



Assessment of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of human health risk

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ABSTRACT

The purpose was to assess of sodium benzoate (SB) and potassium sorbate (PS) preservatives in 103 samples of cake, toast bread, tomato paste, mayonnaise sauce, carbonated soft drink and Olovieh salad in Kashan, by spectrophotometry and high performance liquid chromatography (HPLC) methods. The chronic daily intake (CDI), target hazard quotient (THQ) and hazard index (HI) of SB and PS for Iranian population were calculated. The results showed that SB and PS were not detected in the tomato paste samples. SB and PS concentrations for all samples were less than regulatory limits except for PS in one cake sample (3.57%). CDI and THQ of PS for mayonnaise sauce, Olovieh salad and cake products, except toast bread, were less than the acceptable daily intakes (ADIs) and one, respectively. While HI value of PS for the selected products was more than one, indicating that the non-carcinogenic risk represent a threat to consumers. THQ and HI values of SB for mayonnaise sauce and carbonated soft drink products were more than one through consumption of these products, indicating considerable non-carcinogenic risk. Therefore, the results highlighted the importance of a more attentive monitoring of these preservatives by the public and food health authorities in Iran.

1. Introduction

Benzoic acid (E210), sorbic acid (E200) and their salts (benzoates and sorbates) are widely used preservatives in large-scale foods and drinks as antibacterial and antifungal agents (Piper and Piper, 2017; Mischek and Krapfenbauer-Cermak, 2012). Benzoates and sorbates have a very low mammalian toxicity (Piper and Piper, 2017; Mischek and Krapfenbauer-Cermak, 2012). There is a general agreement that they are intrinsically free of carcinogenicity, but have the potential to undergo a transformation to potential mutagens (Piper and Piper, 2017).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has investigated the safety of these compounds (Mischek and Krapfenbauer-Cermak, 2012). Therefore, the ADIs have reported: 0–5 mg/kg body weight/day for benzoic acid (and benzoate salts) and 0–25 mg/kg body weight/day for sorbic acid (and sorbate salts) (WHO, 2016; Mischek and Krapfenbauer-Cermak, 2012).

Nevertheless, some studies have reported genotoxic effects of benzoates and sorbates. The chromosome aberrations have demonstrated in human lymphocytes, Chinese hamster cells, bone marrow cells of mice that were exposed to sorbate and/or benzoate (Piper and

Piper, 2017; Pongsavee, 2015; Mamur et al., 2012). Saatci et al. (2016) showed that general genomic injuries were present in almost all the liver cell samples of pregnant rats and their fetuses in the SB group compared with the non-treated group. They reported that SB usage may cause DNA damage and increase micronuclei formation. Therefore, they recommended that pregnant women should avoid consuming foodstuffs containing SB as an additive.

Previous studies have focused on the reactions between sorbate and nitrite at pH 2 to 4.2 such as gastric environment. Sodium sorbate could form genotoxic and cell-transforming agents such as 1,4-dinitro-2-methylpyrrole and ethylnitrolic acid under conditions of acidic pH, heating and storage (Pérez-Prior et al., 2008; Schiffmann and Schlatter, 1992). Mamur et al. (2012) indicated that sodium sorbate at 200, 400 and 800 µg/mL concentrations increased the frequency of chromosome aberrations at both 24 and 48 h period compared to control. This additive caused DNA damage at all concentrations in isolated human lymphocytes after 1 h in vitro exposure. They showed that sodium sorbate is genotoxic to the human peripheral blood lymphocytes in vitro at the highest concentrations. Also, adverse effects of benzoates include non-immunological (pseudoallergy) in sensitive patients and hyperactivity in children have reported (Piper and Piper, 2017; McCann

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et al., 2007).

Previous study has demonstrated that the benzoic and sorbic acid contents in all the processed foods such as breads, sauces, beverages and etc. in South Korea were below the Korean maximum permitted levels of 2000 mg/kg and 3000 mg/kg, respectively (Shin et al., 2017). In other study all Iranian yoghurt drink (Doogh) samples were shown to contain SB and PS was not detected in any of them. SB concentration diverse among the samples ranged from 0.94 to 9.77 mg/L. Their results of the exposure estimation showed that no serious public health concern would exist regarding to the mentioned preservatives (Esfandiari et al., 2013).

