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Benzoic Acid and Sorbic Acid Levels in Some Dairy Products Consumed in Turkey

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In this study, white cheese, kashar cheese, yogurt, ayran, mayonnaise and margarine samples of different brands were analyzed for benzoic and sorbic acid levels. They were obtained from retail outlets in Bursa, Turkey. The occurrence and concentration level of benzoic acid and sorbic acid in the samples were investigated using high performance liquid chromatography (HPLC) with diode-array detector (235-254 nm). Chromatographic separation was achieved with a C18 column and acetate buffer (pH 4.74)-methanol mixture (70:30) as a mobile phase. The levels of benzoic acid and sorbic acid in the analyzed samples were in the range of not detected to 466 mg/kg or L and 2.09 to 1133 mg/kg or L, respectively. In 80 and 40 % of the analyzed yogurt samples, 100 and 20 % of the analyzed ayran samples, 80 % of the analyzed white and kashar cheese samples established benzoic acid and sorbic acid, respectively, although not permitted by the Turkish Food Codex. Benzoic acid and sorbic acid levels in the other samples were determined in the maximum tolerable limit of the Turkish Food Codex.

Key Words: Benzoic acid, Sorbic acid, Cheese, Yogurt, Ayran, Mayonnaise, Margarine, HPLC.

INTRODUCTION

Preservatives are defined as chemicals which extend shelf life of foods by preserving them against spoilage caused by microbial, enzymatic and chemical changes. For this purpose widely used preservatives are benzoic acid and sorbic acid and their respective potassium and sodium salts that must be monitored and controlled in dairy products, including cheese, yogurt and ayran[1-4]. These preservatives have been used in the food sector throughout the world over the past 30 years for the preservation of pastries, margarine, cheese, in sour soup tins, beverages, fruit, sausages, fishes, sweets and ground beef[5].

Preservatives are generally used to inhibit mold and yeast growth and they are also effective against many bacteria. Antimicrobial properties of preservatives

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depend on some factors such as antimicrobial spectrum, chemical and physical properties, concentration, affect mechanism of substance and composition, pH, water activity, storage temperature of food. Besides these factors, it must also be paid attention to some other subjects for choosing a preservative to be used for foods, such as genus and microorganism load on food, cost of preservative and its effect on quality of food[1,6,7].

The effect of sodium benzoate against microorganisms is by inactivating cell wall and some enzymes in the cell[8,9]. Sorbic acid is often used as sodium, potassium and calcium salts. However, in use as potassium sorbate is more common because its solubility is more than 50 % in foods[8,10-12]. Sorbic acid becomes effective by inactivating enzymes in cells of microorganisms[8].

Of these antimicrobial substances, that have common use, benzoic acid is metabolized rapidly and moved out from body. It doesn't accumulate in tissues. It's not harmful to health, when added to foods as sodium benzoate at very low levels. However, as the quantity increases, both the nutritional value of food is reduced and some kind of health problems can occur[9]. Very high doses of benzoic acid cause adverse effects such as metabolic acidosis, convulsions and hyperpnoea[4]. Several studies have been reported that benzoates cause asthma and various allergic reactions in human being[4,7,13,14]. Sorbic acid and its salts have less toxic effect than benzoic acid and its salts[9]. Sorbic acid is metabolized rapidly like some fatty acids (as butyric acid, caproic acid) in human and animals and this is shown as a reason for less toxic effect[9,15]. Excessive quantities of these acids cause serious hazards for consumers and they must be strictly controlled[16].

The use of food additives in foods are limited with various laws in every country. Regulations about food additives are made by WHO and FAO in the world[17]. In Turkey, specifications determined and advised by JECFA (The Joint FAO/WHO Expert Committee on Food Additives) that works in the leadership of these two associations are followed. ADI (Acceptable Daily Intake) values about food additives are explained in GRAS (Generally Recognized as Safe) lists prepared by JECFA[1,18,19]. According to the regulations made by JECFA ADIs of benzoic acid and its salts and sorbic acid and its salts are 0-5 and 0-25 mg kg^{-1} of body weight, respectively[20]. Excessive quantities of these acids cause serious hazards for consumers and they must be strictly controlled[16].

