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Sodium Benzoate and Potassium Sorbate content in pickled cucumber supplied in Tehran, Iran

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ABSTRACT

Preservative substances have been used for many years. These materials inhibit the growth of probable microorganisms. However, some of these materials are considered generally regarded as safe (GRAS), and the amount above the standard limits can endanger the health of consumers. Therefore, determining the amounts of chemical conservatives is necessary. In this research, the level of sodium benzoate and potassium sorbet was measured in 60 pickled cucumbers by spectrophotometry. The standard curve of sodium benzoate and potassium sorbet was plotted in five concentrations. Extraction was followed by reading the absorption with the spectrophotometer at 228 nm and 252 nm for sodium benzoate and potassium sorbate, respectively. Results revealed that the average content of sodium benzoate and potassium sorbate were 0.44±0.2 mg/kg and 0.31±0.1 mg/kg, respectively which can be more than the permitted doses. Considering the harmful effects, control precautions must be intensified and the authorities should concern about the quantity of these preservatives in pickled cucumber samples.

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1. Introduction

Benzoic acid, sorbic acid and their salts preservatives are useful in inhibiting yeast and mold growth and also are effective against a variety of bacteria during the storage of food products (1). They are active in acidic foods with low pH and ineffective in food that has a neutral pH value.

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Although, benzoic (<0.1%) and sorbic acid (<0.2%) and their salts are generally recognized as safe (GRAS) excessive use of them in food is harmful to consumers and may cause allergic reactions to benzoate in humans and other disorders (2, 3). The acceptable daily intake (ADI) determined by the joint FAO/WHO expert committee on food additives (JECFA) is 25 mg/kg of body mass for sorbic acid and sorbates salts and 0-5 mg/kg for benzoic acid and sodium benzoate (1).

Benzoic acid ($C_7H_6O_2$) with the molecular weight of 122.12 is an inorganic acid widely used in food preservation. It is present in apple, strawberry, and butter naturally. Benzoic acid shows a low solubility in aqueous phase; therefore, the sodium salt form is usually used which is converted to benzoic acid. Passing across cell membranes is raised in salt type (PKa=4.19) due to increase of unionized form (4).

Risk of using sodium benzoate is known in cooked and heated food. If the food contains ascorbic acid, the sodium benzoate reacts with ascorbic acid and changes to benzene. It has been shown that sodium benzoate up regulates Neuroprotective Parkinson Disease Protein DJ-1 in Astrocytes and Neurons (5). In another research on rats, it was discovered this substance can damage red globules and also hurt to renal cells (6). In Iran, these two materials are permitted to use in legal limit in some kinds of unheated foods, but in foods which are pasteurized or heated, it is banned. Reason for this prohibition is production of benzene which it's carcinogenesis was discovered many years ago. According to Directive 95/2/EC dated on 20.02.1995 of the European Union (EU), the benzoic acid belongs to the preservatives allowed conditionally, and the quantity of benzoic acid added to foods is restricted by upper limits (7). This substance can be absorbed by cell, such as bacteria and molds. If pH decreases to 5 and lower, glucose fermentation by phosphofructokinase decreases to 95%, and this leads to end of ATP and cell death.

However, they are known as preservatives, the carcinogenic effect of them should not be denied, where they can damage DNA (8).

Beezhold et al, 2014 showed that students consuming foods which contain these substances, were hyperactive (9). The amount of benzoate sorbate was determined in some of the most consumed food in schools with spectrophotometry in India (10).

The goal of this study was determination of the amount of sodium benzoate and potassium sorbate by spectrophotometry in different brands of canned and bulk cucumber pickle in Tehran city which are heated under the pasteurized process.

2. Materials and methods

2.1. Materials

Sodium Benzoate, Potassium Sorbate, Petroleum Benzene and hydrochloric acid 6 Normal were HPLC grade and purchased from Merck Company.

2.2. Sampling

Sixty different brands of cucumber pickle samples were collected from various supermarkets of Tehran, Iran. Fifteen samples were bulk cargo and the other samples were canned type.

