Investigation of benzoic acid and sorbic acid in snack foods in Jilin province, China

Chaojian Xu, Jinhong Liu, Chunxue Feng, Hang Lv, Shaowu Lv, Danyang Ge, and Ketong Zhu

College of Food Engineering, Jilin Engineering Normal University, Changchun, Jilin, People’s Republic of China; Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, College of Life Science, Jilin University, Changchun People’s Republic of China

ABSTRACT
A high-performance liquid chromatography method was applied for the determination of the levels of benzoic acid and sorbic acid in 221 snack food samples, including fruit wine, noodle products, pickled products, baking products, cooked meat products, and bean products. Only two cooked food samples in all food samples have a preservative content exceeding the maximum permitted levels (MPL) laid down by the Standardization Administration of China. Among the food samples from which preservatives were detected, 69.6% of the samples contained one type of preservative and 30.4% of the samples detected two preservatives. Among the geometric mean values of preservatives in different types of foods, the highest is benzoic acid in pickled products (136.6 mg/kg) and sorbic acid in baking products (150.0 mg/kg). No preservatives were detected in bean products. Preservatives in packaged foods (21 of 121 samples, 17.4%) have a higher detection rate than bulk foods (2 of 100 samples, 2%), but each has only one piece that exceeds MPL. On the surface of our research, the amount of preservative added to snack foods in Jilin Province of China is relatively safe.

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Introduction
Benzoic acid and sorbic acid are the most common food preservatives used to protect the longevity of food products. [1] Microbial, enzymatic, or chemical changes during storage can decrease the shelf-life of foods. Therefore, the addition of preservative in foods can prolong their shelf-life, protect them against deterioration, and/or prevent the growth of pathogenic microorganisms. The use of preservatives such as benzoic acid and potassium sorbate has become increasingly important in controlling the growth of microbes and extending product shelf life. [2,3]

Both benzoic acid and sorbic acid are widely used in food, beverages, cosmetics, toothpaste, drugs, and so on, but an overdose of the antiseptic agents can cause harmful effects to human health. [4] The maximum permitted levels of preservatives are established by legislation, in order to limit their amount in foods. [5] The daily intakes of benzoic acid and sorbic acid are recommended to be 5 mg/kg d\(^{-1}\) and 25 mg/kg d\(^{-1}\), respectively, according to FAO/WHO Expert Committee on food additives. However, exceeding the permitted levels may cause some adverse reactions, including metabolic acidosis, convulsions, asthma, and allergic reactions. [6,7] There are many analytical techniques for detecting benzoic acid and sorbic acid in foods, such as biosensor [8–10], capillary electrophoresis [11,12], gas chromatography [13–15], high-performance liquid chromatography [16–18]. Currently, the most common method used in the food industry is high-performance liquid chromatography (HPLC). [19,20]
The use of preservatives is not only important to maintain the quality of foods, but also to ensure the health and safety of consumers. Therefore, the aims of this study were to determine the amount of benzoic acid and sorbic acid in 221 samples of 6 types of snack foods by using HPLC, and to analyze the preservatives in these food samples.

Materials and methods

Chemicals and reagents

Ammonia, Potassium ferrocyanide, Zinc acetate, Ethanol, and Hexane were purchased from Beijing chemical plant; Methanol, Ammonium acetate, and Formic acid were purchased from sigma aldrich. All of the reagents used were of Chromatographic purity.

Sample collection

In this study, a total of 221 samples were collected in Jilin province from September 2016 to July 2017. All the samples were categorized into 6 groups, including Fruit wine (n = 39), Noodle products (n = 51), Pickled products (n = 46), Baking products (n = 20), Cooked meat products (n = 44), Bean products (n = 21).

Chromatographic conditions

Chromatographic analysis was carried out on the e2695 series HPLC system (Waters, Milford, MA, USA), equipped with a 10 µL loop injector, UV Detector and an X-SELECT C₁₈ column (150 × 2.1 mm, 5 µm). The mobile phase consisted of an aqueous ammonium acetate buffer (pH = 4.2) and methanol (95:5 v/v) with a flow rate of 1 mL min⁻¹. The injection volume was 10 µL, and the UV detector was set at 230 nm.

