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Assessment of Sodium Benzoate and Potassium Sorbate Preservatives and Artificial Color in Bulk Tomato Paste Samples in Qazvin, Iran

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KEYWORDS

Sodium benzoate;
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Bulk tomato paste

ABSTRACT: Tomato is one of the popular crops in the world and tomato paste is a product of it. Due to its wide application, quality control of this product is important. The goal of our study was to the assessment of potassium sorbate and sodium benzoate preservatives and artificial color in bulk tomato paste samples in Qazvin, Iran. In this study, 45 samples were analyzed by high-performance liquid chromatography (HPLC) to determine the preservatives. Benzoic acid was detected in 17 (37.78%) samples, ranging from nd not detected to 1896 mg kg^{-1} and Potassium sorbate was not detected in any of the samples. The detection of color in samples was done by the thin-layer chromatography method (TLC). Three samples (6.67%) had Pansio 4R artificial color. The mean and standard deviation of sodium benzoate in samples were 990.62 mg kg^{-1} and 396.07 mg kg^{-1}, respectively. According to the national standard of Iran for canned tomato paste, adding any kind of preservative and color to tomato paste is prohibited. The results show the importance monitoring of sodium benzoate and artificial colors in bulk tomato paste by health authorities.

INTRODUCTION

Tomato is one of the most important and nutritious crops. It has desirable organoleptic properties and lycopene is its most abundant antioxidant. A variety of tomato products such as sauces, and canned food are produced, but the most common of them is tomato paste. Local and international laws and standards are legislated to protect consumer health. Adding extra water or cheaper bulk materials, artificial colors, and preservatives to tomato paste is banned [1].

Food additives consist of one substance or a mixture of substances that are not the main component of food. They are added to food for production, processing, storage, and packaging. Additives include preservatives that are intentionally added to the food production process to prevent microbial spoilage and undesirable changes and increase the shelf life of food. Preservatives and antimicrobials play an important role in providing safe and sustainable foods. Some preservatives, such as sulfides, nitrates, and salt have been used for centuries in meat and wine processing. The choice of an antimicrobial agent depends on a variety of factors, such as the antimicrobial spectrum of the preservative, the physical and chemical properties of the food and preservative, and storage conditions [2, 3].

Among the preservatives used in food are benzoate and sorbate. Benzoate at low pH and acidification of the intracellular environment are the most important factors inhibiting the growth and proliferation of yeast and mold.

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Yeast and mold cells need ATP to grow and multiply. Acidification of the intracellular environment inactivates the enzyme phosphofructokinase, which is involved in the ATP production cycle, and ultimately reduces or stops the growth and proliferation of yeast and mold [4, 5].

Sorbate inhibits the enzyme dehydrogenase in fatty acid oxidation. Sulfhydryl enzymes also are the site of the action of sorbate on microorganisms. Sorbate prevents oxidative phosphorylation by inhibiting sulfhydryl-containing enzymes. Increasing hydrogen peroxide in the cell is another method of sorbate activity to inhibition of catalase in molds [6].

Although benzoate is a permitted preservative, it can cause adverse effects such as seizures, urticaria, asthma, and metabolic acidosis, even at low doses. Other adverse effects of benzoate have been reported in skin effects such as urticaria, pimples, and dermatitis. Sorbate can be broken down in the human body like the way fatty acids do. They metabolize water and carbon dioxide. Therefore, its toxic effects are much less than benzoate [7-9].

Other disadvantages of benzoic acid and sodium benzoate are mutations, genetic abnormalities in human blood lymphocytes, and hyperactivity in children [10-14]. These two preservatives are allowed in some foods, but published reports indicate that they are used in the forbidden products or excess in the permitted foods [15].

In 2002, Tfouni and Toledo measured benzoic acid and sorbic acid in various brands of soft drinks, juice, margarine, yogurt, and cheese on the Brazilian market by high-performance liquid chromatography, which under Brazilian law only one sample was above the authorized level [16]. Nour and colleagues measured sorbate and benzoate in tomato paste samples in Romania. This study showed that the amount of benzoate ranged from 348.06 to 472.56 mg kg^{-1}. Also, the amount of sorbate varied from 291.72 to 420.32 mg kg^{-1} [9]. The amount of benzoic acid measured in cheese, yogurt, and buttermilk samples by the HPLC method in Turkey was 3.17 to 56.77, 8.94 to 28.30, and 1.54 to 16.57 mg kg^{-1}, respectively [7]. The amount of benzoic acid measured in cheese, yogurt, and buttermilk samples by the HPLC method in Turkey were 3.17 to 56.77, 8.94 to 28.30, and 1.54 to 16.57 mg kg^{-1}, respectively [17].