The use of benzoic acid and sorbic acid and their salts in processed foods are extremely important. Not using of these antimicrobials may cause microbial activities that lead food poisoning[21]. However there are some limitations in use of these. Fermented products are the foremost food group that have limitations for food additives because of especially their importance in healthy nutrition, preventing and curing effects. Turkish Food Codex that is prepared considering scientific truths and conclusions of Codex Alimentarius and has accordance with European Union directives is effective in such applications in Turkey[22].

The aim of this study is to determine benzoic acid and sorbic acid levels of white and kashar cheese, yogurt, ayran, mayonnaise and margarine that are most common consumed in Turkey and to compare the results with the maximum benzoic acid and sorbic acid tolerance limits which are accepted by Turkish Food Codex. Determining these preservatives as analytically is important not only in aspect of quality specifications but also in consumer health and protection.

EXPERIMENTAL

In this study as an experimental material 5 different brands of white cheese, kashar cheese, yogurt, ayran, mayonnaise and margarine samples purchased from Turkish markets were used. Three samples from each brand were extracted after homogenizing. For extraction 30 mL water was poured into 5 mL or g homogenized sample and shaked for 15-30 s. At the end of this period 60 mL methanol was added and the solution was completed to 100 mL with methanol in a volumetric flask. Samples were firstly filtered through crude filter paper and then through Milipore PVDF 0.45 μm membrane filter[23]. The filtrate was used in the analysis that was done in duplicate.

In the study high purity standards of benzoic acid (Merck, purity: 99.9 %, CAS No: 65-85-0, MW:122.12) and sorbic acid (Fluka, purity: 99.0 %, CAS No: 110-44-1, MW: 112.13) were used. Methanol (Merck) was HPLC quality. Acetic acid (Merck, d = 1.05) that was used for acetate buffer and NaOH (Merck, 5 mol L^{-1}) that was used for regulating pH were analytical purity. Millipore Milli-Q water was used in all stage of the assay.

The analysis was carried out using a diode-array detector (SPM-M10 Avp) connected to HPLC (Shimadzu LC-10 Avp). C18 column (Macharey-Nagel, 250 mm × 4.6 mm i.d., 4 μm particle size) and acetate buffer (pH = 4.74) and methanol mixture (70:30) as mobile phase was used for chromatographic separation of benzoic acid and sorbic acid. The analysis was carried out isocratically at a flow rate of 0.7 mL min^{-1}. The temperature of column oven (CTO-10A) was adjusted to 30 °C and an automatic injection (SIL-10A) system was used. The injection volume was 10 μL into a 50 μL injection block. Wavelength scanning span was between 190-370 nm. The detection of benzoic and sorbic acid were carried out at the wavelengths of maximum absorption of the compounds, 235 and 254 nm, respectively.

For quantitation, first standard stock solutions (1000 mg L^{-1}) were prepared by dissolving benzoic acid and sorbic acid in methanol-water mixture (40:60). Then, standard calibration solutions in concentrations of 0.25-1.25-2.5-12.5-25-50-75 mg L^{-1} were prepared using the standard stock solutions. Primarily retention times (RT) of benzoic acid and sorbic acid were determined for quantitation (Fig. 1). For this reason, 10 μL standard solution was injected to column. Retention time was 8.63 and 9.12 min for benzoic acid and sorbic acid, respectively. After that spectrum scanning of standards was done and UV spectrums were recorded (Figs. 2 and 3). Then samples were injected into column and retention times and spectra of benzoic

acid and sorbic acid in the samples were compared with standards. Thus, the identification of benzoic acid and sorbic acid was corrected with both retention times and spectra.

