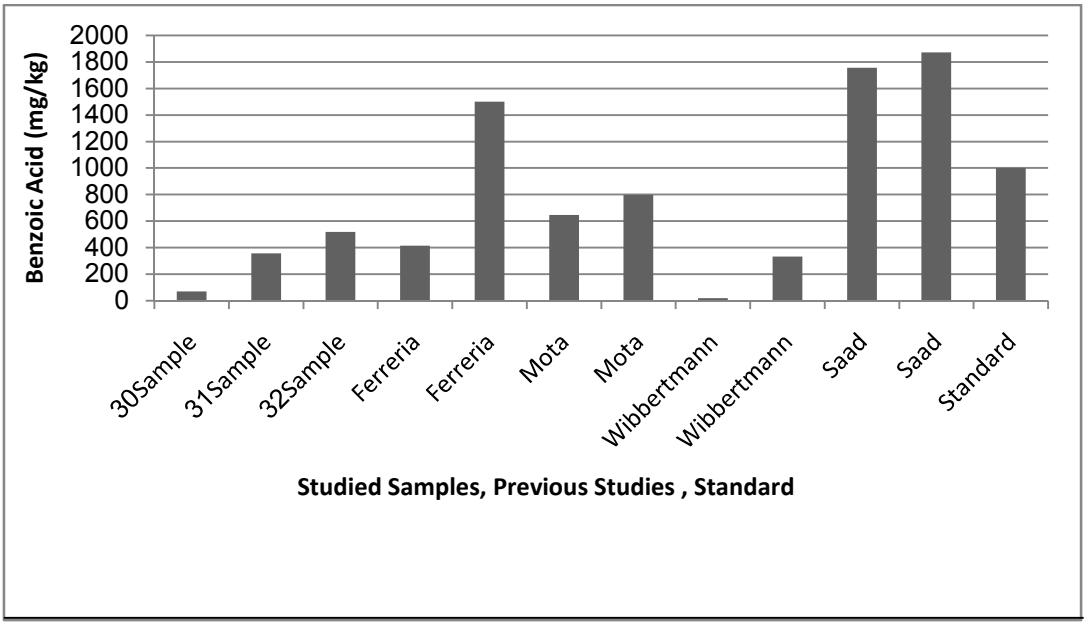

Fig. 5. Category (5) Jam Samples

7. CONCLUSION

The method was found to be suitable for the routine determination of benzoic acid in food samples with acceptable parameters such as the limit of detection, limit of quantitation. The levels of benzoic acid in dry milk and cheese samples were of natural occurrences, small amount, and to some extent, similar to those reported in the previous studies. Yemeni specifications No. 860 issued 2004 only allow addition of benzoic acid as preservatives for this food category. The levels of benzoic acid in samples for infants and young children manufacture from grains, and samples of cake were of natural occurrences, and are a violation of all mentioned specifications. The level of benzoic acid in samples of juices and drinks as well as jams were within the permitted national and international limits, and to some extent, similar to those reported in the previous studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tfouni SAV, Toledo MCF. Determination of benzoic and sorbic acids in Brazilian food. Food Control. 2002;13(2):117-123.
2. European Parliament and council Directive No. 98/72/CE of 20 February 1995 on food additive other than colours and sweeteners; 1995.
3. World Health Organization. Benzoic acid and sodium benzoate. Concise International Chemical Assessment Document No. 26. Geneva; 2000.

4. Fatibello-Filho Orlando, Luiz Humberto, Marcolino-Junior, Airton Vicente Pereira. Solid-phase reactor with copper (II) phosphate for flow-injection spectrophotometric determination of aspartame in tabletop sweeteners. Anal Chim Acta. 1999;384:167-174.
5. Kompany-Zareh Mohsen, Siavash Mirzaei. Spectrophotometric resolution of ternary mixtures of pseudoephedrine hydrochloride, dextromethorphan hydrobromide and sodium benzoate in syrups using wavelength selection by net analyte signals calculated with hybrid linear analysis. Anal Chim Acta. 2004;526(1):83-94.
6. Zen JM, Ting YS. Simultaneous determination of caffeine and acetaminophen in drug formulations by square-wave voltammetry using a chemically modified electrode. Anal Chim Acta. 1997;342:175-180.
7. Nobrega JA, Filho FO, Vieria LC. Flow Injection Determination of Aspartame in Dietary Products. Analyst. 1994;119(9):2101-2104.
8. Frazier Richard A, Elizabeth L Inns, Nicolo Dossi, Jennifer M Ames, Harr E Nursten. Development of a capillary electrophoresis method for the simultaneous analysis of artificial sweeteners, preservatives and colours in soft drinks. Journal of Chromatography A. 2000;876(1-2):213-220.
9. Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives (1). Official Journal of the European Union L. 2011;295:1.
10. Wibbertmann A, Kielhorn J, Mangelsdorf I, Melber C, Koennecker G. Benzoic Acid and Sodium Benzoate. Fraunhofer Institute for Toxicology and Aerosol Research, Hanover, Germany. World Health Organization, Concise International Chemical Assessment Document. 2005;26.
11. Campell RE, Miracle RE, Drake MA. The effect of starter culture and annatto on the flavor and functionality of whey protein concentrate. Journal of Dairy Science. 2011;94(3):1185–1193.
12. Sieber R, Bütikofer U, Bosset JO. Benzoic Acid as a Natural Compound in Cultured Dairy Products and Cheese. Int Dairy Journal. 1995;5:227-246.
13. Urbienė S, Leskauskaitė D. Formation of Some Organic Acids during Fermentation of Milk. Polish J Food Nutrition Sci. 2006;15-56(3):277–281.
14. Ahmet K, Sik B, Muammer D. Determination of Sodium Benzoate, Potassium Sorbate, Nitrate and Nitrite in Some Commercial Dairy Products. Journal GIDA. 2008;33(4):159-164.
15. Smith K. Wisconsin Center for Dairy Research. Bleaching WI-CDR. 2008;1-14.
16. Ishiwata H, SugitaT, Kawasaki Y, Takeda Y, Yamada T, Nishijima M, Fukasawa Y. Estimation of Preservative Concentrations in Food and Their Daily Intake based on Official inspection results in Japan in fiscal year 1996. J Food Hygiene Soc Japan. 1999;40(3):246-258.
17. Ping Qi, Hong Hong, Xiaoyan Liang, Donghao Liu. Assessment of benzoic acid levels in milk in China. Food Control. 2009;20:414–418.
18. Yemen Standardization, Metrology and Quality Control. Milk food for babies. The Republic of Yemen. 2003;11:442.
19. Yemen Standardization, Metrology and Quality Control. Benzoic acid and sodium benzoate and potassium benzoate used in food preservation. The Republic of Yemen. 2003;586:8.