For the measurement of potassium sorbate and sodium benzoate, several analysis methods have been reported, which can be referred to as high-performance liquid chromatography, gas chromatography, and spectrophotometry. These methods can be used to measure potassium sorbate and sodium benzoate. Chromatography is a fast and reliable method that can measure these two preservatives simultaneously [18-20].

Several studies in Iran have measured the amount of sodium benzoate and potassium sorbate in various food products (Packaged and industrial products) such as buttermilk and soft drinks, etc. In some cases level of their preservative has exceeded the allowed level [21-24].

Colors have been added to food for many years and they are divided into artificial and natural. In the 19th century, a wide range of synthetic colors were used, but the toxicity of these dyes led to the development of legislation regarding the type and amount of them in food products. Food and Agricultural Organization (FAO), World Health Organization (WHO), and local legislation and standards have restricted the use of artificial colors in food products [25].

Tomato paste is used as a seasoning to improve the taste and color of food. Due to its high consumption by Iranian families, quality control of this product is of particular importance. One of the important items that should be considered is the use of preservatives in it. According to Iranian National Standard No. 761 related to canned tomato paste, the use of any kind of preservative and color in this product is prohibited [25].

Industrial products are constantly monitored by health experts; however, bulk tomato pastes are stored in large amounts in sales centers without any signs of microbial contamination, so preservatives might be used in these products. Since there is no report about the use of preservatives and artificial colors in bulk tomato paste, in this study, we decided to investigate the number of preservatives and type of artificial colors in these samples by the high-performance liquid chromatography and TLC method in Qazvin.

MATERIALS AND METHODS

Sampling

45 samples of bulk tomato paste were purchased from Qazvin's stores and sales centers and were stored in the refrigerator until analysis. Samples were collected and analyzed in 2020.

Materials

Sodium benzoate, potassium sorbate, ammonium acetate, methanol, and acetonitrile HPLC grade were purchased from Merck (Germany). Deionized water was prepared directly from a Millipore (Direct-Q 3 UV System). Samples of synthetic food colorants (Tartrazine (E102), Quinoline yellow (E104), Sunset yellow (E110), Ponceau 4R (E124), Azorubine (E122), Allura red (E129), Brilliant Blue (E133), Indigotine (E132),), n-Butanol, Acetic acid, Ammonia were purchased from Merck. Plate-silica gel (20 × 20 cm; Merck,) for thin-layer chromatography (TLC), Chromatography tank, Glass capillary tubes, Bain-Marie bath, and white wool used for detection of color.

Mobile Phase Preparation

The mobile phase consisted of 20% acetonitrile and 80% ammonium acetate buffer (pH=4.2). After the preparation of the mobile phase, it was degassed, filtered, and used as the HPLC mobile phase.

Standard preparation

Exactly 100.0 mg of each potassium sorbate and sodium benzoate in a 100.0 ml volumetric flask were diluted to the mark line with HPLC-grade water. The mixed standard solutions were prepared by diluting the stock solution with methanol: water (65:35) to yield 2.5, 5, 10, 20, 30 and 40 mg L^{-1} of potassium sorbate and sodium benzoate solutions.

Sample preparation

About, 10 g of the sample was accurately weighed and 20 ml of methanol: water (65:35) solution was added to it. The resulting solution is stirred for 60 minutes. Then the sample solution transfererred to a 50 ml volumetric flask and diluted to the mark line with methanol: water (65:35). Then 9 ml of solvent (methanol: water) was added to 1 ml of solution. First, all solutions and samples were filtered by a 0.45 µm nylon membrane filter, and then they were injected into the HPLC Column [26].

HPLC apparatus

The analysis was carried out by waters 1515 Series HPLC system equipped with a 2489 Diode Array Detector. The chromatographic separation was performed with a WAT036975 PAK C18 column (3.9×150mm) at room temperature (30°C). The peaks of potassium sorbate and Sodium benzoate were measured at a wavelength of 220 nm. The analysis was performed using a flow rate of 1 ml min^{-1}. The injection volume was 200µL. By plotting the average area of the peaks versus the concentration of the standards, the line equation and the value of the coefficient of determination were estimated [26].

