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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 μL 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 μL 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 μm 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 μm 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 μm: 25 cm × 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;

$$HI = THQ_{FS\ 1} + THQ_{FS\ 2} + \ldots + THQ_{FS\ n}$$

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 ± 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/