SHORT COMMUNICATION

STATUS OF BENZOIC ACID AMOUNT DURING PROCESSING FROM YOGHURT TO ITS BY-PRODUCT DRINK (DOOGH)

ZAHRA ESFANDIARI[1]*, MOHAMMAD SARAJI[2], ROYA ALSADAT MADANI[1] and ELHAM JAHANMARD[1]

[1] Department of Research and Development, Department of Food and Drug, Isfahan University of Medical Sciences, Isfahan, Iran

[2] Department of Chemistry, Faculty of Chemistry, Isfahan University of Technology, Isfahan, Iran

*Corresponding author: research_esfandiary@mui.ac.ir

ABSTRACT

The addition of benzoic acid is forbidden in by-product drink of yoghurt "Doogh" in Iran. However, this preservative can be naturally found in milk and its products. A total of 24 and 48 samples of yoghurt and Doogh were analyzed by HPLC method to assess the natural and permitted amount of benzoic acid. All samples of yoghurt and Doogh contained benzoic acid in mean amount of 1.5-5.0 mg/kg and 0.8-4.7 mg/ L, respectively. These findings showed that the amount of 6 mg/L can be defined for benzoic acid as admissible limit for Doogh in Iran.

Keywords: benzoic acid, yoghurt, Doogh, admissible limit, HPLC, Iran

1. INTRODUCTION

Doogh is a traditional fermented drink which is widely used in Asia and produced from salt, set yoghurt and water (ZAMANI MAZDEH *et al*., 2014). This product is known with different names like "Ayran" in Turkey and "Lassi" in India (YILDIZ *et al*., 2012; HINGMIRE *et al*., 2009). Similar to other acidified drinks, one of the main problems with Doogh is microbial contamination leading to the spoilage and safety reduction of this product (ELVIRA *et al*., 2014; SOSPEDRA *et al*., 2012). For this purpose, the antimicrobial preservatives such as benzoic acid and/or its salts are usually added to Doogh to impart many advantages including increasing their shelf life, but the unpleasant effects of these additives have been reported in some surveys (ZENGIN *et al*., 2011; KAMANKESH *et al*., 2013; ZAMANI MAZDEH *et al*., 2014). The use of chemical additives in different countries is restricted by the specific policies adopted. In Iran, the scientific panel on the food additives in " Food and Drug Administration: FDA" and "Institute of Standards and Industrial Research of Iran: ISIRI" has prohibited the use of benzoic acid in the dairy products. The existence of benzoic acid in those products leads to fine payment by the producers and suspension of the production licenses (ISIRI, 2008). Recent evidences suggest the natural occurring of benzoic acid in yoghurt and its transfer to diluted and salted formulation prepared thereof (Doogh) (ESFANDIARI *et al*., 2013; ZAMANI MAZDEH *et al*., 2014; AMIRPOUR *et al*., 2015). This can cause the misinterpretation of the inspected results regarding the existence of the natural or added benzoic acid to Doogh. In fact, the use of benzoic acid is not allowed in the dairy products in Iran even though the presence of the naturally-occurring benzoic acid in the fermented dairy products has been reported by many workers (SIEBER *et al*., 1995; IAMMARINO *et al*., 2011; HORNICKOVA *et al*., 2014). The source of benzoic acid can be hippuric acid as the natural compound in milk that changes to benzoic acid through the fermentation of the lactic acid bacteria in yoghurt (SIEBER *et al*., 1999). To author's knowledge, there have been no controlled study which determines the natural amount of benzoic acid in Doogh. Therefore, this project was undertaken to assess the level of benzoic acid as the natural and permitted value in Doogh. These findings could help FDA and ISIRI to reassess the status of the enacted rules for benzoic acid in Doogh and resolve the legislative issue in Iran.

2. MATERIALS AND METHODS

2.1. Samples

Set yoghurt samples (n=24) were taken from three commercial brands of the dairy processing plants (A, B and C; for each 4 samples) and small scale brands (D, E and F; for each 4 samples) with high sale in Isfahan, Iran. A total of 12 Doogh samples were manually prepared by mixing 0.7, 40 and 59.3 g of salt (NaCl), yoghurt sampled in the previous stage (for each brand 2 samples) and water, respectively. A sum of 36 Doogh samples from three aforementioned brands (A, B and C; for each 12 samples) were purchased from the local food stores located in Isfahan, Iran. All samples were kept at 4° C before the examination would be begun. The analysis was performed in two replicates to determine the mean of the measurements for benzoic acid.