Preparation of standards and samples

Individual standard stock solutions of sodium benzoate and potassium sorbate were prepared in distilled water (1000 mg L⁻¹). Working standard solutions of each preservative were prepared by diluting the stock solutions appropriately with distilled water to give a concentration between 0 mg L⁻¹ and 200 mg L⁻¹. Mixed standard solutions, which contained two preservatives at concentrations between 0 mg L⁻¹ and 200 mg L⁻¹ (0 mg L⁻¹, 1 mg L⁻¹, 5 mg L⁻¹, 10 mg L⁻¹, 20 mg L⁻¹, 50 mg L⁻¹, 100 mg L⁻¹ and 200 mg L⁻¹) were also prepared by mixing and appropriately diluting the working solutions.

A sample having a volume of 2 mL or g was shaken in a 50 mL centrifuge tube with 25 mL of deionized water for 2 min and then sonicated in a 50°C water bath for 20 min. After the ultrasonic product is cooled to room temperature, 2 mL of potassium ferrocyanide solution and 2 mL of zinc acetate solution are added and mixed, and then centrifuged at 8000 r/min for 5 min to collect the aqueous phase. Transfer the aqueous phase to a 50 mL volumetric flask and add 20 mL of water. After vortexing, the mixture was sonicated for 5 min and centrifuged at 8000 r/min for 5 min. Pour the new aqueous phase back into the volumetric flask and dilute to 50 mL with deionized water and mix. The mixtures were then filtered through a 0.22 µm cellulose acetate syringe filter and 20 µL was injected into the HPLC.

Method verification

The method was validated by international guidelines (International Conference on Harmonization (ICH)), as described by Walfish. The calibration curves of each salt were constructed over a range of 0–200 mg L⁻¹. Linearity between the concentration of both benzoate and sorbate salt and the peak areas was obtained, and the correlation coefficient for each standard curve was calculated. The limit
of detection (LOD) and quantification (LOQ) were determined by considering, respectively, 3.3 and 10 times the residual standard deviation of a regression line to the slope of the calibration curve. 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. In addition, to evaluate the intraday precision (as RSD r), each sample was extracted three times and each sample extract was analyzed on the same day. Matrix-match calibration curves were constructed using blank food samples spiked with standard solutions. The representative matrices, i.e., fruit wine, noodle products, pickled products, baking products, cooked meat products, and bean products, were previously confirmed to be free of the target analytes. All food samples were screened based on a calibration curve for the corresponding type of food. The representative matrices were evaluated for three times in 3 days. Thereafter, twice the standard deviation was reported as measurement uncertainty at a confidence level of 95%.

**Statistical analysis**

Data arrangement was performed using Excel (Microsoft, Redmond, WA, USA). The differences in benzoic acid and sorbic acid levels among the six types of foods were evaluated by ANOVA (Analysis of Variance). The results for the analytical samples were expressed as geometric mean, mean ± SD, and range in all tables. Statistical significance was set at p < 0.05.

**Results and discussion**

**Method validation**

Figure 1 gives a typical chromatogram, showing retention times for benzoic acid and sorbic acid at 8.0 and 11.5 min, respectively. Peak identification was achieved by comparison to the retention time of standard compounds, and quantification was performed based on an external standard method using a calibration curve fitted by linear regression analysis. The peak area response and concentration were well linear in the range of 5.1–50 mg kg\(^{-1}\) under optimized experimental conditions. The results of the calibration data, LOD, LOQ, and recovery are listed in Table 1. Recoveries ranged from 91% for sorbic acid in meat products to 105% for benzoic acid in drink indicating acceptable accuracy and suitability for simultaneous determination of these preservatives in various types of food.

**Overall occurrence of benzoic acid and sorbic acid**

Table 2 shows occurrence of benzoic acid and sorbic acid in food from Jilin province, China. 89.6% of the samples were found to have no preservatives, and 10.4% of the samples detected preservatives. 0.9% of samples had preservatives higher than maximum permitted levels (MPL) laid down by the Standardization
Another report from Iran shows that there is a big difference of preservatives added to foods from various countries and regions. Therefore, the health risks associated with any food additive are based on an accurate assessment of the average levels of food containing that additive consumed by the public. As shown in Table 3, the high frequency of benzoic acid is Baking products (20%), followed by fruit wine (15.4%), Pickled products (13.0%) and Cooked meat products (2.3%). Then, the high frequency of sorbic acid is Pickled products (10.9%), followed by Fruit wine (7.7%), Cooked meat products (6.8%), Baking products (5%) and Noodle products (2%). In all of these foods, the benzoic acid detection rate of the package (15.4%) is higher than that of the bulk product (2.0%), which was the same in sorbic acid. Only two food samples contained more preservatives than MPL, one was benzoic acid in bulk cooked meat products (12.4%) is higher than that of the bulk product (2.0%) and the other was sorbic acid in packaged cooked food (2.3%). Among the food samples from which preservatives were detected, 69.6% of the samples contained one type of preservative and 30.4% of the samples detected two preservatives. Benzoic acid and sorbic acid are often used as preservatives in foods, sometimes used alone and sometimes in combination. In the past, it was generally believed that benzoic acid/benzoate did not accumulate in the body and the risk of acute toxicity was low, but recent studies have found that college students drinking benzoic acid-containing beverages cause attention-deficit/hyperactivity disorder. At the same time, EFSA believes that the genetic toxicity of sorbic acid cannot be ignored, so the ban on sorbic acid remains. Therefore, the health risks associated with ingesting foods containing preservatives such as benzoic acid and sorbic acid require attention.