The goal of the present work was to assess of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of daily intakes using Iranian food consumption patterns in selected food categories. Estimated intakes were compared to the respective ADIs. Additionally, the human health risk through their consumption for consumers was estimated.

2. Materials and methods

2.1. Sample collection

The present cross-sectional study was conducted from July 2015 to January 2017. The food categories for analysis of SB and PS were selected based on regulations of food additives in the Institute of Standards and Industrial Research of Iran (ISIRI, 2008). A total of 103 samples in six categories including cake, toast bread, tomato paste, mayonnaise sauce, carbonated soft drink and Olovieh salad were collected on random basis from different local supermarkets in Kashan, Iran. These food samples were all processed foods and were selected from various production batches and produced by various manufactures in all over Iran and distributed in Kashan.

2.2. Analytical methods

2.2.1. Spectrophotometry method

Sodium benzoate in carbonated soft drink samples were determined using method of Institute of Standards and Industrial Research of Iran (ISIRI, 2003). Standard solutions with concentration of 2 ppm–12 ppm prepared from SB (Merck Co., Darmstadt, Germany). 5 mL of each solution was transferred to a test tube and 400 μ L HCL 6 N was added and brought to 50 mL with petroleum benzene and shake vigorously for 1 min. The optical absorption was measured at a wavelength of 227 nm by a spectrophotometer (CECIL, 2021; England) and a standard curve was prepared ($y = 0.133x - 0.054$; $r^2 = 0.997$). Then, carbonated soft drink samples degassed in ultrasonic bath and then 5 mL of solutions were caught and added 400 μ L HCL 6 N as well as brought to 50 mL with petroleum benzene and shake vigorously for 1 min. The obtained absorbance rate was posed at the standard curve and thus the SB content of the samples in mg/L SB equivalent was estimated.

2.2.2. Determination SB and PS by high performance liquid chromatography (HPLC)

In order to extract of SB and PS, the solid samples were prepared as follows: 1 g of the sample was weighed and transferred to into a screw-capped test tube. Then, 10 mL of methanol, 2.5 mL sodium hydroxide (0.2 mol/L), 5 mL of deionized water, 0.5 mL of Carrez I (potassium hexaciano ferrate: deionized water; 15% w/v), and 0.5 mL of Carrez II (22 g of zinc acetate was mixed with 3 mL of acetic acid in 100 mL of deionized water) were added. Then, the suspension placed in an ultrasonic bath for 10 min. Then, it was centrifuged for 10 min at 4000 rpm. Afterwards, the supernatant was collected and filtered through a 0.45 μ m nylon membrane syringe filter, and the clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done. The liquid extract samples were filtered through a 0.45 μ m nylon membrane and the clear filtrate

was injected into the HPLC column.

SB and PB was separated by HPLC (Knauer, Germany), on a Supelcosil LC-18 column (Supelco, Bellefonte, PA, USA C18 5 μ m: 25 cm \times 4.6 mm), measuring the absorbance at 225 and 254 nm for SB and PB, respectively (UV detector). A solution of methanol/phosphate buffer (pH = 4.4; 70:30 v/v) was used as eluent in an isocratic mode and quantification was made by standard external method (sorbic acid and benzoic acid reagent, Sigma-Aldrich). The mobile phase flow rate was set to 1.0 mL/min. Results were expressed as sorbic or benzoic acid equivalents (Faraji and Rahbarzare, 2016). The limit of detection (LOD) and limit of quantification (LOQ) for benzoic acid were 0.6 and 4 mg/L or mg/kg, respectively. The LOD and LOQ for sorbic acid were 0.2 and 2 mg/L or mg/kg, respectively.

2.3. Health risk assessment for SB and PS

The daily food consumption data was considered for bread 286 g/day (NNFTRI, 2004), cake 8.3 g/day (Abdollahi et al., 2014), mayonnaise sauce 3.4 g/day, carbonated soft drink 144 mL/day and Olovieh salad 3.4 g/day (ISIRI, 2008). Chronic daily intake (CDI) of SB and PS were obtained by the mean contents of SB and PS found in each food category with food consumption data for consumers with average consumption. If SB and PS were not detected, the content of the sample was assigned a value of zero. For the worst case scenario, the maximum level of determined preservatives was used (Alif Adham and Shaharuddin, 2014; USEPA, 1989).

$$CDI = \Sigma c \times DI/BW$$

Where, *CDI* is chronic daily intake of additive for average consumer (mg/kg body weight/day), *c* is mean additive content (mg/kg), *DI* is mean food consumption (g/day), *BW* is average body weight set to 60 kg in this study.