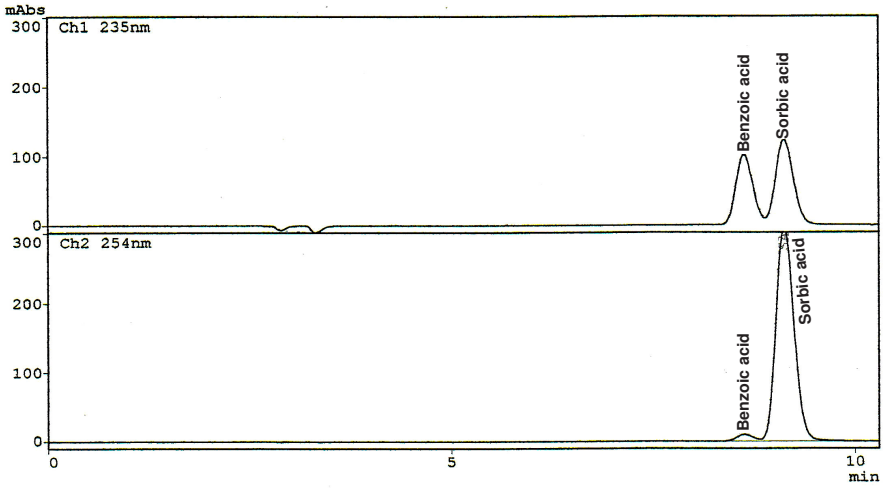

Fig. 1. HPLC chromatogram of benzoic acid and sorbic acid

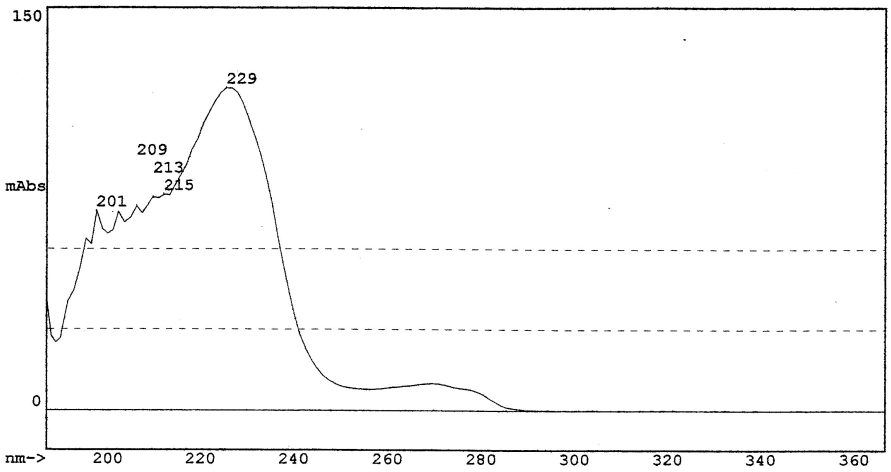

Fig. 2. Spectrum of benzoic acid

Calibration curves belonging to benzoic acid and sorbic acid were drawn by external standard method using standard calibration solutions. The R^2 values of benzoic acid and sorbic acid were found 0.9998 and 0.9999, respectively (Fig. 4). The detection limit (LOD) was found 0.19 mg kg^{-1} and the quantitation limit (LOQ) was found 0.64 mg kg^{-1} for both benzoic acid and sorbic acid. Recovery values of both preservatives were determined in between 72-105 %.