2.3. Preparation of standard curve

2.3.1. Preparation of stock solution: After accurate weighing of 0.2 g and 0.1 g of sodium benzoate and potassium sorbate powders, respectively, they were adjusted with distilled water into separate volumetric flask (100 mL).

2.3.2. Preparation of working solution

One to five ml of stock solution content of sodium benzoate was transferred to five volumetric flasks (100 mL). Moreover, five concentrations of potassium sorbate (0.5- 2.5 mL) of stock solution that used in

previous stage was poured to another five volumetric flasks. Finally, all of these contents were diluted with distillated water.

2.3.3. Extraction

After homogenizing the obtained solution, 5 mL of every solution was poured in volumetric flask (50 mL), and 0.4 mL of hydrochloric acid (6 N) was added to the content. At the end, the level of contents was adjusted to 50 mL by petroleum benzene. The contents were mixed well.

2.3.4. Blank solution

About 0.4 mL of hydrochloric acid 6 N was added to 5 mL of distilled water, following by adjusting to 50 mL by petroleum benzene. As the petroleum benzene is toxic and volatile, it was done under vent. At last, the leap of volumetric flask was closed tightly, and the contents were homogenized for an hour.

2.3.5. Standard curve

The wavelength was adjusted on 228 nm for sodium benzoate and on 252 nm for potassium sorbate. The content of blank solution was poured in both of cells and put on the spectrophotometer. Then the cells were washed and filled with each of concentrations of the last volumetric flask (50 mL). The curve standard was figured and the equation of curve obtained (Fig. 4). All experiments were conducted triplicate.

2.3.6. Sample preparation and extraction

As the amount of sodium benzoate and potassium sorbate in aqueous part is as much as solid part after two weeks of production date, so in this test, the aqueous phase was used. After filtering the solution of the sample to increase the accuracy of the measurement, 5 mL of each sample was accurately transferred to volumetric flask (50 mL), then 0.4 mL of hydrochloric acid 6 N was added, and adjusted to 50 mL by adding petroleum benzene. At last, the leap of volumetric flask was closed tightly, and the contents were homogenized for an hour. After that the absorption of samples was read by the double beam UV/Visible spectrophotometer. To obtain the concentration of samples, the value of absorptions was located in the equation in relation to the standard curve, and the concentration of sodium benzoate and potassium sorbate was calculated.

Statistical Data analysis was done by SPSS 24 using ANOVA and t-test and p value less than 0.05 was considered as significant.

3. Results

The standard curves of the absorbance of the five concentrations for both additives, sodium benzoate and potassium sorbate were measured at 228 nm and 252 nm, respectively, with a double beam UV/Visible spectrophotometer. These curves are shown in Fig. 1 and Fig. 2. As indicated, the calibration graphs for both potassium sorbate and sodium benzoate were linear, with 5 calibration points. The equation for sodium benzoate was $y = 0.1096x-0.0314$ and the correlation coefficient was $R^2=0.9930$. The equation for potassium sorbate was $y = 0.0873x-0.0211$ and the correlation coefficient was $R^2 = 0.9993$.

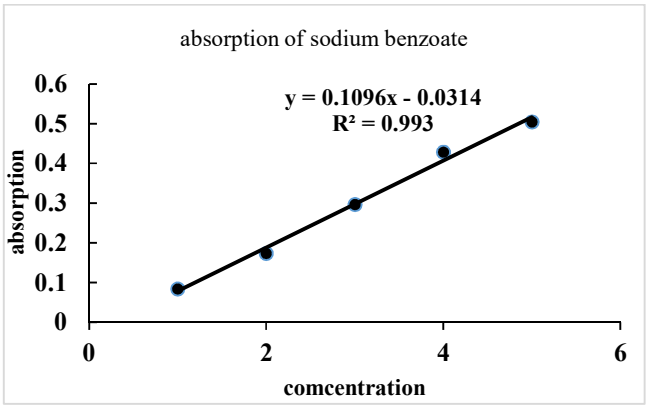


Figure 1. The curve standard of sodium benzoate on 228 nm.