2.2. Instrumentation and quantification

A Hewlett–Packard 1090-II liquid chromatograph (now Agilent, Waldbronn, Germany) equipped with a diode array detector was used. The system was equipped with a

Rheodyne 7725i injector with a 20-μL loop. The separations were carried out at the room temperature on a 3-μm, 150 × 3 mm I.D. Hector-A C18 reversed-phase column (RStech Co., South Korea) was preceded by a guard column (4 ×4 mm, 5 μm) of the same packing material from Merck (Darmstadt, Germany). The separation was performed isocratically using a mobile phase consisting of HPLC grade methanol (40%, v/v) and 0.25 mM ammonium acetate aqueous solution (60%, v/v at pH = 4.5). The mobile phase flow rate was 0.4 mL/min and the detection was performed at 230 nm.
The stock standard solution of benzoic acid (99%, Merck) was prepared in methanol at 1000 mg/L concentration level. More diluted standard solutions were prepared in the pure water at the concentrations ranging from 1 to 50 mg/L. Pure water was prepared by Overseas Equipment & Services water purification system (OK, USA).
Estimation of benzoic acid amount was performed according to a previous reported method (GUARINO *et al*., 2011). A 2-g yoghurt sample was mixed with 5 mL methanol/water (35/65, v/v). For Doogh analysis, 3 mL of the sample was mixed with 2 mL methanol. The mixture was ultrasonicated for 5 min. It was then heated at 50 ºC for 5 min and centrifuged in 2000 rpm (5 min). The clear supernatant solution was injected into HPLC.

3. RESULTS AND DISCUSSIONS

The results corresponding to benzoic acid level in the industrial (A, B and C) and small scale brands (D, E and F) yoghurt samples are listed in Table 1. The mean values of benzoic acid were in the range of 1.5-2.9 and 3.6-5.0 mg/kg in the samples collected from three yoghurt processing plants and small scale brands, respectively. Our findings imply to the presence of benzoic acid in all samples of yoghurt. The surveys on the presence of benzoic acid in all yoghurt samples in various studies, performed by MIHYAR *et al*. (1999), EL ZEINY (2009), YILDIZ *et al.* (2012), CAKIR and CAGRI-MEHMETOGLU (2013) and AMIRPOUR *et al*. (2015), revealed that the ranges found were higher than those described in the current study.
MIHYAR *et al*. (1999) showed that the content of benzoic acid was in the range of 10.6 to 1998.8 mg/Kg of Labaneh yoghurt in Amman. EL-ZEINY (2009) also pointed out high concentration level of benzoic acid in the amount of 921 mg/Kg in yoghurt ice dressing in Saudi Arabia. Furthermore, the amount of benzoic acid is stated from 9.36 to 26.21 in all 25 yoghurt samples gathered from five cities in Turkey (YILDIZ *et al*., 2012). In another study in Turkey, a mean of 35.2 mg/kg of benzoic acid found in all 21 yoghurt (CAKIR and CAGRI-MEHMETOGLU, 2013). In a recent experiment by AMIRPOUR *et al*., (2015) in Iran, the mean amount of 29.3±8.6 mg/kg of benzoic acid detected in all yoghurt samples collected from four different brands of the dairy processing plants.
In contrast, the detection of benzoic acid was reported in 80% of yoghurt samples in Turkey (KOYUNCU and UYLASER, 2009). Additionally, Benzoic acid was not observed in yoghurt samples examined in Spain, Brazil and China (GONZALEZ *et al*., 1998; TFOUNI and TOLEDO, 2002; WANG *et al*., 2006).
Table 1 illustrates the benzoic acid value in the manually manufactured Doogh. Benzoic acid was found in the range of 0.8-2.2 and 2.6-4.0 mg/kg in Doogh made from the yoghurt collected from the dairy processing plants and small scale brands, respectively. Since Doogh is a diluted and salted formulation prepared from yoghurt, processing yoghurt to Doogh leads to a decrease in the benzoic acid content with amount of around 60-75%
As table 1 shows the amount of benzoic acid was higher in yoghurt and Doogh samples collected from the small scale brands. A probable explanation could be related to the lack

of controlling the quality criteria in the laboratory and proper hygiene practices as observed by the researchers.

Table 1: Mean of benzoic acid concentration (mg/kg) in yoghurt and manually manufactured Doogh (mg/L).

Sample Brand	Yoghurt [a] (Mean±SD)	Manually manufactured Doogh[b] (Mean±SD)
A	2.9±0.13	2.2±0.34
B	1.7±0.14	1.2±0.02
C	1.5±0.10	0.80±0.10
D	4.0±0.39	2.6±0.34
E	3.6±0.12	2.6±0.21
F	5.0±0.21	4.0±0.25

[a]Standard deviation calculated for 4 samples of yoghurt.
[b]Standard deviation calculated for 2 samples of manually manufactured Doogh.

