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Surveillance of sodium benzoate and potassium sorbate preservatives in dairy products produced in Hamedan province, north west of Iran

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Abstract

The aim of this study was to consider the presence of sodium benzoate and potassium sorbate preservatives in 40 samples of UF cheese, 45 samples of yogurt, and 45 samples of Doogh (The Iranian traditional dairy drink) by high-performance liquid chromatography (HPLC). All of the samples were collected from 14 different brands producing in dairy factories in Hamedan province in North West of Iran. The results showed that sodium benzoate and potassium sorbate were not detected in the yogurt and Doogh samples and the minimum and maximum concentration of preservative in various UF cheese brands were 239.68 and 534.26 mg/kg for benzoate and 101.59 and 996.29 mg/kg for sorbate, respectively. The mean and standard deviation of benzoate and sorbate in cheese samples were 366.73 ± 108.70 mg/kg and 499.56 ± 361.74 mg/kg, respectively. The results highlight the importance of a more rigorous monitoring of these preservatives due to the well-established carcinogenic and toxic effects of these chemicals by the public health authorities.

Introduction

The preservative is described in Food and Agricultural Organization (FAO) as any substance added to food to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods within the extension of the product shelflife, so that the milk protects its original wholesomeness and nutritional value. In dairy products such as cheese, benzoic acid, sorbic acid and their salts chemical preservatives are used to inhibit yeast and mould growth and being also effective against a wide range of bacteria during storage (Kucukcetin *et al.*, 2008; Santini *et al*., 2009) and they are active in foods with low pH value and ineffective at neutral pH value (Tfouni and Toledo, 2002; Santini *et al.*, 2009). Caleja *et al*. (2016) compared the effects of natural and synthetic antioxidants in yogurt. They showed that the fortification of yogurts with natural and synthetic antioxidants did not cause significant changes in the yoghurt pH and nutritional value, in comparison with control samples (yogurt without any additive). Also this study indicated that the fortified yogurts had higher antioxidant activity, mainly the yogurts with natural additives. In general, they concluded that plant decoctions can be used to develop novel yogurts, by replacing synthetic preservatives and improving the antioxidant properties of the final product, without changing the nutritional profile. Although benzoic (<0.1%) and sorbic acid (<0.2%) and their salts are generally recognized as safe (GRAS) but excessive addition of these preservatives in milk are harmful for the consumers and continuous consumption of such milk may cause health hazards to society such as: the development of allergic reactions to benzoate in humans, urticaria, non-immunological contact urticaria, metabolic acidosis, convulsions, hyperpnoea, weak clastogenic activity and asthma has been reported in some studies (Tfouni and Toledo, 2002; Jahed, 2007; Wen *et al.*, 2007; Santini *et al*., 2009; Lino and Pena, 2010). The acceptable daily intake (ADI) values, determined by the joint FAO/WHO expert committee on food additives (JECFA) is 25 mg/kg of body mass for sorbic acid and sorbates salts. This value for benzoic acid and sodium benzoate has allocated per body weight as 0-5 mg/kg (WHO, 1996). According to ISIRI, Potassium sorbate and sodium benzoate usage in dairy products is prohibited. This is the reason that the uses of food additives in different countries have been limited

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by specific regulations. In Iran the producers use these preservatives according to legislation and follows regulations of Institute of Standard and Industrial Research of Iran (ISIRI) on the safe use of food additives (Kucukcetin *et al.*, 2008). So, the development of convenient and inexpensive analytical methods for these preservatives is of great importance for food safety (Fang *et al.*, 2008). The most common analytical method for the determination of benzoic acid (BA), sorbic acid (SA) or sodium benzoate (E211) and potassium sorbate (E202) has been based on reversed-phase HPLC (Saad *et al.*, 2005; Zamani Mazdeh *et al*., 2014).

The purpose of this study was to investigate the existence of benzoate and sorbate salts in ultra-filtration (UF) cheese, yogurt, and Doogh (diluted yogurt drink), as the main dairy products manufactured in Hamedan province in Iran by high performance liquid chromatography (HPLC).

Material and Methods

Materials

A total of 45 Doogh samples, 45 yogurt samples and 40 UF cheese from 14 different Iranian brands were collected from supermarkets located in Hamedan (North West of Iran). HPLC grade acetonitrile and other reagents such as ammonium acetate, glacial acetic acid, hydrochloric acid and petroleum benzene (analytical grade) were purchased from Merck Ltd. (Darmstradt, Germany). Commercial standards of sodium benzoate and potassium sorbate were supplied by Sigma chemical (St. Louis, USA). Deionised water used for chromatography processing was obtained from a Millipore Milli-Q water purification system (ELGA, UHQ-II-MK3, UK). A Millex HV 0.45 µm filter (Millipore) was used for the filtration of samples prior to injection into the HPLC system.