Then, to conclude the significant different exposure and overall potential for non-carcinogenic health effects caused by SB and PS in each food category, the target hazard quotient (THQ) was calculated using the following equation:

$$THQ = CDI/RfD$$

Where, *THQ* is target hazard quotient, *CDI* is chronic daily intake of additive for average consumer (mg/kg bw/day), *RfD* is reference dose (mg/kg bw/day).

A *THQ* value more than 1 ($THQ > 1$) will show a significant risk level, where the higher the value, the greater the likelihood of adverse non-carcinogenic health impact (Alif Adham and Shaharuddin, 2014; USEPA, 1989).

The hazard index (HI) from the consumption of SB and PS by various food obtained from Kashan was calculated as the sum of *THQs* of all the food samples (FS) and was expressed as follows;

$$HI = THQ_{FS_1} + THQ_{FS_2} + \dots + THQ_{FS_n}$$

The *RfD* values that were used in this study were 4 and 25 (mg/Kg bw/d) for SB and PS, respectively (EU, 2012; Mischek and Krapfenbauer-Cermak, 2012; USEPA, 1988).

2.4. Statistical analysis

Data was analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and descriptive statistic was used. The results of all experiments were expressed as the mean \pm standard deviation (SD).

3. Results

The results of SB and PS content in different foods are shown in Table 1. PS with concentration $>$ LOQ was found in toast bread (ranging from 2.5 to 415 mg/kg), cake (ranging from 5.4 to 700 mg/

Table 1
Concentrations of preservatives in different foods (mg/kg or mg/L).

| Food | Sodium benzoate | | | | | | Potassium sorbate | | | | | |
|------------------------------------|-----------------|----------------------|-----------------|-------|----------------|------------------|-------------------|----------------------|--------|--------|----------------|------------------|
| | No. of samples | No. of samples > LOQ | Min | Max | Mean ± SD | MPL ^a | No. of samples | No. of samples > LOQ | Min | Max | Mean ± SD | MPL ^a |
| Cake | – | – | – | – | – | – | 28 | 18 | ND | 700 | 127.93 ± 167.5 | 500 |
| Toast bread | – | – | – | – | – | – | 20 | 8 | ND | 415 | 56.41 ± 130.2 | 500 |
| Tomato paste | 15 | 0 | ND ^b | ND | ND | unallowable | 15 | 0 | ND | ND | ND | unallowable |
| Mayonnaise sauce | 15 | 15 | 161.68 | 296.2 | 243.42 ± 42.22 | 750 | 15 | 15 | 125.92 | 334.99 | 232.47 ± 52.56 | 750 |
| Carbonated soft drink ^c | 19 | – | 2.12 | 130 | 61.75 ± 55.53 | 150 | – | – | – | – | – | – |
| Olovieh salad | – | – | – | – | – | – | 6 | 6 | 104.6 | 144.2 | 119.4 ± 19.33 | 150 |
| Total | 49 | 15 | – | – | 98.46 ± 108.65 | – | 84 | 47 | – | – | 106.71 ± 138.5 | – |

^a MPL: Maximum permitted level of Institute of Standard and Industrial Research of Iran (mg/kg or mg/L).

^b ND: Not detected (< LOQ).

^c Sodium benzoate was determined by spectrophotometry.

kg), mayonnaise sauce (ranging from 125.92 to 334.99 mg/kg) and Olovieh salad (ranging from 104.6 to 144.2 mg/kg) samples. The detection rates of PS in the 4 food products were in the following order: mayonnaise sauce (100%), Olovieh salad (100%), cake (64.28%) and toast bread (40%). PS was not detected in tomato paste samples (< LOQ 2 mg/kg or L). PS concentrations for all samples were acceptable according to ISIRI, except for one cake sample (3.57%).

SB was found in 15 (100%) mayonnaise sauce samples ranging from 161.68 to 296.2 mg/kg and 19 (100%) carbonated soft drink samples ranging from 2.148 to 131 mg/kg. Also, SB was not detected in tomato paste samples (< LOQ 4 mg/kg or L). SB concentrations for all samples were acceptable according to ISIRI.

