



Research Note

Evaluation of Sodium Benzoate and Potassium Sorbate Preservative Concentrations in Different Sauce Samples in Urmia, Iran



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ABSTRACT

Sodium benzoate and potassium sorbate are relatively common preservatives used in a wide range of foods including flavoring products like sauces. The potential health risks arising from these preservatives along with the high-consumption rate of these flavoring products worldwide highlight the importance of the quality and safety assurance of these products. So, this study aimed to evaluate the concentrations of these two common preservatives (i.e., sodium benzoate and potassium sorbate) in different sauce samples, including mayonnaise, salad dressings, Caesar sauce, Italian dressing, Ranch dressing, French dressing, using high-performance liquid chromatography (HPLC) and to compare them with the acceptable level of Codex standard. For this purpose, 49 samples, including three to five samples of each type of different brands of sauce samples, were randomly collected from supermarkets in Urmia, Iran. Based on the results, the mean concentrations \pm standard deviation of sodium benzoate and potassium sorbate in the collected samples were found to be 249.9 ± 157 and 158.0 ± 131 ppm, respectively, which were lower than the general standard of the Codex Alimentarius and the European legislation. Due to the importance of hazardous side effects of these preservatives for consumers, regular and accurate evaluation of these preservatives in sauces as highly consumed food products is still recommended for consumer safety.

Mayonnaise and salad dressings are semisolid emulsion food products, which are used extensively as food flavoring worldwide (Guzmán-Gerónimo et al., 2022; Raghunath et al., 2021). Mayonnaise is generally comprised of different ingredients including egg (yolk or whole egg), vegetable oil, milk or skimmed milk powder, sugar, salt, vinegar, spices, and food additives (Raghunath et al., 2021). Food additives are allowed only if they are considered safe and harmless for consumer health (Sen, 2021). One of the highly consumed food additives is food preservatives. Preservatives are used to increase the shelf life of foods by preventing microorganism growth and to control food spoilage (Kefi et al., 2022; Zhang et al., 2021). Sorbates and benzoates are the most common preservatives that are used in a wide range of foods including mayonnaise and salad dressings (Amirpour et al., 2015; Chaleshtori et al., 2018). Despite their protective properties on food stability, different evidence has shown the adverse effects

of sodium sorbate (including genotoxicity, DNA damage, and increased micronucleus) in almost all hepatocyte samples from pregnant rats (Saatci et al., 2016). Moreover, an in vitro study reported the genotoxic effects of high concentrations of sodium sorbate on human peripheral blood lymphocytes (Mamur et al., 2012). Furthermore, another survey revealed that a nonimmunological contact reaction (pseudo-allergy) could occur in sensitive patients when exposed to doses lower than the tolerance limit (5 mg/kg body weight per day) of benzoate (Chaleshtori et al., 2018; Salehi et al., 2017; Magomya et al., 2020). Hence, it is recommended to limit the consumption of foods containing sorbates and benzoates in most vulnerable groups such as pregnant women, immunocompromised individuals, and children (Saatci et al., 2016; Zhang et al., 2021). Due to their usage in a wide variety of foods, their acceptable levels in each food have been defined by different international laws, which may vary in different countries

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according to their national guidelines (Amirpour et al., 2015; Azuma et al., 2020; Esfandiari et al., 2017; Kefi et al., 2022). The Codex Alimentarius and the European legislation on food additives have set a maximum level of 1,000 ppm for benzoate and sorbate in different sauces and like products, so higher amount of these preservatives is not only against the rules but also dangerous to human health (Alimentarius, 2015; Laganà et al., 2017). Consequently, due to the potential side effects of these preservatives, it is better to control their amount in highly consumed food products to prevent an increase in their intake from the usual diet in the community (Zhang et al., 2021).

To the best of our knowledge, the number of these studies at the national level is limited. There is particularly no study investigating the amount of these preservatives in sauces and like products in Iran. Given that sauces are among the highly perishable food categories due to their ingredients, preservatives are commonly used in these products to ensure consumer safety (Mumtaz et al., 2018). Therefore, the present study was designed to evaluate the concentration of potassium sorbate and sodium benzoate in various brands of sauce samples (including mayonnaise, salad dressings, Caesar sauce, Italian dressing, Ranch dressing, French dressing) collected from supermarkets in Urmia, Iran, using the high-performance liquid chromatography (HPLC) method, as well as comparing them with the standard level set by Codex Alimentarius and the European legislation.