The detected two preservatives obtained for the geometric mean, mean ± SD, maximum and minimum values (mg kg⁻¹) are listed in Table 3. The geometric mean concentrations of preservatives in total samples were the highest for sorbic acid (72.1 mg kg⁻¹), followed by benzoic acid (46.3 mg kg⁻¹). In this study, ingestion of foods that exceed MPL poses a certain health risk. A report from Iran’s Sari showed that 75% of the 400 food samples surveyed detected benzoic acid or sorbic acid, the most commonly detected preservative being benzoic acid. Another report from Iran’s Arak showed that the benzoate content of canned kimchi and pickled cucumber from producing factories was close to zero. In a report in South Korea, its benzoic acid level was 0.001 mg kg⁻¹. In the past, it was generally believed that benzoic acid/benzoate did not accumulate in the body and the risk of acute toxicity was low, but recent studies have found that college students drinking benzoic acid-containing beverages cause attention-deficit/hyperactivity disorder. At the same time, EFSA believes that the genetic toxicity of sorbic acid cannot be ignored, so the ban on sorbic acid remains.

Administration of China. The percentages of preservatives above MPL was cooked meat products (4.5%). At the same time, both the package and the bulk products have a detected amount exceeding MPL. In view of the observed genotoxicity of benzoic acid/benzoate and sorbic acid/sorbate, ingestion of foods that exceed MPL poses a certain health risk. A report from Iran’s Sari showed that 75% of the 400 food samples surveyed detected benzoic acid or sorbic acid, the most commonly detected preservative being benzoic acid. Another report from Iran’s Arak showed that the benzoate content of canned kimchi and pickled cucumber from producing factories was close to zero. In a report in South Korea, its benzoic acid level (9.2%) is closer to this study. Reports from Iran, South Korea, and this study show that there is a big difference of preservatives added to foods from various countries and regions. Therefore, the health risks associated with preservatives in foods vary from region to region, as the health risks associated with any food additive are based on an accurate assessment of the average levels of food containing that additive consumed by the public.

As shown in Table 3, the high frequency of benzoic acid is Baking products (20%), followed by fruit wine (15.4%), Pickled products (13.0%) and Cooked meat products (2.3%). Then, the high frequency of sorbic acid is Pickled products (10.9%), followed by Fruit wine (7.7%), Cooked meat products (6.8%), Baking products (5%) and Noodle products (2%). In all of these foods, the benzoic acid detection rate of the package (12.4%) is higher than that of the bulk product (2.0%), which was the same in sorbic acid. Only two food samples contained more preservatives than MPL, one was benzoic acid in bulk cooked meat products (2.3%), and the other was sorbic acid in packaged cooked food (2.3%). Among the food samples from which preservatives were detected, 69.6% of the samples contained one type of preservative and 30.4% of the samples detected two preservatives. Benzoic acid and sorbic acid are often used as preservatives in foods, sometimes used alone and sometimes in combination. In the past, it was generally believed that benzoic acid/benzoate did not accumulate in the body and the risk of acute toxicity was low, but recent studies have found that college students drinking benzoic acid-containing beverages cause attention-deficit/hyperactivity disorder. At the same time, EFSA believes that the genetic toxicity of sorbic acid cannot be ignored, so the ban on sorbic acid remains. Therefore, the health risks associated with ingesting foods containing preservatives such as benzoic acid and sorbic acid require attention.