The exposure estimation, THQ and hazard index of SB and PS intake via different foods intake in Iranian community are expressed in Table 2. It was observed that the CDI of PS per day for mayonnaise sauce, Olovieh salad and cake products were lower than ADI (25 mg/kg bw/day), but the greatest contributor to the CDI of PS was for toast bread. In addition, the CDI of SB per day for mayonnaise sauce and carbonated soft drink products were much higher than ADI (5 mg/kg bw/day). The results showed that in the worst case condition of PS per day for mayonnaise sauce and Olovieh salad products were lower than ADI and this demonstrated the safety concern correspond to the utilized preservative is negligible. But the worst case condition of PS per day for cake and toast bread products and SB per day for mayonnaise sauce and carbonated soft drink products were much higher than ADI and therefore, the results showed the potential adverse risks due to exposure of PS and SB from these products.

Our results demonstrated that the THQ values of PS for mayonnaise sauce, Olovieh salad and cake products were less than 1 and this

indicated the risk of adverse effect of PS in these products was negligible. However, THQ value of PS for toast bread was more than 1. The THQ values of SB for mayonnaise sauce and carbonated soft drink products were more than 1 and are hence at a harmful level.

The total non-carcinogenic hazard index (HI) for the consumption of food samples containing PS and SB were more than 1. This indicates potential health risk hazard to the exposed population at the moment.

4. Discussion

In this work, the mean SB values were below the limit of 750 and 150 mg/kg established by the ISIRI, for mayonnaise sauce and carbonated soft drink samples, respectively (ISIRI, 2008). Also, the mean content of PS preservative was below the limit of 500, 500, 750 and 150 mg/kg established by the ISIRI, for cake, toast bread, mayonnaise sauce and Olovieh salad samples, respectively (ISIRI, 2008). In tomato paste products, ISIRI issues production licenses for the registered processing units provided that they do not use chemical preservatives such as SB and PS in their processed products (ISIRI, 2008). In this study, PS and SB were not detected in tomato paste samples.

Also, it is evident that mayonnaise sauce samples were the main source of SB and PS. Similar conditions were in other works in Iran, where the main source of SB (ranging from 330.01 to 660.02 mg/kg) and PS (ranging from 18.9 to 214.5 mg/kg) were mayonnaise sauces samples (Heshmati et al., 2018; Faraji and Rahbarzare, 2016). Our results showed that mean levels of SB and PS in cake, toast bread, tomato paste, mayonnaise sauce and carbonated soft drink samples were lower than those found in some of other studies from south east Serbia (Lazarevic et al., 2011), Austria (Mischek and Krapfenbauer-Cermak,

Table 2
Exposure estimation of preservatives in different foods intake in Iranian community.

| Food | Sodium benzoate | | | Potassium sorbate | | |
|-----------------------|--|--|------------------------|--|--|------------------------|
| | Chronic daily intake (mg/kg bw/day) ^a | Worst case condition (mg/kg bw/day) ^b | Target hazard quotient | Chronic daily intake (mg/kg bw/day) ^a | Worst case condition (mg/kg bw/day) ^b | Target hazard quotient |
| Cake | – | – | – | 17.7 | 688.33 | 0.71 |
| Toast bread | – | – | – | 268.9 | 1978.2 | 10.75 |
| Tomato paste | – | – | – | – | – | – |
| Mayonnaise sauce | 13.79 | 16.78 | 3.45 | 13.17 | 18.92 | 0.52 |
| Carbonated soft drink | 148.2 | 314.4 | 37.05 | – | – | – |
| Olovieh salad | – | – | – | 6.76 | 8.17 | 0.27 |
| Total | 81 ± 95.04 | 165.6 ± 210.45 | 40.5 ^c | 76.63 ± 128.26 | 673.4 ± 122.9 | 12.25 ^c |

^a The average concentration of each preservative was taken as the residue levels.

^b The maximum concentration of each preservative was taken as the residue levels.

^c Hazard index.

2012), Macedonia (Kostik, 2014) and Iran (Javanmardi et al., 2017).

In the study from South Korea (Shin et al., 2017) and Pakistan (Ahmed et al., 2013) PS in bread and sauce products were lower than our results study. Ulca et al. (2013) reported that a total of 23 samples (2.3%), some syrups, tomato sauces and fruit contained individual or combined levels of sorbic and benzoic acids above regulatory limits. In the present study all of the samples (except for one cake sample 3.57%) contained SB and PS less than regulatory limits.