Materials and Methods

Reagents, solutions, and instrument

Sodium benzoate, potassium sorbate, ammonium acetate, and glacial acetic acid were purchased from Merck (Darmstadt, Germany). Acetonitrile, HPLC-grade water, and other solvents were prepared from Samchun Chemical Co. . Moreover, syringe filter and filter paper were purchased from Whatman (United States). An analytical precision scale (Sartorius), a vortex shaker (IKA), a Metrohm 781 pH meter (Herisau Switzerland), and a Persia Ind group centrifuge (Tehran, Iran) were used. Chromatographic analysis was performed on an Agilent HPLC system 1200 series equipped with a diode array detector (DAD). All chemical materials were of the highest purity.

Sodium benzoate and potassium sorbate were extracted from 5 g sauce samples under acidic conditions using a mixture of ammonium acetate and acetonitrile buffer (as reported below) (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018). The concentration of preservatives was determined by HPLC using a reverse phase column and a suitable detector (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Solutions

Ammonium acetate Buffer

The amount of 0.3 g of ammonium acetate (98%) was carefully weighed and dissolved in 900 mL of deionized water. After adjusting the pH to 2.4 by adding 0.5 mL of glacial acetic acid (100%), the volume of the solution was increased to 1,000 mL by deionized water (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Liquid phase chromatography (Mobile phase)

A volumetric mixture (vv^{-1}) of ammonium acetate and acetonitrile was prepared in a ratio of 80:20 vv^{-1} (800 mL of ammonium acetate buffer and 200 mL of acetonitrile) (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Standard storage solutions (500 mg L^{-1})

The amount of 50 mg of sodium benzoate or potassium sorbate was carefully weighed and transferred to a 100-mL volumetric flask, and the solution was prepared by adding HPLC-grade deionized water.

To prepare 0.5, 1.0, 2.5, 5, 10, 20, 50, and 100 mg L^{-1} standard concentrations, 0.1, 0.2, 0.5, 1, 2, 4, 10, and 20 mL of the storage solution was diluted, respectively, by the mobile phase in a 100-mL volumetric flask (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Chromatographic analysis

Chromatographic analysis was performed on an Agilent HPLC system 1200 series equipped with a diode array detector (DAD). Separations were conducted using an Eclipse-XDB-C18 analytical column (250 mm by 4.6 mm, 5 μm). The mobile phase was a volumetric mixture (vv^{-1}) of ammonium acetate and acetonitrile which was prepared in a ratio of 80:20 vv^{-1} and flowed at the rate of 1.0 mL min^{-1} as an isocratic elution. The injection volume was 20 μL using an Agilent 1200 HPLC G1329A Autosampler. The working wavelengths were 225 nm for sodium benzoate and 255 nm for potassium sorbate (Fig. 1).

Sample collection and preparation

A total number of 49 samples including three to five samples of each type of different brands of sauces, including mayonnaise, salad dressings, Caesar sauce, Italian dressing, Ranch dressing, French dressing, etc. (Table 2) were randomly collected from local supermarkets in Urmia, Iran. The concentration of sodium benzoate and potassium sorbate was evaluated in collected samples using high-performance liquid chromatography (HPLC) and compared with the general standard of Codex Alimentarius and the European legislation. The analysis was performed in triplicate for each sample. For this purpose, the contents of the sauces were mixed and homogenized using an 800 W hand blender for one min. Then, an amount of 5 g of the homogenized sample was poured into a 50 mL volumetric balloon, and it reached the volume by adding the mobile phase. The resulting solution was then centrifuged at 4,000 $\times g$ for 10 min. The solution was filtered in the first step with filter paper, and then, the clear solution was passed through a 0.45- μm syringe filter. Finally, a clear solution was used for injection into the HPLC column (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Calibration curve

An amount of 20 μL of different concentrations of standards prepared under the test conditions was injected into the HPLC column. By integrating peaks, a calibration curve was obtained for the standard concentrations of sodium benzoate and potassium sorbate (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Determination of sodium benzoate and potassium sorbate concentrations

An amount of 20 μL of the prepared sample was injected into the column under the described test conditions, and the results were compared with the peaks of the calibration curve. Concentration was calculated by obtaining the area below the peak from the graph and equation. The concentration of preservatives in the prepared sample was calculated by considering the amount and volume prepared from the sample (Amirpour et al., 2015; Esfandiari et al., 2017; ISIRI, 2018).