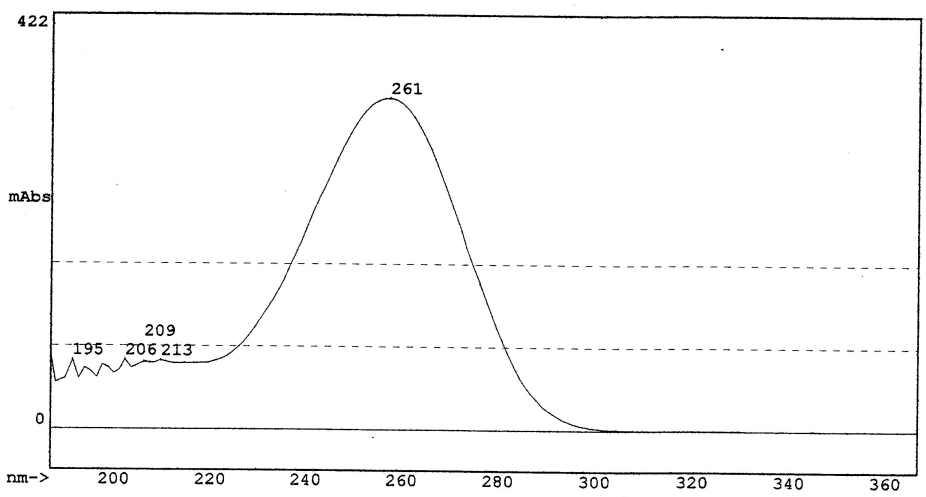

Fig. 3. Spectrum of sorbic acid

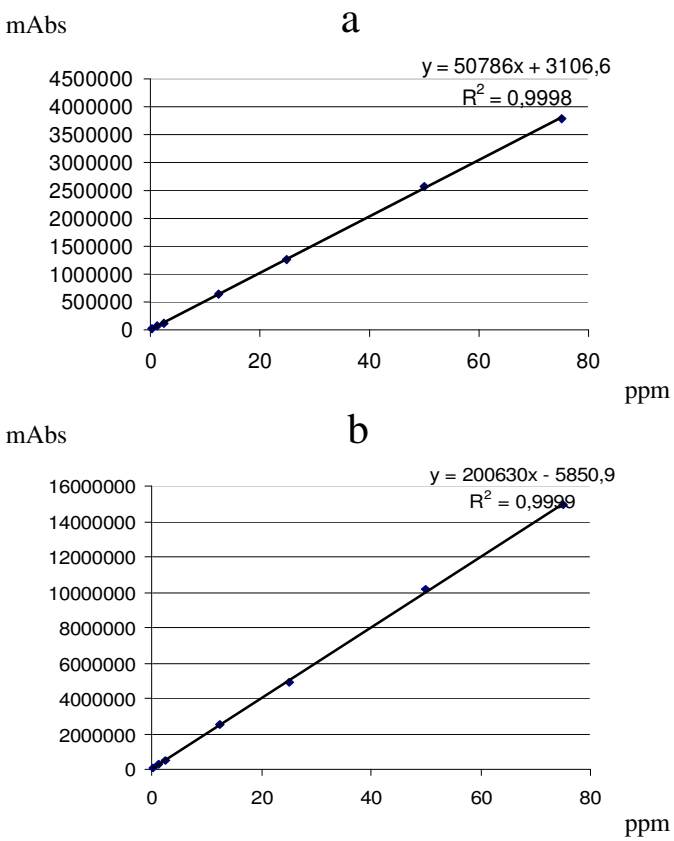

Fig. 4. Calibration curves of benzoic acid (a) and sorbic acid (b)

The following formula was used to calculate levels of preservatives in analyzed samples:

$$W = Cc \times V/m \times 1/R$$

W= Amount of preservative in sample (mg kg^{-1} or mg L^{-1}); Cc = Amount of preservative read on device (mg L^{-1}); V = Total volume of sample solution (mL); m = Amount of sample (g or mL); R = Recovery.

The following linear modeling was used to evaluate data.

$$y = \mu + a_i + e$$

y = Data belonging to parameter that is evaluated in sample; μ = Expected average of population; a_i = effect of sample i; e = Showing normal distribution, random mistake effect of that average is zero and variance σ^2_e, N (0, σ^2_e); Statistical analysis of data was done by SPSS 10.0.1 program[24].

RESULTS AND DISCUSSION

The occurrence and concentration level of benzoic acid and sorbic acid in the analyzed samples are given Table-1. As shown in Table-1, in all of the analyzed white and kashar cheese samples were determined the detectable level of benzoic acid and sorbic acid. As the Turkish Food Codex, the use of benzoic acid in the white and kashar cheese not allowed. Although, benzoic acid was established in all the analyzed samples. Sorbic acid levels in the cheese samples were found to be lower than the maximum acceptable limits (matured cheese: 1000 mg kg^{-1} or L^{-1}) of the Turkish Food Codex[25]. In one of the studies conducted on the subject in Turkey, 500 mg kg^{-1} sorbic acid was detected in one of the 25 kashar cheese samples [26],