Extraction and detection of color

Detection of artificial colors in the samples was performed by thin-layer chromatography. First, under acidic conditions, the color is separated from the sample by natural wool and absorbed. Then the color is separated from the wool under alkaline conditions. The resulting solution (after condensation) and synthetic color standards are spotted on chromatographic paper. Finally, the Rf (Rf is equal to the distance traveled by the substance divided by the total distance traveled by the mobile phase) obtained from the sample is compared with the standard Rf and finally, the type of artificial color is identified [27].

RESULTS AND DISCUSSION

In the present work, we determined the concentrations of potassium sorbate and sodium benzoate preservatives in bulk tomato paste samples in Qazvin. An isocratic HPLC technique is described for the determination of potassium sorbate and sodium benzoate in samples. Peaks were identified by comparing retention times and quantification of them was determined based on the calibration curve. The HPLC chromatogram is shown in Figure 1. The retention time of sodium benzoate and potassium sorbates were 3.06 and 3.96 min, respectively. The limits of detection (LOD) and limits of quantification (LOQ) were calculated with the relationship (3.3 * S / m) and (10 * S / m), respectively, where S is the standard deviation and m is the slope of the calibration curve. The LOD for benzoate and sorbate were 0.79 and 0.73 mg kg^{-1}, respectively. The LOQ for benzoate and sorbate were 2.37 and 2.2 mg kg^{-1}, respectively. The method with brief modifications was according to the method of Pylypiw et al [26].

Of 45 samples analyzed, 17(37.78 %) samples contained sodium benzoate. The levels of benzoic acid ranged from not detected to 1896 ppm. Potassium sorbate was not detected in any of the samples (Table 1).

Another analysis performed for samples was the detection of artificial color and its type. Of the 45 samples, 3 samples (6.67%) had Pansio 4R artificial color, Table 2. Of course, these samples also contained benzoate. Table 3 and Figure 2 show the samples that have benzoate and color. 6.67% of the samples had artificial color and benzoate. The only artificial color observed in samples was the Pansio 4R.

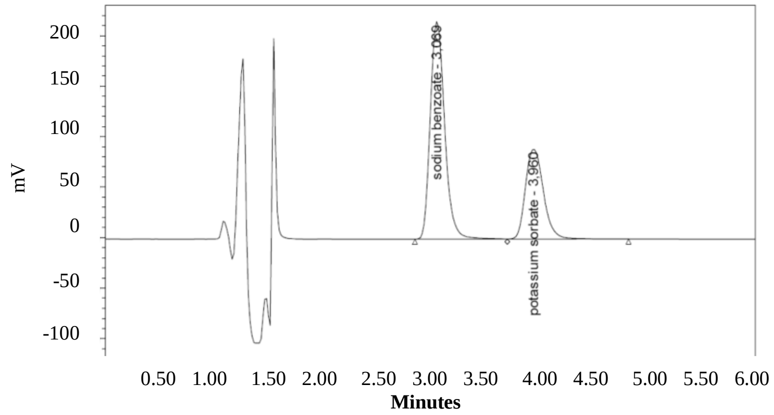

Figure 1. HPLC chromatogram of solution of sodium benzoate and potassium sorbate.

Table 1. Sodium benzoate and potassium sorbate levels in bulk tomato paste samples

Preservatives	n	Mean (mg L^{-1})	Range (mg L^{-1})	% Samples with preservative
Sodium Benzoate	45	990.62	nd - 1896	37.8
Potassium Sorbate	45	0	Nd(not detected)	0

n: number of analyzed samples; The mean value is calculated assuming that nd is zero.

Table 2. Percentage of samples with artificial color and without artificial color

n	Artificial colors Number (%)	Without artificial colors Number (%)
45	3 (6.66%)	42 (93.33%)

n: number of analyzed samples

Table 3. The concentration of benzoate and type of artificial color in positive samples.