The potential risk of contaminants such as PS and SB is depended on various factors, such as toxicity, per capita consumption of food product, body weight, exposure time and etc. Therefore, to better and more accurate interpretation of the obtained results, non-carcinogenic risk assessment was done (Rahmani et al., 2018; Shahrabakki et al., 2018).

Mean chronic daily intakes of SB and PS in the average intake of all the samples were 81 ± 95.04 and 76.63 ± 128.26 mg/kg bw/day, respectively. The results showed that Iranian people have an intake higher than the ADI for SB and PS.

However, CDI of PS for mayonnaise sauce, Olovieh salad and cake products were lower than ADI but the greatest contributor to the CDI of PS was for toast breads. The reason might be that there is no information about the amount of consumption of different Iranian breads such as toast breads. Because of the absence of this information, the CDI for the general population was derived from Iranian total consumption of all breads. Therefore, mean CDI of PS in the average intake of all the samples was higher than ADI. Similar results of estimation intakes of PS were reported from studies conducted in Australia and New Zealand (FSANZ, 2005), Austria (Mischek and Krapfenbauer-Cermak, 2012) and Iran (Esfandiari et al., 2013) that were well below the ADI.

CDI of SB for mayonnaise sauce and carbonated soft drink samples were higher than ADI. Therefore, mayonnaise sauces and carbonated soft drinks are the largest contributors to the intake of SB by consumers with both average and high rates of these foods consumption in Iran. Previous studies in Australia and New Zealand (FSANZ, 2005) and South Korea (Shin et al., 2017) reported that carbonated beverages were major contributors to the consumption of benzoic acid. Also, the WHO, 2000 reported that beverages were major contributors to the intake of benzoic acid. The results of this study are similar to these reports.

Based on the results of this study, the THQ values of PS for mayonnaise sauce, Olovieh salad and cake products were less than 1 and therefore, dietary intake of PS of these products in population of Kashan, Iran does not represent a public health risk. However, THQ value of PS for toast breads was more than 1. Mischek and Krapfenbauer-Cermak (2012) demonstrated that dietary intake of PS of toast bread, mayonnaise and tomato ketchup products did not represent a public health risk.

The THQ values of SB for mayonnaise sauce and carbonated soft drink products were more than 1 and therefore, dietary intakes of SB of these products in population of Kashan, Iran represent a public health risk. Ling et al. (2015) demonstrated that use of benzoic acid and sorbic acid as preservatives in the Taiwanese diet does not constitute a public health and safety concern.

The comparison of dietary intakes in this study and various studies is difficult due to differences of regional dietary and in the methodology used as well as assumptions made in the exposure assessment.

According to HI values our results indicated potential health risk hazard to the exposed population at the moment, suggesting that more attention should be directed toward the ingestion of SB and PS. Therefore, where possible, it is recommended pasteurization of various food samples and using high-pressure sterilization, replacing SB and PS with plant essential oils and extracts and finally reduce consumption of foods containing SB and PS. In the last few decades, nonthermal pasteurization methods have been considered, including pulsed electric field, high-pressure homogenization, high hydrostatic pressure, and ultrasound (Souza et al., 2016; Rupasinghe and Yu, 2012). In addition, accurate monitoring of preservatives amounts by regulatory agencies,

application of good manufacturing practices (GMP), good hygienic practices (GHP) and cold chain management during storage, distribution and retail are warranted to reduce and/or remove food preservatives usage in food industry as to assure human safety.

5. Conclusion

In the current study concentrations of SB and PS in some products from Kashan city, Iran were measured using HPLC and spectrophotometry methods. Also, the estimation of daily intakes using Iranian food consumption patterns in selected food categories and non-carcinogenic risk for consumers exposed to these products were calculated. Based on the obtained results, all of the samples (except for one cake sample 3.57%) contained SB and PS less than regulatory limits. These results indicated that CDI and THQ of PS for mayonnaise sauce, Olovieh salad and cake products, except toast bread, were less than the ADIs and one, respectively. While HI value of PS for these products was higher than one and its risk assessment showed that the non-carcinogenic risk represent a threat to consumers. THQ and HI values of SB for mayonnaise sauce and carbonated soft drink products were higher than one for consumers, indicating considerable non-carcinogenic risk. Therefore, the consumption of these products is considered to be unsafe and their consumption on a regular basis is not recommended. It is also suggested monitoring the PS and SB concentrations in future studies on other processed products in Iran.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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