TABLE-1
OCCURRENCE AND CONCENTRATION LEVEL OF BENZOIC ACID (BA) AND SORBIC ACID (SA) IN THE ANALYZED SAMPLES

Sample	n	Legal limit[a] (mg kg^{-1} or L^{-1})		No. of samples (ND)		Conc. of benzoic acid (mg kg^{-1} or L^{-1})			Conc. of sorbic acid (mg kg^{-1} or L^{-1})		
		BA	SA	BA	SA	Min.	Max.	$\overline{X} \pm S_{\overline{X}}$	Min.	Max.	$\overline{X} \pm S_{\overline{X}}$
White cheese	15	-	1000	3	6	3.72 ± 0.186	18.12 ± 0.779	8.44 ± 1.748	2.09 ± 0.098	55.18 ± 0.761	12.80 ± 5.550
Kashar cheese	15	-	1000	3	3	3.93 ± 0.201	8.35 ± 0.130	5.10 ± 0.786	2.93 ± 0.268	393.0 ± 5.044	78.87 ± 40.696
Yogurt	15	-	-	3	9	19.90 ± 1.021	29.50 ± 0.616	20.09 ± 2.807	26.33 ± 0.639	186.0 ± 2.906	41.77 ± 18.873
Ayran	15	-	-	0	12	5.20 ± 0.391	13.21 ± 0.407	9.17 ± 0.615	122.0 ± 7.000	146.0 ± 7.000	26.60 ± 14.267
Mayonnaise	15	1000	2000	12	6	415.0 ± 14.731	466.0 ± 14.731	88.20 ± 47.211	805.0 ± 26.028	1133.0 ± 41.968	551.93 ± 122.768
Margarine	15	-	1000	15	0	ND	ND	-	79.40 ± 5.999	698.20 ± 9.492	246.73 ± 58.722

[a]Anonymous 2003; n = number of samples analyzed; ND = not determined.
In calculation ND was assumed as zero.

while in another study, 0.3 mg kg^{-1} sorbic acid was observed in again only one among 10 kashar cheese samples[27]. It is observed that the amount of sorbic acid in the kashar cheese samples used in the present study is lower than the finding of other studies. However, the increased number of samples in which this food additive was observed has been evaluated as an indication of the gradually increasing use of such additives.

It was determined that the white cheese samples contained benzoic acid, though an violation of the Turkish Food Codex and their sorbic acid contents were under the tolerance limits (1000 mg kg^{-1}) (Table-1). Küçükçetin *et al.*[28] reported that the amount of sodium benzoate found in the samples of white cheese sold in Antalya, Turkey, was 50.9 mg kg^{-1}. These results obtained in this study are in parallel with the findings of some previous reports[4,28-30].

No sign of benzoic acid was observed in 3 of the yogurt samples analyzed and no sign of sorbic acid in 9 of them. In the other samples, the amounts of benzoic acid and sorbic acid were found to be 19.90-29.50 mg kg^{-1} and 26.33-186.00 mg kg^{-1}, respectively. Gonzalez *et al.*[29] reported that the sorbic acid content of yogurt samples was between 175-280 mg kg^{-1}, while Tfouni and Toledo[4] determined it to be 126-213 mg kg^{-1}. Küçükçetin *et al.*[28] found that the yogurt samples sold in Antalya market contained a maximum amount of 233.9 mg kg^{-1} benzoic acid and 557.9 mg kg^{-1} sorbic acid. Generally, benzoic acid and sorbic acid levels in the yogurt samples analyzed are lower than the findings of other studies. However, the Turkish Food Codex[25] does not allow the use of these preservatives in yogurt production. Hence, it was observed that the legal limits had been breached in these 12 yogurt samples. Similarly, in all the ayran samples analyzed, benzoic acid was detected, though its use is prohibited and in 3 of the samples, sorbic acid was found. Benzoic acid and sorbic acid levels of mayonnaise and margarine were found to be within the legal limits (Table-1).

Consequently, it is evident that the presence of benzoic acid and sorbic acid in some dairy products in Turkey, such as white cheese, kashar cheese, yogurt and ayran, though their use is a violation of the Turkish Food Codex, is among the significant factors threatening public health. Therefore, the authorities should urgently take necessary measures to ensure the continuous monitoring of the presence of food additives in dairy products and to inform dairy producers.

Conclusion

The method used to extract samples and chromatographic conditions enabled to get analysis results in a short time, confidently. The analysis period including extraction and analysis at HPLC was totally 11 min.

Benzoic acid and sorbic acid used illegally in some dairy products that constitutes a human health risk in Turkey. Therefore dairy companies need to be about utilization of these preservatives and also the continuous monitoring of the benzoic and sorbic acid levels must be supplied by the Turkish public health authorities.

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