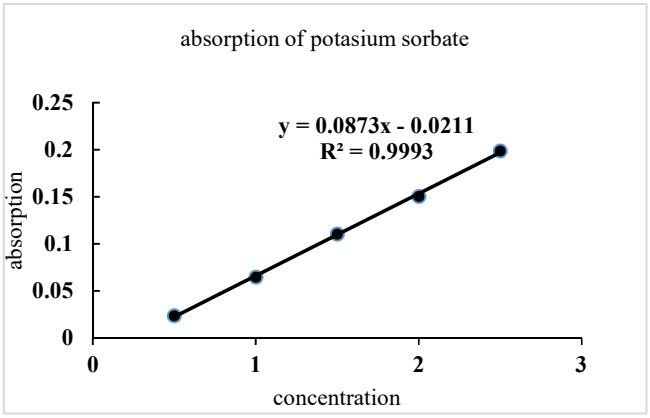
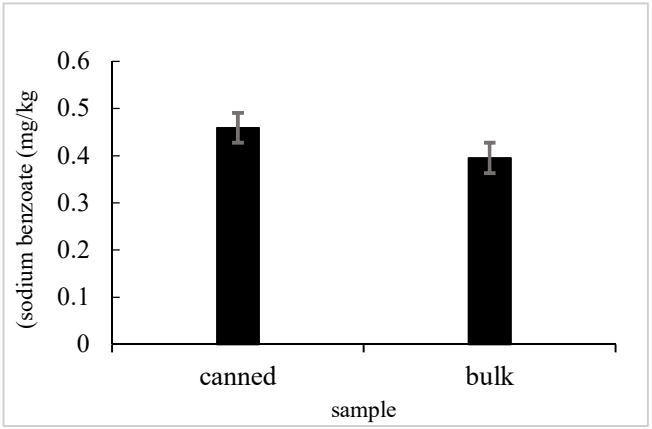


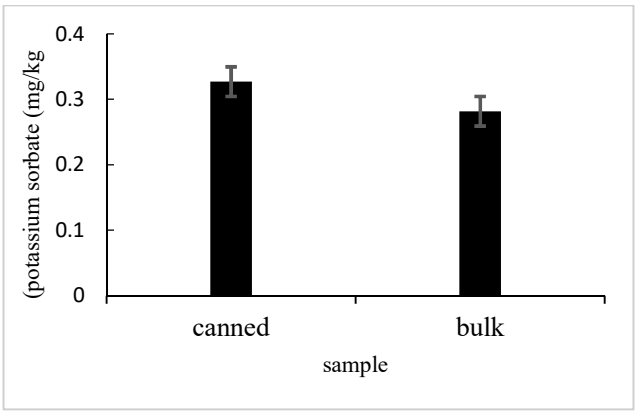
Figure 2. The standard curve of potassium sorbate on 252 nm

In this research the absorption of extracting sodium benzoate and potassium sorbate was read. The values were put in y = 0.1096x-0.0314 instead of x, and then y was obtained as the concentration of sodium benzoate. Besides, the absorption of each sample was read on the spectrophotometer, and again, the values were placed instead of x in the related correlation, y = 0.0873x-0.0211, and y was calculated and the concentration of sodium benzoate.

To statistically compare the means of normally distributed numbers between groups independent sample t- test was used (significance level $p < 0.05$). Fig. 3 demonstrates the results of the amount of sodium benzoate (a) and potassium sorbate (b) in canned and bulk samples. Obtained P-value between the amount of sodium benzoate and potassium sorbate in canned and bulk samples was 0.039 and 0.008 which illustrates that there was a significant difference ($p < 0.05$).



(a)



(b)

Figure 3. The comparison between the amount of sodium benzoate (a) and potassium sorbate (b) in canned and bulk samples.

The result of the analysis with the software SPSS 24, one-way ANOVA showed that there was a significant

difference between the amount of potassium sorbate in canned and bulk samples ($p<0.01$).