Preparation of standards and samples

Individual standard stock solutions of sodium benzoate and potassium sorbate were prepared in deionised water (1000 mg/kg). The working standard solutions of each preservative were prepared by diluting the stock solutions appropriately with deionised water to give a concentration between 20 and 120 mg/kg. The mixed standard solutions, which contained two preservatives at concentrations between 2 and 12 mg/kg with 2 mg/kg of sodium salicylate as the internal standard, were also prepared by mixing and appropriately diluting the working solutions (Akbari-adergani *et al*., 2013).

Exactly 10 g of each sample was dissolved and diluted with deionised water up to 50 ml in a volumetric flask and shaked for 20 min. Then 2.0 mL of each diluted sample was mixed with 200 µL of the internal standard (1000 mg/kg), further diluted by mobile phase to 10 mL and shaken vigorously for 2 min. The mixtures were then filtered through a 0.45 µm cellulose acetate syringe filter and an aliquot of 20 µL was injected into the HPLC.

HPLC analysis

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 to 20^{o}C. The chromatography column was a Supelcosil LC-18: 25 cm × 4.6 mm, 5 mm, Supelco, Bellefonte, PA, USA. Sample runtime 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 composed of a 90:10 degassed solution of ammonium acetate buffer (pH=4.1):Acetonitrile which was set at flow rate of 0.5 mL/min (Akbari-adergani *et al.*, 2013)

Statistical analysis

The acquired data were analyzed using the SPSS statistical package version 16 (SPSS Inc., Chicago, IL, USA).

Result and Discussion

The chromatographic results revealed that sodium benzoate and potassium sorbate were not detected in the yogurt and Doogh samples. About 60 percent of UF cheese samples contained benzoate with concentrations ranging from 239.68 to 534.26 mg/kg and sorbate with concentrations ranging from 101.59 to 996.29 mg/kg (Table 1). Due to the harmful effects of chemical preservatives for human health, these concentrations are not acceptable according to Institute of Standard and Industrial Research of Iran (ISIRI). Esfandiari *et al.* (2013) showed several adverse health effects are associated with the uncontrolled consumption of chemical food preservatives. For example, the presence of sodium benzoate in diet led to hyperactivity in children (McCann *et al.*, 2007) and some allergic reactions such as urticaria (Juhlin, 1981). It has also been reported that sodium benzoate and potassium sorbate could suppress some immune responses (Maier *et al*.,

Table 1. Mean and concentration range of sodium benzoate and potassium sorbate in cheese samples by HPLC method

Cheese preservative type	Number sample	Min (mg/kg)	Max (mg/kg)	Mean (mg/kg)±SD
Potassium sorbate	20	101.59	996.29	499.56 ± 361.74
Sodium benzoate	10	239.68	534.26	366.73 ± 108.70

2010).

There are also some other reports about the presence of artificial preservatives in dairy products. Amirpour *et al.* (2015) used HPLC method for determining the levels of benzoate and sorbate in 400 food samples, including pickled cucumbers, canned tomato pastes, sour cherry jams, soft drinks, fruit juices and dairy products (UF-Feta cheeses, Lighvan cheeses, lactic cheeses, yogurts and Doogh). Their results illustrated that 270 (67.5%) of all samples contained benzoate ranging from 11.9 to 288.5 mg/kg in lactic cheese and fruit juice, respectively. The levels of sorbate in 98 (24.5%) of the samples were 20.1 to 284.3 mg/kg in Doogh and fruit juice, respectively. Moreover, benzoate was detected in all dairy products ranging from 11.9 mg/kg in lactic cheese to 91.2 mg/kg in UF-Feta cheese. Akbari-adergani *et al.* (2013) also showed all of the Doogh samples in Iran contained sodium benzoate in the range of 18.3 to 2345.1 mg/kg and 25.9 percents of samples contained concentrations of potassium sorbate between 0.6 to 4961.3 mg/kg which was not in compliance with the ISIRI legislations. Rahimirad *et al.* (2014) reported on the level of potassium sorbate in Doogh whereby 14.6% of their samples were contaminated with potassium sorbate and the maximum and average concentration of this preservative in analyzed samples was 722.4 and 27.136 mg/kg, respectively during the past 3 years and these results are comparable to the results of previous studies performed in Iran by Gholipour *et al.* (2014), they reported that 32% of analyzed Doogh samples were contaminated with potassium sorbate.