Statistical analysis

The data obtained from the study were analyzed by descriptive tests in Excel software version 2013. Measurements of the concentration of preservatives in each sample were repeated three times, and their mean was reported as the final concentration.

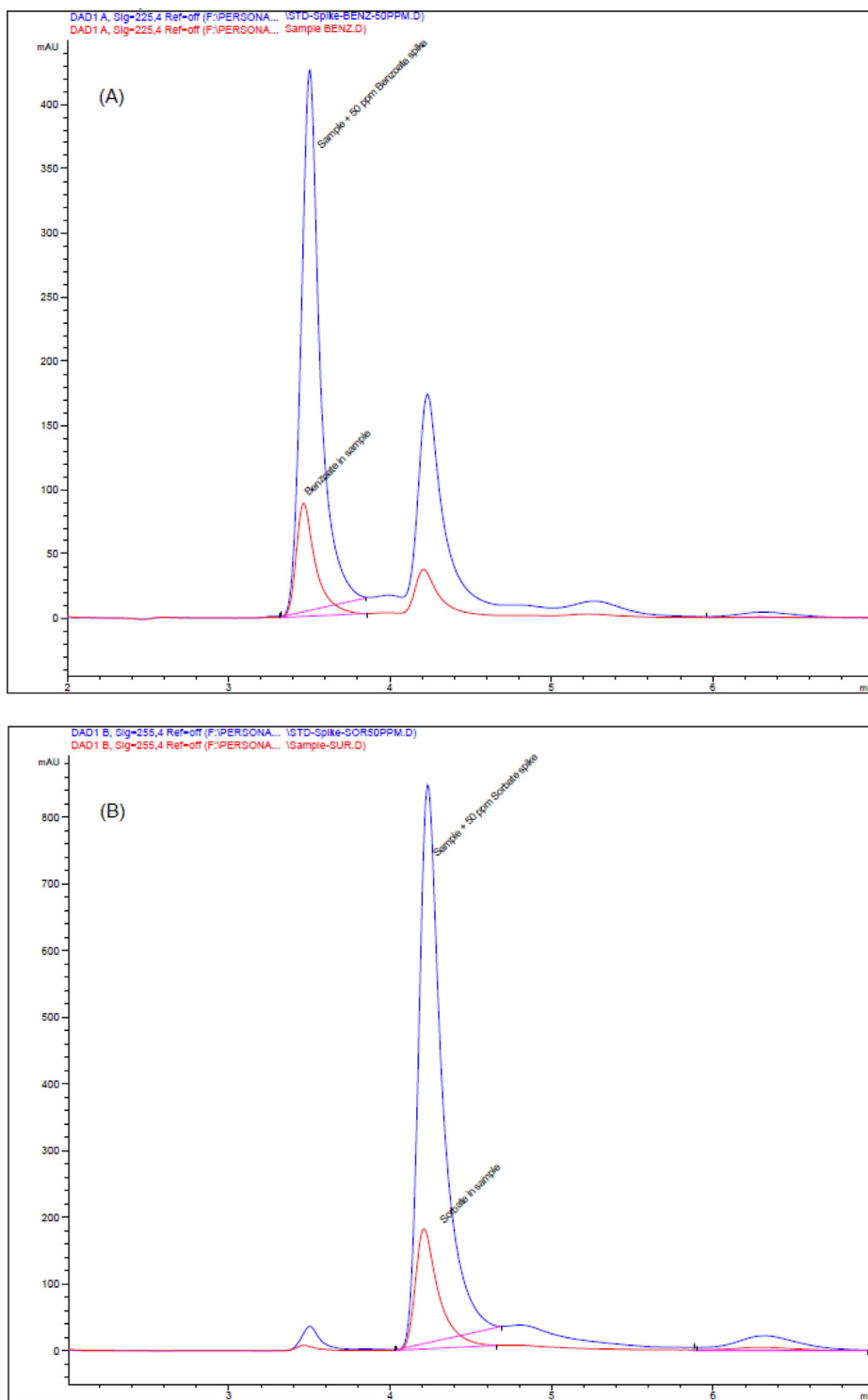


Figure 1. The chromatograms of HPLC-DAD of benzoate (A), sorbate (B); real samples (red chromatograms) and spiked samples with 50 mgkg⁻¹ of benzoate and sorbate (blue chromatograms)

Results

Method validation procedure

The linearity was observed over the range of 1.0–100.0 mg L⁻¹ with a coefficient of determination (r^2) of 0.9998 for benzoate and 0.5–100.0 mg L⁻¹ with a coefficient of determination (r^2) of 0.9998 for sorbate. Limit of detection (mg L⁻¹) and limit of quantification

(mg L⁻¹) were 0.05 and 0.5 for benzoate and 0.005 and 0.01 for sorbate, practically based on signal-to-noise (S/N) ratio of >3 and >10 for LOD and LOQ, respectively. S/N values for LOD of benzoate and sorbate were 3.66 mg L⁻¹ (Height of signal = 0.26 and Height of noise 0.07) and 4.23 mg L⁻¹ (Height of signal = 0.11 and Height of noise 0.02), respectively. Moreover, S/N values for LOQ of benzoate and sorbate were 12.40 mg L⁻¹ (Height of signal = 3.1 and Height of noise 0.25) and 10.71 mg L⁻¹ (Height of signal = 0.15 and Height

of noise 0.01), respectively. At 5 and 50 mg kg⁻¹ levels, the intraday and interday precisions were investigated by five replicate extractions. The interday calculations were performed on three continuous days. RSDs% obtained were in the satisfactory range of 0.05%–0.29%. Both sodium benzoate and potassium sorbate were detected at 255 nm wavelength.

Concentrations of preservatives in sauces

In the present study, the concentration of sodium benzoate and potassium sorbate in 49 sauce samples collected from supermarkets in Urmia, Iran was evaluated using HPLC, and the results are presented in Table 1. According to general standards of the Codex Alimentarius and the European legislation, the maximum allowable concentration of sodium benzoate and potassium sorbate (alone or in combination) in sauces is set as 1,000 ppm (Alimentarius, 2015; Laganà et al., 2017). The findings showed that all the sauce samples studied in this survey were safe in terms of benzoate and sorbate content, and their concentrations did not exceed the acceptable level of Codex standard. The samples' mean concentrations of potassium sorbate and sodium benzoates were 158.0 ppm and 249.9 ppm, respectively. In more detail, none of the samples contained higher amounts of sodium benzoate and potassium sorbate than the Codex standard level. Moreover, the number of 2 (4.08%) and 4 (8.16%) sauce samples out of 49 samples did not contain detectable levels of sodium benzoate and potassium sorbate, respectively (Table 2).

Discussions

Preservatives play an important role in the food industry as they prolong the shelf life of food, so special attention has recently been paid to these antimicrobial agents in terms of their quality safety and quality assurance in different foods. Sodium benzoate and potassium sorbate are among the widely used preservatives in the food industry, reducing the microbial load and increasing the shelf life of food products (Amirpour et al., 2015; Chaleshtori et al., 2018). Despite their low toxicity and carcinogenic effects in low doses, their excessive use in the food industry may lead to potential health risks to humans (Chaleshtori et al., 2018; Pereira et al., 2021; Piper & Piper, 2017). On the other hand, complications such as asthma, seizures, and urticaria have been observed at low doses of benzoate in sensitive persons (Gholipour et al., 2014). It should be noted that sorbate is metabolized and decomposed in the body like fatty acids and is less toxic than benzoate; however, in a few cases, it can lead to idiosyncratic intolerance in the consumers (Hong et al., 2009; Saad et al., 2005). Considering their potential hazardous health risks for humans like hyperactivity in children, suppression of the immune system, pseudo-allergy, as well as chromosomal aberrations, genotoxic and mutagenic effects, strict monitoring of their concentrations to be within the permissible limits is needed (Beezhold et al., 2014; Chaleshtori et al., 2018; Zengin et al., 2011).

