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Determination of Sodium Benzoate and Potassium Sorbate in "Doogh" Samples in Post Market Surveillance in Iran 2012

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Abstract: Sodium benzoate and potassium sorbate are two major chemical preservatives which are used in Doogh (Iranian traditional dairy drink). In this study, a total of 27 commercial brands of highly consumed of Doogh samples were analyzed. The means and standard deviation for concentration of these preservatives based on HPLC results for analysis of benzoate and sorbate were 195·9 (SD 1·8) and 328·8 (SD 2·1) $mg.Kg^{-1}$ respectively. The minimum and maximum of benzoate content in various brands were 18.3 and 2345.1 $mg.Kg^{-1}$ and for sorbate were not detected and 4961.3 $mg.Kg^{-1}$ respectively. The study revealed that there was not significant difference in preservative concentration in the samples that belonged to various dates. However, a few samples had a high preservative concentration, which could be a risk factor for human health, especially when their intake was being occurred by various foodstuffs simultaneously.

Keywords: Preservative, Sodium benzoate, Potassium sorbate, Doogh, post-market

INTRODUCTION

Doogh as an old and traditional drink of Iran has high value of nutrients (same as fermented milk and Yoghurt) and remedial property and has plenty full of healthfulness effects include: Improving lactose digestion in individuals that have this difficulty; lowering serum cholesterol levels; stimulating the immune system [1]. Although using common salt is a major preservative for such a pleasure drink, but health legislations and restrictions caused the use of chemical preservatives will be developed. Chemical preservation have become an increasingly important practice in modern food technology with the increase in the production of processed and convenience foods. These preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods and to prolong shelf life and quality of foods; these also prevent consumer hazards due to the presence of microbial toxin or pathogenic microorganisms and economic losses due to spoilage. The most commonly used preservatives in many types of foods are benzoic and sorbic acids, nitrate and nitrite [2-3]. Benzoic and sorbic acids and their respective sodium, potassium and calcium salts are the most commonly used preservatives in food stuffs. These chemicals are generally used to inhibit yeast and mould growth, being also effective against a wide range of bacteria. These compounds are most active in foods of low pH value and essentially ineffective in foods at neutral pH value [3-4]. At acidic pH, where sorbic and benzoic acids and their salts

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effective, the lipophilic un-dissociated molecule is freely permeable across the cell membrane. Subsequently upon encountering the higher pH inside the cell, the molecule dissociates resulting in the release of changed anions and protons, which cannot cross the plasma membrane [5]. The importance of food preservatives to consumers has always been a health safety issue [2]. Although benzoic and sorbic acids and their salts are generally recognized as safe (GRAS) but the development of allergic reactions to benzoate in humans, such as uriticaria, non-immunological contact urticaria, metabolic acidosis, convulsions, hyperpnoea, weak clastogenic activity and asthma has been reported in some studies [3-4, 6-7]. Other studies showed that sorbic acid has low toxicity, explained by the fact that it is rapidly metabolized by path ways similar to those of other fatty acids. In humans a few cases of idiosyncratic intolerance to sorbic acid and sorbate salts have been reported (non-immunological contact urticaria and pseudo-allergy) [3-4]. For the above mentioned reasons, sorbic acid and sorbate salts (especially potassium sorbate) have become the leading preservatives for a wide variety of food products [3]. For these reasons, the use of food additives in different countries is limited by specific regulations. These preservatives are allowed by legislation but their use demands special care. Iran follows regulations of Institute of Standard and Industrial Research of Iran (ISIRI) on the safe use of food additives [2]. The acceptable daily intake (ADI) values, determined by the joint FAO/WHO expert committee on food additives (JECFA) is 25mg/Kg of body mass for sorbic acid and sorbates salts. According to ISIRI, usage of potassium sorbate and sodium benzoate in dairy products is prohibited. The analytical determination of these preservatives is not only important for quality assurance purposes but also for consumer interest and protection. The most common analytical method for the determination of benzoic acid (BA) and sorbic acid (SA) or sodium benzoate (E211) and potassium sorbate (E202) has been reversed-phase HPLC [8-9]. Some other analytical methods such as Capillary Electrophoresis [10], Spectrophotometry [11], Gas Chromatography-Mass Spectrometry [12], liquid chromatography [4-5, 12-15] and SPME-HPLC [6] have also been reported. As can be seen, there are various methods for the analysis of these preservatives in foodstuffs, but a rapid and reliable method for identification and quantization of these preservatives in Doogh is procedure that mainly utilizes high performance liquid chromatography (HPLC) followed by ultraviolet detection. High sensitive chromatographic method is important as there seem to be an increasing trend in using combination of preservatives and their control in food stuffs. The purpose of this study was to quantify sodium benzoate and potassium sorbate in Doogh samples commercially available on the local markets in Tehran, Iran in 2012.