Bahremand and Eskandari (2013) also reported that 100% of Doogh samples contained sodium benzoate and about 25.92 percent of samples contained potassium sorbate which was not in compliance with the ISIRI legislations. Mazdeh *et al.* (2016) indicated that the mean concentrations of sodium benzoate and potassium sorbate in Iranian commercial sports drinks were 147.72 and 11.54 ppm, respectively. These values were significantly higher than imported brands (19.43 and 4.91 ppm, respectively, $p < 0.05$). Koyuncu and Uylaser (2009) determined benzoic and sorbic acid levels in different brands of jam, candied chestnut, carbonated drink, pickle, black table olive, green table olive, whole meal/brown bread and white

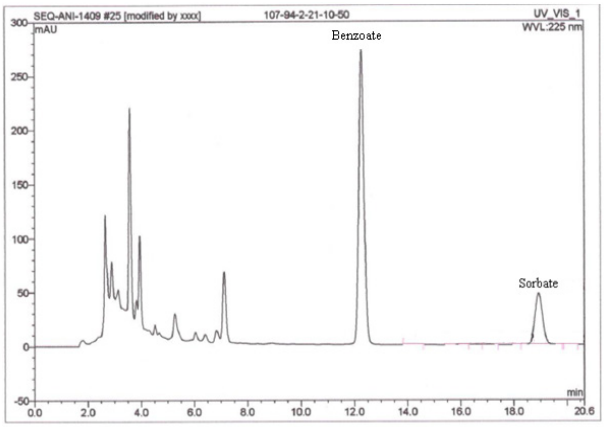

Figure 1. Typical HPLC chromatogram for determination of sodium benzoate and potassium sorbate in UF cheese sample

bread, by high-performance liquid chromatography. They indicated that benzoic and sorbic acid were used lower than the certain legal limitations permitted by legislation.

Also in this study, the developed HPLC method was validated with regard to linearity and range, precision, accuracy, LOD and LOQ following the International Conference on Harmonization (ICH) (Akbari-adergani *et al*., 2013). Duplicate injections of 20 µL sodium benzoate and potassium sorbate standard solutions were used to create linear regression lines. The peaks were identified based on the retention time. The standard curves were constructed at five concentration levels for both of sodium benzoate and potassium sorbate. The concentration of these solutions was 2, 4, 8, 12 mg/L and a zero as blank sample. The determined coefficient (r^2) obtained for the regression line shows the excellent relationship between peak area and the concentration of benzoate and sorbate (r^2>0.999). The mean regression equations for concentrations of sodium benzoate and potassium sorbate versus arbitrary units of peak area were y= 41.83 x + 3.44 and y= 114.24 x + 2.27, respectively (Y represents peak area, X represents concentration in mg/L). The correlation coefficients for standard curves of sodium benzoate and potassium sorbate were more than 0.999. In order to verify the feasibility of the method, sample recovery was used by analyzing samples before and after the addition of known quantities of benzoate and sorbate. The calculation

of accuracy was carried out as the percentage of preservatives recovered from the mixture. Mean recovery for benzoate and sorbate was between 95.2-97.1% and 93.4-98.9%, respectively (n=5) indicating that the developed method was accurate for the determination of the preservatives in samples. Figure 1 illustrates the typical chromatogram, showing retention times for benzoate and sorbate at 12.5 and 19.1 min, respectively. Peak identification was achieved by comparing with retention times of standard compounds and quantification was based on the internal standard and using calibration curves fitted by linear regression analysis. The limits of detection (LOD) and quantification (LOQ) were defined as 3 and 10 times the value of noise, respectively. The standard deviation of the estimated concentration values of the lowest calibration point was used as a measure of the noise. The LOD for benzoate and sorbate were 0.13 and 0.21 mg/kg in the samples, respectively. The LOQ for sodium benzoate and potassium sorbate were 0.40 and 0.62 mg/kg in the samples, respectively.

Conclusion

In this research a reverse phase HPLC method has been used for detection of sodium benzoate and potassium sorbate in some highly consumed brands of dairy products in Hamedan province. As it can be seen about 60 percent of the collected cheese samples are not acceptable by Institute of Standard and Industrial Research of Iran and it seems that in general, sodium benzoate and potassium sorbate in most cheese samples are commonly used as preservatives. The results also indicated that no potassium sorbate and sodium benzoate were detected in Doogh and yoghurt samples but the use of sorbate and benzoate in cheese should be controlled and more cooperation among producers, processors and the regional administration is needed. Moreover, it is recommended instead of artificial preservatives, natural products should be used in foodstuffs.

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