It is obvious that unconscious and excessive use of preservatives can cause various side effects as described above. Therefore, the present study was designed to test if the amount of these two most common preservatives (i.e., benzoate and sorbate) in one of the most favorable food items named sauces in Iran were above the standard

Table 1
The concentration (ppm) of sodium benzoate and potassium sorbate in the sauce samples

Preservatives	Mean ± SD*	Min	Max	Standard limit**
Sodium benzoate	249.9 ± 157	<LOD	673.0	≤1,000
Potassium sorbate	158.0 ± 131	<LOD	460.3	≤1,000

* SD: Standard Deviation.

** Codex standard level (Alimentarius, 2015).

Table 2
The concentration of sodium benzoate (SB) and potassium sorbate (PS) in the samples

No.	Samples	Mean ± SD of SB (ppm)	Mean ± SD of PS (ppm)
1	Aloe vera sauce	267.9 ± 12.7	300.8 ± 100.9
2	Mayonnaise with reduced fat	75 ± 123.6	55.1 ± 72.7
3	Low-fat mayonnaise	54.5 ± 138.1	90.6 ± 47.6
4	Low-fat mayonnaise	257.3 ± 5.2	97.7 ± 42.6
5	French mayonnaise	415.94 ± 117.4	162.1 ± 2.9
6	High-fat mayonnaise	<LOQ	<LOQ
7	Cucumber and garlic salad dressing	145.7 ± 73.6	301.4 ± 101.4
8	mayonnaise sauce	516.5 ± 188.5	90.6 ± 47.6
9	Low-fat mayonnaise	<LOQ	<LOQ
10	Low-fat salad mayonnaise	341.7 ± 64.9	98.4 ± 42.1
11	Mayonnaise with reduced fat	118.8 ± 92.7	383.6 ± 159.5
12	Lemon salad sauce	91.9 ± 111.7	284.4 ± 89.4
13	Italian sauce	276 ± 18.4	137.7 ± 14.3
14	Aloe vera sauce	267.9 ± 12.7	300.8 ± 100.9
15	Caesar sauce	362.8 ± 79.8	371.2 ± 150.7
16	Balsamic sauce	292.8 ± 30.3	294.4 ± 96.4
17	Italian sauce	363.7 ± 80.4	190.1 ± 22.7
18	Ranch sauce	450.2 ± 141.6	202.9 ± 31.7
19	Low-fat mayonnaise	346 ± 67.9	355 ± 139.3
20	Thousand Island Salad Sauce	207 ± 30.3	14 ± 101.8
21	Low-fat lettuce salad dressing	209.2 ± 28.7	14 ± 101.8
22	French salad dressing	450 ± 141.4	48 ± 77.8
23	Multi-layer wrapped low-fat mayonnaise	120 ± 91.8	15 ± 101.1
24	Mayonnaise with reduced fat	673 ± 299.1	<LOQ
25	Mayonnaise with reduced fat	10.3 ± 169.4	0.005 ± 111.7
26	Mayonnaise with reduced fat	495.35 ± 173.5	0.005 ± 111.7
27	Mayonnaise	239.2 ± 7.5	93.6 ± 45.5
28	Low-fat mayonnaise	307.1 ± 40.4	111.8 ± 32.6
29	Mayonnaise with reduced fat	283 ± 23.4	112.2 ± 32.4
30	Low-fat mayonnaise	98 ± 107.4	348 ± 134.3
31	Salad mayonnaise	252 ± 1.4	72 ± 60.8
32	Thousand Island Salad Sauce	189.9±42.4	345. ± 2132.3
33	Mayonnaise with reduced fat	232.5 ± 12.3	349.5 ± 135.4
34	French salad dressing	227.08 ± 16.1	204.95 ± 33.2
35	Mayonnaise with reduced fat	256.7 ± 4.8	265.1 ± 75.7
36	Mayonnaise salad dressing	171.3 ± 55.5	227.6 ± 49.2
37	French Mayonnaise	375 ± 88.4	151.8 ± 4.3
38	Fat-free mayonnaise sauce	177.1 ± 51.4	62.12 ± 67.7
39	Low-fat mayonnaise	156.4 ± 66.1	56.6 ± 71.7
40	Low-fat mayonnaise	97.2 ± 107.9	460.3 ± 213.7
41	French salad mayonnaise	612.6 ± 256.4	<LOQ
42	French salad dressing	477 ± 160.5	95 ± 44.5
43	Sauce of a thousand islands	205.5 ± 31.3	314.2 ± 110.4
44	Low-fat mayonnaise	199.3 ± 35.7	249.3 ± 64.5
45	mayonnaise with reduced fat	155.3 ± 66.8	216 ± 41.0
46	Fat-free mayonnaise sauce	426.12 ± 124.6	98.87 ± 41.8
47	Low-fat mayonnaise	36.84 ± 150.6	46.84 ± 78.6
48	Mayonnaise with reduced fat	208.6 ± 29.2	47.9 ± 77.8
49	Low-fat mayonnaise	54.8 ± 137.9	8.5 ± 105.7