Benzoic acid concentrations quantified in the industrially made Doogh are revealed in Table 2. The results demonstrated all samples presented benzoic acid with the levels ranging between 3 and 5.6 mg/L. The presence of benzoic acid in our study was near agreement with (YILDIZ *et al*., 2012 and ESFANDIARI *et al*., 2013) that reported the content of benzoic acid in Ayran and Doogh samples in Turkey and Iran ranged from 1.54 to 16.57 and 0.94 to 9.77 mg/ L, respectively. AKBARI-ADERGANI *et al*. (2013) and ZAMANI MAZDEH *et al*., (2014) pointed out the high concentration of benzoic acid with the mean of 195.9 and 21.3 in 27 and 130 Doogh samples in Iran. In similar study in Iran, benzoic acid content in Doogh samples purchased from four dairy brands including A, B, C and D were 22.2, 20.0, 19.4 and 21.5 mg/kg, respectively (AMIRPOUR *et al*., 2015).

Table 2: Benzoic acid (mg/ L) level in the investigated Doogh.

Sample brand	Concentration range	Mean±SD (n=12)
A	3.9-4.2	4.0±0.1
B	3.0-5.2	4.3±0.7
C	4.0-5.6	4.7±0.5

The amount of benzoic acid in the industrially manufactured Doogh was higher than manually ones. Whereas manually manufactured Doogh was prepared under the laboratory conditions and the industrially manufactured Doogh was purchased from the local stores, it seems that the storage, handling and hygiene conditions in the dairy processing plants is plausible justification for this status. Therefore, this part of the food chain requires continuous monitoring for good hygienic practice in the milk processing (Smith, 2003).
Totally, the reason for the difference in the amount of benzoic acid in yoghurt and Doogh of the present study and others is not clear but it may be attributed to the several factors including the feed of the milk- producing animal, the season of milking, the breeding

conditions, the content of hippuric acid in the raw milk, samples size, type of commercial lactic acid bacteria starter, processing technique, storage condition and type of yoghurt analyzed as mentioned in other studies (SIEBER *et al*., 1999; GARMIENE *et al*., 2008; QI *et al*., 2009; HORNICKOVA *et al*., 2014; JAVANMARDI *et al*., 2015).
The low level of benzoic acid found in the manually and industrially manufactured Doogh indicates that this compound is as the indigenous constituent transferring from yoghurt to Doogh. Regarding with the codex standard act, the permitted amounts of benzoic acid as preservative used in the fermented milk drink is 300 mg/kg (Codex, 2003). Therefore the amount of benzoic acid detected in present study should not affect the public health. To sum up, the permissible amount of benzoic acid could define less than 6 mg/L in Doogh. Below this value, samples can be considered "acceptable" in Iran because such a low concentration could originate from the natural endogenous formation of benzoic acid and not from the fraudulent addition.

4. CONCLUSIONS

An investigation of 72 yoghurt and Doogh samples was carried out to define the natural occurring amount of benzoic acid. In the current study, it was found that all yoghurt and Doogh analyzed contained benzoic acid in low level at less than 6 mg/L. This amount can be a permitted amount without having any harmful effect on the human health and considered as the admissible level for benzoic acid in Doogh in the national act by Iranian supervision authorities. Moreover, it is suggested further studies on the continuous monitoring and the measuring of benzoic acid content in milk and its products to declare the amount of this compound on the labels of packaging. The inserted data on the labels of packaging can be useful for the exposure estimation of the consumers with this compound.

ACKNOWLEDGEMENTS

Sincere thanks are forward to Research Council of Isfahan University of Medical Sciences for supporting the research project number 292227.

REFERENCES

Akbari-adergani B., Eskandari S. and Bahremand N. 2013. Determination of sodium benzoate and potassium sorbate in "Doogh" samples in post market surveillance in Iran 2012. Journal of Chemical Health Risks 3:65-71.

Amirpour M., Arman A., Yolmeh A., Akbari Azam M. and Moradi-Khatoonabadi Z. 2015. Sodium benzoate and potassium sorbate preservatives in food stuffs in Iran. Food Additives and Contaminants: Part B 8:142-148.

Cakir R. and Cagri-Mehmetoglu A. 2013. Sorbic and benzoic acid in non-preservative-added food products in Turkey. Food Additives and Contaminants: Part B 6: 47-54.

Codex Standard. 2003. "Codex standard for fermented milks. http://www.codexalimentarius.org/input/download/standards/CXS_243e.pdf. Accessed 26 July 2015.

Elvira L., Duran C.M., Urrejola. J. and Espinosa F.R.M. 2014. Detection of microbial contamination in fruit juices using non-invasive ultrasound. Food Control 40:145-150.

El-Zieny M. 2009. GC-MS analysis of benzoate and sorbate in Saudi dairy and food products with estimation of daily exposure. Journal of Food Technology 7:127-134.