EXPERIMENTAL SECTION

Samples

The highly consumed samples of Doogh with different brands produced from cow's milk were purchased from vendors in Tehran, Iran. A total of 27 samples were collected to be representative of what a consumer would find in market-basket. Sample size ranged from 500 mL to 1.0 Liter. Each sample was tested for the two preservative, sodium benzoate and potassium sorbate.

HPLC grade acetonitrile and other reagents such as ammonium acetate, glacial acetic acid, hydrochloric acid and petroleum benzene (analytical grade) were purchased from Merck (Darmstradt, Germany). Commercial standards of sodium benzoate and potassium sorbate were supplied by Sigma chemical Co. Deionised water used for chromatography processing was obtained from a Millipore Milli-Q water purification system (ELGA, UHQ-II-MK3, UK). For the filtration of

samples prior to injection into the HPLC system, a Millex HV 0.*45µm* filter (Millipore) was used. The mobile phase consisted of 90% ammonium acetate buffer with 10% HPLC-grade acetonitrile was prepared in two steps (Pylypiw and Grether, 2000):

Step1: *Acetate Buffer*: Exactly 0.30 gr of ammonium acetate were dissolved in approximately 900 mL of deionised water in a 1.0 L beaker. To this solution were added approximately 0.5mL of glacial acetic acid and the pH adjusted to 4.2. The buffer solution was then transferred to 1.0 L volumetric flask, brought to volume and filtered through a 47mm×0.45µm nylon filter.

Step2: *Completion*: Exactly 900 mL of the acetate buffer solution was mixed with 100mL of HPLC grade acetonitrile. This was mixed, degassed in degasser (ultra sonic clear sweep system) and used for sample dilution, standard dilution and as HPLC mobile phase.

Apparatus

The chromatographic analysis was carried out in a high-performance liquid chromatography from Dionex equipped as follows: ultimate 3000 pump, ASI-100 Automated sample injector, Dionex UVD 170U detector, thermostatted column compartment oven TCC-100. The HPLC operating mode was isocratic, the injection volume was 20µL and the column temperature was adjusted at 20^0C. The chromatography column was a Supelcosil LC-18: 25cm × 4.6mm, 5mm, Supelco, Bellefonte, PA, USA. Sample data collection was optimized to 30 min per sample with UV detection at wavelength of maximum absorption of the compounds, 225 nm for sodium benzoate and potassium sorbate. The optimal mobile phase flow rate was determined to be 0.8mL.min^{-1}.

INSTRUMENTATION

Analysis of Sodium Benzoate and Potassium Sorbate

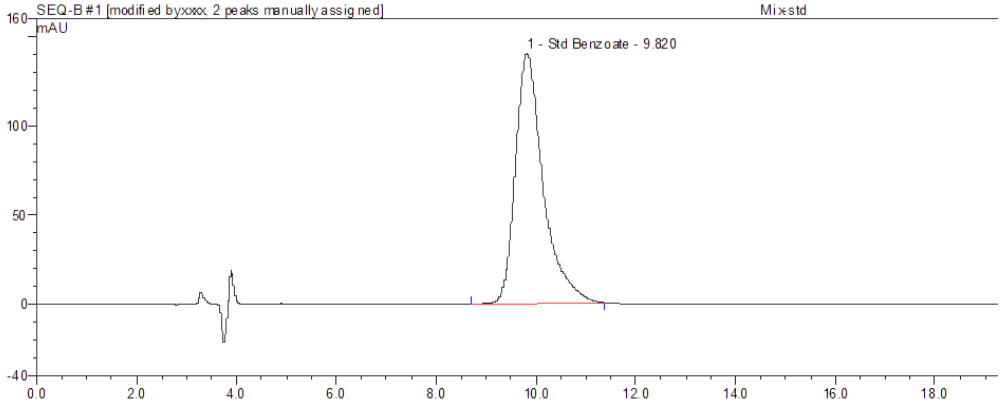
A high performance liquid chromatography technique was used to determine the concentrations of sodium benzoate and potassium sorbate in the samples following the procedures described by Pylypiw and Grether, 2000. Each of Doogh samples were degassed in an ultra sonic bath and 1.0 mL of sample was diluted 1:10 with mobile phase. After that, obtained aqueous phase solution was transferred into dry falcon and put it in centrifuge (biofuge primco 6000 Heraeus) for 6000 rpm for 15 min. The clear aqueous solution on top of samples in falcons were caught with pipettes and filtered through a 25mm×0.45µm nylon Acrodisk filter to remove particulate matter from the samples and to prevent these particles from damaging the pumping or injection system or clogging the column. After that, aqueous phase solution was transferred to the dry HPLC vials and put on auto sampler of HPLC for detection and quantification.

