SHORT COMMUNICATION

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

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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

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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 sample 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 4º 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 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).

<table>
<thead>
<tr>
<th>Sample Brand</th>
<th>Yoghurt (^a) (Mean±SD)</th>
<th>Manually manufactured Doogh (^b) (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.9±0.13</td>
<td>2.2±0.34</td>
</tr>
<tr>
<td>B</td>
<td>1.7±0.14</td>
<td>1.2±0.02</td>
</tr>
<tr>
<td>C</td>
<td>1.5±0.10</td>
<td>0.80±0.10</td>
</tr>
<tr>
<td>D</td>
<td>4.0±0.39</td>
<td>2.6±0.34</td>
</tr>
<tr>
<td>E</td>
<td>3.6±0.12</td>
<td>2.6±0.21</td>
</tr>
<tr>
<td>F</td>
<td>5.0±0.21</td>
<td>4.0±0.25</td>
</tr>
</tbody>
</table>

\(^a\) 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.

<table>
<thead>
<tr>
<th>Sample brand</th>
<th>Concentration range</th>
<th>Mean±SD (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.9-4.2</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>B</td>
<td>3.0-5.2</td>
<td>4.3±0.7</td>
</tr>
<tr>
<td>C</td>
<td>4.0-5.6</td>
<td>4.7±0.5</td>
</tr>
</tbody>
</table>

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.

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REFERENCES


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