20. Yemen Standardization, Metrology and Quality Control. Chemical methods to test the cheese. The Republic of Yemen. 2004; 10:740.
21. Yemen Standardization, Metrology and Quality Control. Methods of test for milk powder. The Republic of Yemen. 2005;15:10-91.
22. Yemen Standardization, Metrology and Quality Control. Processed cheese and paste cheese. The Republic of Yemen. 2008;373:9.
23. Codex Standard for food Additives. (Codex Stan192. 1995;80-82:23.
24. European Parliament and Council Directive No 95/2/EC on food additives other than colors and sweeteners. Official Journal. 1995;61-1:61-40.
25. Yemen Standardization. Metrology and Quality Control, flavored milk. The Republic of Yemen. 2004;9:860.
26. Yemen Standardization. Metrology and Quality Control. Methods of test for biscuits. The Republic of Yemen. 2004;10-28:11.
27. Yemen Standardization, Metrology and Quality Control, food for infants and young children mainly manufactures grain. The Republic of Yemen. 2009;22:14.
28. Standards and Metrology Organization for the Cooperation Council for the Arab Gulf States Food infants and children. Foods for infants and young children mainly manufactures grain. 2007;677:9.
29. Codex Standard Specification for Canned Baby Foods. Codex Stan. 1981;73.
30. Codex Standard Specification for Processed Cereal-Based Foods for Infants and Children. Codex Stan; 1981;74.
31. Yemen Standardization, Metrology and Quality Control. Cake. The Republic of Yemen. 2006;104:8.
32. Yemen Standardization. Metrology and Quality Control. Drinks and juices. The Republic of Yemen. 2007;1859:11.
33. Gardner Lalita K, Lawrence Glen D. Benzene production from decarboxylation of benzoic acid in the presence of ascorbic acid and a transition-metal catalyst. J Agric Food Chem. 1993;41(5):693–695.
34. Pylypiw HM, Grether MT. Rapid High-Performance Liquid Chromatography Method for the Analysis of Sodium Benzoate and Potassium Sorbate in Foods. Journal of Chromatography A. 2000;883:299–304.
35. Mota Fernando JM, Ferreira IMP, Sara LVO, Cunha C, Beatriz M, Olivera PP. Optimisation of extraction procedures for analysis of benzoic and sorbic acids in foodstuffs. Food Chemistry. 2003;82(3):469–473.
36. Visti A, Viljakainen S, Laakso S. Preparation of fermentable lingonberry juice through removal of benzoic acid by Saccharomyces cerevisiae yeast. Food Research International. 2003;36(6):597–602.
37. Oladipo IC, Adeleke DT. Adebiyi AO. The Effect of pH and Chemical Preservatives on the Growth of Bacterial Isolates from Some Nigerian Packaged Fruit Juices. Pakistan Journal of Nutrition. 2009;8(11):1796-1801.
38. Cornelia Petronela ENE, Elena DIACU, High-Performance Liquid Chromatography Method For The Determination of benzoic acid In Beverages. U.P.B Sci. Bull Series B. 2009;71(4):81-88.
39. Khosrokhavar R, Sadeghzadeh N, Amini M, Ghazi-Khansari M, Hajiaghaee R, Ejtemaei Mehr Sh. Simultaneous Determination of Preservatives (Sodium Benzoate and Potassium Sorbate) in Soft Drinks and Herbal Extracts Using High- Performance Liquid Chromatography (HPLC). Journal of Medicinal Plants. 2010;9(35):81-87.

40. Ferreira MPLVO Isabel, Mendes Eulalia, Brito Paula, Ferreira Margarida A. Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC. Food Research International. 2000;33:113-117.
41. Saad Bahr, Md. Fazlul Bari, Muhammad Idiris Saleh, Kamarudzaman Ahmad, Mohd. Khairuddin Mohd. Talib Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using High-Performance Liquid Chromatography. Journal of Chromatography A. 2005;1073:393–397.
42. Codex Standard for Jams. Jellies and Marmalades. CODEX STAN. 2009;296.
43. Scientific Committee on Consumer Products (SCCP). Opinion on Benzoic Acid and Sodium Benzoate; 2005.

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