Esfandiari Z., Badiey M., Mahmoodian P., Sarhangpour R., Yazdani E. and Mirlohi M. 2013. Simultaneous determination of sodium benzoate, potassium sorbate and natamycin content in Iranian yoghurt drink (Doogh) and the associated risk of their intake through Doogh consumption. Iranian Journal of Public Health 42:915-920.

Garmiene G., Salomskiene J., Jasutiene I., Macioniene I. and Miliauskiene I. 2010. Production of benzoic acid by lactic acid bacteria from Lactobacillus, Lactococcus and Streptococcus genera in milk. Milchwissenschaft 65:295-298.

Gonzalez M., Gallego M. and Valcarcel M. 1998. Simultaneous gas chromatographic determination of food preservatives following solid-phase extraction. Journal of Chromatography A 823:321-329.

Guarino C., Fuselli F., La Mantia A. and Longo L. 2011. Development of an RP-HPLC method for the simultaneous determination of benzoic acid, sorbic acid, natamycin and lysozyme in hard and pasta filata cheeses. Food Chemistry 127:1294-1299.

Hingmire S.R., Lembhe A.F., Zanjad P.N., Pawar V.D. and Machewad G.M. 2009. Production and quality evaluation of instant lassi. International Journal of Dairy Technology 62:80-84.

Hornickova S., Dragounova H., Hejtmankova K., Michlova T. and Hejtmankova K. 2014. Production of benzoic acid in feremented goat's and sheep's milk. Scientia Agriculturae Bohemica 4: 247-253.

Iammarino M., Taranto A.D., Palermo C. and Muscarella M. 2011. Survey of benzoic acid in cheeses: contribution to estimation of an admissible maximum limit. Food Additives and Contaminants: Part B 4:231-237.

ISIRI. 2008. "Institute of Standards and Industrial Research of Iran" 2th revision. no. 2453. Doogh-Specification and test method.

Javanmardi F., Nemati M., Ansarin M. and Rafie S.A. 2015. Benzoic and sorbic acid in soft drink, milk, ketchup sauce and bread by dispersive liquid-liquid microextraction coupled with HPLC. Food Additives and Contaminants: Part B 8:32-39.

Kamankesh M., Mohammadi A., Modarres Tehrani Z., Ferdowsi R. and Hosseini H. 2013. Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography for determination of benzoate and sorbate in yogurt drinks and method optimization by central composite design. Talanta 109: 46-51.

Koyuncu N. and Uylaser V. 2009. Benzoic acid and sorbic acid levels in some dairy products consumed in Turkey. Asian Journal of Chemistry 21: 4901-4908.

Mihyar G.F., Yousif A.K. and Yamani M.I. 1999. Determination of benzoic and sorbic acids in Labaneh by high-performance liquid chromatography. Journal of Food Composition and Analysis 12:53-61.

Qi P., Hong H., Liang X. and Liu D. 2009. Assessment of benzoic acid levels in milk in China. Food Control 20:414-418.
Sieber R., Bütikofer U. and Bosset J.O. 1995. Benzoic acid as a natural compound in cultured dairy products and cheese. International Dairy Journal 5:227-246.

Sieber R., Bütikofer U. and Bosset J.O. 1995. Benzoic acid as a natural compound in cultured dairy products and cheese. International Dairy Journal 5:227-246.

Smith G. 2003 “Dairy processing, Improving quality”. Woodhead Publishing Limited, CRC Press, New York Washington, DC.

Sospedra I., Rubert J., Soriano J.M. and Manes J. 2012. Incidence of microorganisms from fresh orange juice processed by squeezing machines. Food Control 23:282-285.

Tfouni S.A.V. and Toledo M.C.F. 2002. Determination of benzoic and sorbic acids in Brazilian food. Food Control 13:117-123.

Wang L., Zhang X., Wang Y. and Wang W. 2006. Simultaneous determination of preservatives in soft drinks, yogurts and sauces by a novel solid-phase extraction element and thermal desorption-gas chromatography. Analytica Chimica Acta 577:62-67.

Yildiz A., Erdogan S., Saydut A. and Hamamci C. 2012. High-performance liquid chromatography analysis and assessment of benzoic acid in yogurt, ayran, and cheese in Turkey. Food Analytical Methods 5:591-595.

Zamani Mazdeh F., Esmaeili Aftabdari F., Moradi-Khatoonabadi Z., Shaneshin M., Torabi P., Shams Ardekani M.R. and Hajimahmoodi M. 2014. Sodium benzoate and potassium sorbate preservatives in Iranian doogh. Food Additives and Contaminants: Part B 7:115-119.

Zengin N., Yuzbasioglu D., Unal F. and Aksoy H. 2011. The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food and Chemical Toxicology 49:763-769.

Paper Received November 5, 2016 Accepted April 11, 2016