The detected two preservatives obtained for the geometric mean, mean ± SD, maximum and minimum values (mg kg⁻¹) are listed in Table 3. The geometric mean concentrations of preservatives in total samples were the highest for sorbic acid (72.1 mg kg⁻¹), followed by benzoic acid (46.3 mg kg⁻¹).
| Food                  | Number of samples | Detection | Benzoic acid (mg/kg) | Sorbic acid (mg/kg) | MPL mg/kg | Number of above MPL |
|----------------------|-------------------|-----------|-----------------------|---------------------|-----------|---------------------|
|                      |                   | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range | Benzoic acid | Sorbic acid | Geomean ± SD | Mean ± SD | Range |
| Fruit wine           | 39                | 6 (15.4%) | 3 (7.7%) | 2.0–50.0 | 18.1 ± 17.3 | 136.1 ± 82.0 | 800 | 600 | 0 | 0 |
| Noodle products      | 51                | 0 (0.0%) | 1 (2.0%) | 0          | -        | 82.0 ± 82.0 | 0 | 1000 | 0 | 0 |
| Pickled products     | 46                | 6 (13.3%) | 5 (10.9%) | 2.0–780.0 | 136.6 ± 327.0 | 42.6 ± 123.0 | 1000 | 1000 | 0 | 0 |
| Baking products      | 20                | 4 (20%)  | 1 (5%) | 5.0–370.0 | 79.1 ± 175.0 | 150.0 ± 150.0 | 1000 | 1000 | 0 | 0 |
| Cooked meat products | 44                | 1 (2.3%) | 3 (6.8%) | 2.3 | 2.3 | 68.7 ± 141.7 | 5 | 75 | 1 | 1 |
| Bean products        | 21                | 0 (0.0%) | 0 (0.0%) | 0 | - | - | 1000 | 1000 | 0 | 0 |
| Packaged             | 121               | 15 (12.4%) | 11 (9.1%) | 2.0–780.0 | 65.6 ± 187.5 | 75.8 ± 138.4 | 1000 | 1000 | 0 | 1 |
| Bulk                 | 100               | 2 (2.0%) | 2 (2.0%) | 2.3–5.0 | 3.4 ± 3.7 | 54.8 ± 85.0 | 1000 | 1000 | 0 | 1 |
| Total                | 221               | 17 (7.7%) | 13 (5.9%) | 2.0–780.0 | 46.3 ± 165.9 | 72.1 ± 130.2 | 1000 | 1000 | 1 | 1 |
Among the geometric mean of benzoic acid in different types of foods, pickled products (136.6 mg kg\(^{-1}\)) were the highest, followed by baking products (79.1 mg kg\(^{-1}\)), fruit wine (18.1 mg kg\(^{-1}\)) and cooked meat products (2.3 mg kg\(^{-1}\)). Then, in the geometric mean of sorbic acid from different types of foods, baking products (150.0 mg kg\(^{-1}\)) were the highest, followed by fruit wine (136.1 mg kg\(^{-1}\)), noodle products (82.0 mg kg\(^{-1}\)), cooked meat products (68.7 mg kg\(^{-1}\)) and pickled products (42.6 mg kg\(^{-1}\)). In this study, not only the detection amount of benzoic acid and sorbic acid in the same type of food differed greatly, but also the detection amount of the same preservative in different types of foods was quite different. In a study in Portugal\(^{[40][38]}\), the average values of benzoic acid and sorbic acid in traditional beverages were 158 ± 39 mg L\(^{-1}\) and 148 ± 18 mg L\(^{-1}\), respectively, and in soft drinks, 172 ± 96 mg L\(^{-1}\) and 188 ± 66 mg L\(^{-1}\), respectively. This is closer to the results of the fruit wine in this study. In a study in Korea\(^{[41][15]}\), benzoic acid was detected in 5.6% of fish samples and no sorbic acid was detected in any of the samples. The Korean study is similar to the one found in the use of preservatives in the same food.

**Conclusion**

This is the first representative study to monitor sorbic acid and benzoic acid in snack foods such as fruit wine, noodle products, pickled products, baking products, cooked meat products, and bean products in Jilin Province, China. The detection rate of benzoic acid (7.7%) was higher than that of sorbic acid (5.9%), and 30.4% of the samples in which the preservative was detected simultaneously detected two preservatives. In this study, the preservatives added to most foods were below the minimum permitted levels (MPL), however, 0.9% of the food samples exceeded the MRL by the Standardization Administration of China. In addition, since the detection rate of packaged foods (17.4%) is much higher than that of bulk foods (2%), it is recommended to strictly implement national intensive inspections to ensure that the amount of preservatives added to foods remains within the safe level of human consumption. The results of this study can be used to provide a status of preservative addition and to assess human exposure to preservatives in food products.

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