RESULTS AND DISCUSSION

Method Validation

The developed HPLC method was validated with respect to linearity and range, precision, accuracy, limit of detection and limit of quantitation following the International Conference on Harmonization (ICH) [16]. The external standard plot method was used for evaluation of linearity and range. Duplicate injections of 20 µL sodium benzoate and potassium sorbate standard solutions were used to construct linear regression lines (peak area versus concentration). The peaks were identified based on the retention time. The standard curves were obtained at five concentration levels for both of sodium benzoate and potassium sorbate. The concentration of these

solutions was 5, 10, 20, 40 mg.L^{-1} and a zero as blank sample. The determination coefficient (r^2) obtained for the regression line (Table 1) demonstrates the excellent relationship between peak area and the concentration of benzoate and sorbate. The mean regression equations for concentrations of sodium benzoate and potassium sorbate versus arbitrary units of peak area were Y= 48.92 X + 34.51 and Y=134.04 X + 11.30, respectively (Y represents peak area, X represents concentration in mg.L^{-1}). The correlation coefficients for standard curves of sodium benzoate and potassium sorbate were more than 0.999 and 0.998, respectively. In order to verify the accuracy and precision of this analytical procedure, the recovery studies and relative standard deviations were carried out. The recovery of sodium benzoate and potassium sorbate added to the samples free of the two preservatives was calculated (Table 2). Samples of Doogh were analyzed before and after addition of 10 and 20 mg of sodium benzoate and potassium sorbate to 500 ml of the samples. The calculation of accuracy was carried out as the percentage of preservatives recovered from the mixture. Mean recovery for benzoate and sorbate was between 93.1-96.3 and 92.9-99.7 respectively (n=5) indicating that the developed method was accurate for the determination of the preservatives in Doogh samples. The overlaid UV spectrum showed good response at 225 nm for these preservatives. Therefore, this wavelength was used for simultaneous determination of both compounds. In optimized conditions, benzoate and sorbate were separated with a resolution of more then 7 and the mean retention times were found to be 9.80 and 26.50 min respectively (figure 1).



No.	Ret. Time min	Peak Name	Height mAU	Area mAU*min	Rel. Area %	Amount	Type
1	9.82	Std-Benzoate	138.565	86.854	83.23	n. a.	BMB
2	26.48	Std-Sorbate	29.601	17.501	16.77	n. a.	BMB
Total			168.166	104.355	100.00	0.000	

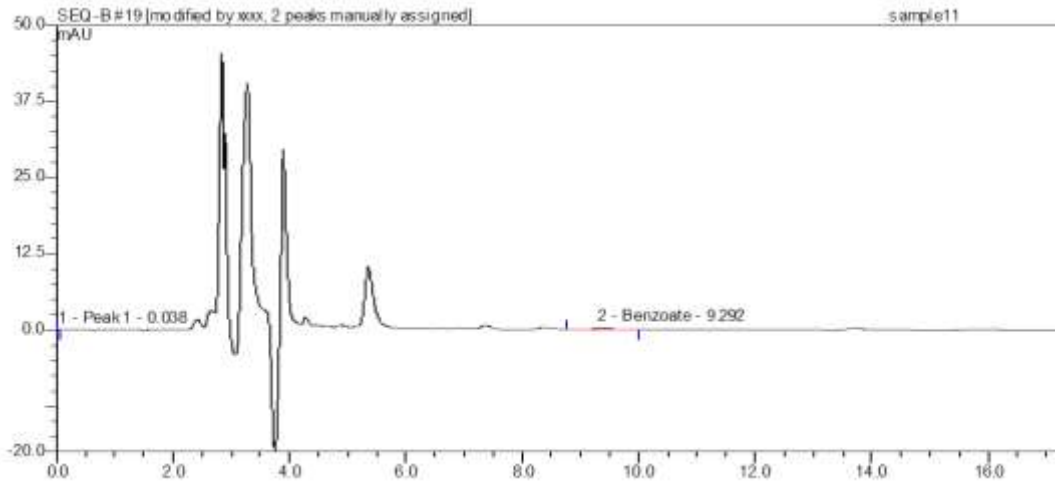
Figure 1. Typical HPLC chromatogram of standard solutions of preservatives containing 40 mg.$L^{-1}$of sodium benzoate and potassium sorbate, Conditions: column Supelcosil LC-18: 25cm × 4.6mm, 5mm, eluted with a mixture of ammonium acetate buffer and acetonitrile as described in experimental section with a flow rate of 0.8 mL.min^{-1} and detected at 225 nm.