Number	C Sodium benzoate (mg l^{-1}) [1]	Type of artificial color	Number	C Sodium benzoate (mg l^{-1}) [1]	Type of artificial color
1	1214.09	-	**10**	1161.991	-
2	992.49	ponceau 4R	**11**	1081.48	-
3	380.6	-	**12**	993.55	-
4	1246.97	-	**13**	973.5397	-
5	1303.38	ponceau 4R	**14**	1094.37	-
6	305.12	-	**15**	860.32	-
7	1072.2	ponceau 4R	**16**	986.7387	-
8	242.02	-	**17**	1896	-
9	1035.77	-			

1. C Sodium benzoate (mg l^{-1}): Concentration of Sodium benzoate (mg l^{-1}: ppm)

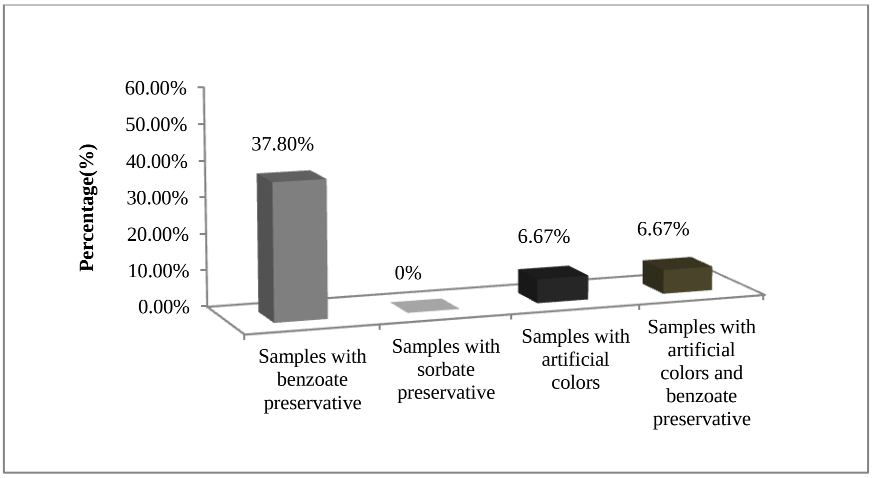

Figure 2. Percentage of samples contaminated with artificial color and benzoate and sorbate preservatives

The estimated amount of benzoate in this study was % 37.8 with a 95% confidence interval (23.76, 53.45). Also, the point estimate of the amount of sorbate was equal to zero on average with a confidence interval (0, 7.87). This study showed that in samples with preservatives, the average of benzoate is 990.62 with a standard deviation of 396.07 units and its median is 1035.77 with an interquartile range (973.53, 1161.99).The only synthetic dye observed was ponceau 4R in 6.67% of the samples.

Due to the harmful effects of preservatives and artificial colors on human health and according to the national standard of Iran, the use of colors and preservatives in tomato paste is prohibited. For example, the presence of sodium benzoate in the diet led to hyperactivity in children [13] and some allergic reactions such as urticaria [28].

The presence of these preservatives in ketchup samples available on the Romanian market was determined. The results showed that the concentration of benzoate ranged from 348.06 to 472.56 mg kg^{-1} and sorbate varied from 291.72 to 420.32 mg kg^{-1} [9]. Also, Javanmardi et al. reported the highest amount of benzoate in tomato paste samples of 27.7 mg kg^{-1}, which indicated the low amount of this preservative in the samples [24]. In the study of Faraji et al., the average amount of sodium benzoate in 4 samples of tomato paste in Karaj was reported to be 2.18 mg kg^{-1} [29]. The average sodium benzoate content in tomato sauce samples in Gilan and Tabriz was reported at 120 and 13.02 mg kg^{-1}, respectively.

In our study, the amount of benzoate in the positive samples varied from 242.02 to 1896 mg kg^{-1} (Table 3). Some of these values are even higher than the permitted limits for other packaged products [30]. This indicates that benzoate has been added to the tomato pastes in bulk by unaware persons. They are unaware of the dangers of these preservatives and their permitted limits, while many people trust household products more than industrial ones. While industrial products are supervised by relevant organizations. Also, the results of Delavar et al.'s research in the determination of benzoate levels in bulk and factory pickled cucumber showed that factory products follow national standards, but bulk products are not [31].

CONCLUSIONS

Due to the high consumption of tomato paste by Iranian families, the quality control of this product is important. Therefore, it is necessary to manage the continuous monitoring of sodium benzoate amount in bulk tomato paste samples by health organizations. Also, more monitoring is needed so that the percentage of colored samples does not increase and it decreases to zero percent.

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Conflict of interest

No conflict.

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