* SD: Standard Deviation.

level to prevent potential associated health issues as discussed in the manuscript (Yazdanfar et al., 2023). Our findings indicated that the concentration of sodium benzoate and potassium sorbate in mayonnaise sauce and salad dressings complied well with the standard limit. Similarly, the concentration of sodium benzoate and potassium sorbate

in several Iranian food products including mayonnaise, cake, olive salad, and toast was in the acceptable standard range, except for the concentration of potassium sorbate in one sample of cakes (Chaleshtori et al., 2018). In addition, the level of sodium benzoate and potassium sorbate in mayonnaise in another research study in Iran was reported to be below the acceptable level, which is in a good agreement with the result of the present study (Faraji & Rahbarzare, 2016; Heshmati et al., 2018). However, they detected higher levels of sodium benzoate and lower levels of potassium sorbate compared to the present study. Furthermore, a study performed in Belgium revealed that the average concentration of benzoic acid in the different sauce samples like emulsified sauces, light sauces, light mayonnaise, dressings, and nonemulsified sauces was recorded as 819.0, 844.6, 498.6, 768.6, and 594.3 ppm, respectively, which was less than the recommended limit (Vandevijvere et al., 2009). In another study, the concentrations of benzoic and sorbic acids were evaluated in different brands of sauces available in Istanbul market. The amounts of benzoic and sorbic acids were reported as 0.00–0.00 and 0.00–1279.89 ppm in mayonnaise, 0.00–838.09 and 0.00–227.27 ppm in ketchup, 0.00–22.28 and 0.00 ppm in industrial tomato paste, 0.00–2417.65 and 0.00–1820.31 ppm in traditional tomato paste, 0.00 and 0.00 ppm industrial pepper paste, 0.00–4613.84 and 0.00–1071.92 ppm in traditional pepper paste, 0.00–176.47 and 0.00–169.36 ppm carbonated drinks, respectively. The results showed a high level of sodium benzoate in mayonnaise samples compared to the present study. On the other hand, the level of benzoic and sorbic acids in traditional samples (i.e., samples produced by small and local producers) was significantly higher compared to industrial samples (i.e., samples produced by larger companies) (Karataşlı et al., 2016). It showed the uninformed use of these compounds in traditional products due to the impossibility of monitoring the traditional products in the sales market. In line with the hypothesis, an investigation showed that the level of sodium benzoate in bulk pickled cucumber samples was much higher than the standard range compared to an industrial pickled cucumber (Delavar et al., 2012). Likewise, the amount of benzoate and sorbate in bulky tomato paste samples was reported to be in a range of 242.0–1896 ppm and 0 ppm, respectively. However, the amount of benzoate was more than the Iranian acceptable level (750ppm) in some cases (Ghajarbeygi et al., 2022). It can be concluded that traditional products are more likely to have higher amounts of preservatives than their industrial counterparts, due to lesser monitoring. Moreover, the levels of sodium benzoate and potassium sorbate in different brands of fruit juice and soft drinks were reported in another study from Nigeria (Magomya et al., 2020). This study showed that all the analyzed samples contained sodium benzoate at concentrations ranging from 25.80 to 245.10 mg L⁻¹ (Magomya et al., 2020). Additionally, 35% of samples had higher amounts of sodium benzoate than the permitted limits. On the other hand, potassium sorbate was observed in 75% of samples in a concentration of 1.36–158 mg L⁻¹, which was within the permissible range (Magomya et al., 2020). In another study conducted in Iran, all the samples of Doogh contained sodium benzoate which could be due to the presence of sodium benzoate in yogurt and salt used in Doogh's formula, but potassium sorbate was not detected in any of the Doogh samples (Esfandiari et al., 2013). On the other hand, in the study of a recent researcher, 205 samples out of a total of 983 Turkish retail food-stuff samples were found to contain benzoate and sorbate (Ulca et al., 2013). It was stated that the concentration of these two preservatives was above the acceptable range only in 11% of positive samples (Ulca et al., 2013). In addition, a recent study conducted in Hamedan, Iran showed that sodium benzoate and potassium sorbate were not detected in Doogh and yogurt samples. The LOD for benzoate and sorbate were 0.13 and 0.21 mg kg⁻¹ in the samples, respectively (Salehi et al., 2017). On the other hand, the mean concentrations of sodium benzoate and potassium sorbate in cheese samples were reported as