The limit of detection (LOD) for benzoate and sorbate were 0.15 and 0.24 mg.kg^{-1} in the samples, respectively. The limit of quantitation (LOQ) for sodium benzoate and potassium sorbate were 0.5 and 0.8 mg.kg^{-1} in the samples, respectively.

ASSAY RESULTS

The results of the preservative analysis in the highly consumed Doogh samples are demonstrated in Table 2. The mean of sodium benzoate in all of the samples was 195.9 mg.kg^{-1} with standard deviation of 1.8 mg.kg^{-1}. The mean of potassium sorbate in all the samples was 328.8 with standard deviation of 2.1 mg.kg^{-1}. The highest concentration

of benzoate was found in a flavored Doogh sample and the lowest concentration was found in a Doogh sample which was containing any flavor. There was no significant difference in preservative concentration in the samples that belonged to various production date. All of the Doogh samples contained sodium benzoate in the range of 18.3 to 2345.1 $mg.kg^{-1}$, which are not acceptable according to Institute of Standard and Industrial Research of Iran (ISIRI). About 25.9 percents of samples contained concentrations of potassium sorbate between 0.6 to 4961.3 $mg.kg^{-1}$, which was not in compliance with the ISIRI legislations. Exactly 25.9 percents of Doogh samples contained both of sodium benzoate and potassium sorbate and there was not any potassium sorbate in 74.9 percent of samples. A typical HPLC chromatogram for a flavored Doogh sample was illustrated in figure 2. As can be seen in this figure, this sample containing sodium benzoate and potassium sorbate and two well separated peaks could be seen in 9.29 and 26.78 min for these preservatives.



No.	Ret. Time min	Peak Name	Height mAU	Area mAU*min	Rel. Area %	Amount	Type
1	9.29	Benzoate	0.159	0.081	9.37	n. a.	BMB
2	26.78	Sorbate	1.293	0.787	90.63	n. a.	BMB
Total			1.452	0.869	100.00	0.000	

Figure 2. A typical HPLC chromatogram of Doogh sample containing sodium benzoate and potassium sorbate. These preservatives were detected at 9.29 and 26.78 min respectively. All other conditions is the same as in figure 1.

Table 1. The linearity results of the HPLC method

Preservative	Concentration (µg/mL)	Equation for regression line	r^2
Sodium benzoate	5.0-40.0	Y= 48.92 X + 34.51	0.999
Potassium sorbate	5.0-40.0	Y=134.04 X + 11.30	0.998

Table 2. Mean and concentration range of sodium benzoate and potassium sorbate in Doogh samples by HPLC method

Preservative	Mean (mg/Kg)	Range (mg/Kg)	S.D. (mg/Kg)	Mean Recovery (%)
Sodium benzoate	195.9	18.3 - 2345.1	1.8	95.7
Potassium sorbate	328.8	0.0 - 4961.3	2.1	96.1

CONCLUSION

In this research a reverse phase HPLC method has been developed and validated for detection and quantification of sodium benzoate and potassium sorbate in some highly consumed brands of Doogh samples which were assigned as representative of what a consumer would find in market-basket. Due to the positive results for sodium benzoate and potassium sorbate in most of the Doogh samples it can be concluded that about 25.9 percent of the collected samples are not acceptable Institute of Standard and Industrial Research of Iran. Many of the reported methods use complicated and labor-intensive pre-treatment procedures such as steam distillation multiple steps and solid-phase extractions. Comparing to the previous methods [4, 6], the presented analysis method simplifies considerably the analysis, reducing its cost and time encompasses higher level of sensitivity. This information about general detections of sodium benzoate and potassium sorbate in most of the samples shows that these chemicals are commonly used as preservatives in Doogh sample. Recently the use of sodium benzoate and of potassium sorbate in Doogh is prohibited by the Institute of Standard and Industrial Research of Iran (ISIRI). Therefore, the use of sorbate and benzoate should be regulated and more cooperation among producers, processors and the regional administration is essential.

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