Fig. 4 compares the concentration of sodium benzoate and potassium sorbate in all samples. The result of analysis showed that amount of sodium benzoate is more than potassium sorbate.

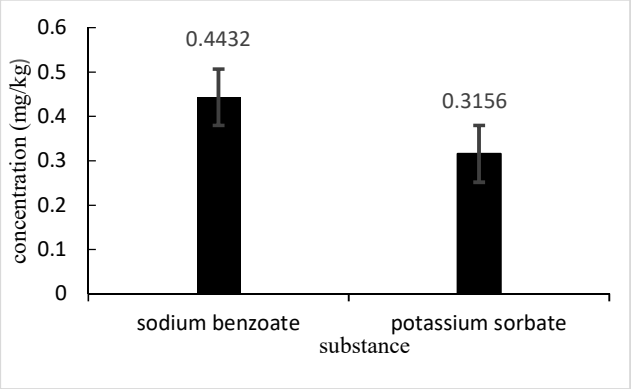


Figure 4. Comparison between sodium benzoate and potassium sorbate between samples.

The consequence of the survey with ANOVA showed the amounts of sodium benzoate and potassium sorbate did not show significant difference in the samples.

4. Discussion

The contents of sodium benzoate and potassium sorbate contents in pickled cucumber was determined by spectrophotometry method**.** The quantity of sodium benzoate in the most samples of the pickled cucumber was more than potassium sorbate. This is because of the more inhibitory effect of sodium benzoate than potassium sorbate on bacteria and molds. Moreover, the quantity of sodium benzoate, which is more harmful than potassium sorbate, was more than the allowable dose (the maximum limit is 150 ppm).

In our study, the amount of sodium benzoate in canned samples was more than bulk cargo samples. In another study on pickle and pickled cucumber products in Markazi province of Iran, sodium benzoate was determined using UV-VIS spectrophotometry method. The results showed sodium benzoate level was almost zero in the samples of canned pickles and pickled cucumbers. This was 200-400 ppm in 7 samples from bulk cargo pickled cucumbers which was more eminent than the allowable dose. There was not a statistically important deviation between the mean benzoate level of canned pickles and pickled cucumbers produced in Markazi province factories and other food manufacturing plants. Benzoate level was significantly higher than permissible dose in bulk cargo pickled cucumbers (4). In other research, near-infrared spectroscopy (NIRS) was employed to assess the quantity of potassium sorbate in the muddy flesh (pork). The results demonstrated that near-infrared spectroscopy can be used to quantify the amount of potassium sorbate, which avoids the pollution of the gas and high-performance liquid chromatography column (12). In a research spectrophotometry and HPLC were compared with each other. According to this research the former was more rapid and less expensive than the other, however the HPLC was more precise And also more expensive than the first one (12, 13). Ren et al, 2014 used both of this method to regulate these materials. They perceived that spectrophotometry was easy, rapid that can be used in screening projects (14).

This method has additional advantages in terms of simple sample preparation, reduced sample consumption, rapid measurement, provisional qualification data, involved structural information, and non-destructive analysis (15). In another study which compared the HPLC method and spectrophotometry, the latter was more accurate than the former. Although the HPLC was much more costly than the spectrophotometry. The advantages of HPLC was higher sensitivity. By contrast, the time of experiment in this method was more than UV spectrophotometry (12). Another comparison between HPLC and spectrophotometry was in a study on "Doogh" in Iran. The results of spectrophotometry in low concentrations, showed high values in comparison to what had been observed by HPLC. In high concentration, spectrophotometry showed the low value in comparison to HPLC (16).

5. Conclusions

Additives such as sodium benzoate and potassium sorbate are being used in pickled cucumber supplied in Tehran, Iran. The sum of these additives in all samples was more than the permissible limit. Consequently, these substances, sodium benzoate particularly, can endanger the health of consumers. Spectrophotometry method can detect and assess the quantity of these preservatives. The authorities should concern about them in food products more than ever. In summation, the consumers also should be aware about the effects of these materials on health, and choose the products with the least amount of them.

Conflict of interest

The authors declare they have no conflict of interest.

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