366.73 and 499.56 ppm, respectively. It should be noted that the addition of any amount of potassium benzoate and sorbate to dairy products is not allowed according to the ISIRI (Institute of Standards and Industrial Research of Iran) (Salehi et al., 2017). A recent study also analyzed 400 food samples including canned tomato paste, cherry jam, pickled cucumbers, soft drinks, fruit juice, and some dairy products (i.e., yogurt, Doogh, lactic cheese, Liqvan cheese, and UF-feta cheese) in terms of benzoate and sorbate content. According to their results, 67.5% of all samples contained benzoate, and its level varied from 11.9 ppm in Doogh to 288.5 ppm in lactic cheese. In addition, benzoate was observed in all the investigated dairy products in a range from 11.9 ppm in lactic cheese to 91.2 ppm in feta cheese samples. Sorbate was detected in 24.5% of the samples ranging from 1.20 ppm in Doogh to 3.284 ppm in fruit juice. In addition, none of the pickled cucumber samples contained these preservatives (Amirpour et al., 2015). In addition, in several other studies, high levels of benzoate and sorbate have been reported in Doogh samples examined in Iran (Bahremand & Eskandari, 2013; Eskandari & Bahremand, 2013; Gholipour et al., 2014; Rahimirad, 2014). As mentioned above, according to the Iranian standard, adding benzoate to milk and dairy products is prohibited. However, due to the hippuric acid present in milk, some benzoate is always observed naturally in dairy products. Indeed, it is considered an integral part of the natural composition of milk and dairy products. Therefore, the standard limit of benzoate in milk should be defined to detect intentionally added benzoate at higher levels (Amirpour et al., 2015).

Conclusion

Despite the dangerous side effects of the excessive use of preservatives, the safe use of preservatives can control the microbial spoilage of food and increase its shelf life. Therefore, monitoring the use of preservatives in the food industry and controlling their exact amount in high-consumption foods such as mayonnaise sauces and salad dressings, as highly consumed flavor-enhancer products, is essential for both human health and economics. In the present study, the amounts of two most common preservatives namely benzoate and sorbate were evaluated in different sauce samples in Uremia, Iran. Based on the study results, the mean concentrations of sodium benzoate and potassium sorbate in the 49 collected samples were 249.9 and 158.0 ppm, respectively, which were lower than the general standards of the Codex Alimentarius and the European legislation (1,000 ppm). Although sodium benzoate and potassium sorbate were in the safe ranges in our study and they would not raise any safety risks for human health, regular and accurate evaluation of these preservatives in the aforementioned products is still recommended to provide consumer safety.

Data Availability

The dataset used is available from the corresponding author (saramohamadi12@yahoo.com), upon judicious request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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