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Quantitatation of Benzoic and Sorbic Acids in Some Processed Foods of Korea

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일부 한국 가공식품의 벤조산과 소르빈산의 함량 조사

미트라 카니카[1] · 한장일[1] · 이기택[2] · 김성애[1†]
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Abstract

Benzoic acid (BA) and sorbic acid (SA) are the preservatives most commonly used in food. Although BA and SA are generally safe, some previous studies have shown that consumption of excessive amounts of these food additives can be a health hazard. The aim of this study was to determine the amounts of BA and SA in processed foods in Korea. Different brands of fruit juice, yogurt, cheese, dried fruits, jam, and margarine were purchased at a local market in Daejeon, Korea. Samples were analyzed by high-performance liquid chromatography (HPLC) using a UV detector. Chromatographic separation was achieved with a C18 column. Methanol acetate buffer (pH 4.4) at a 35:65 v/v ratio was used as the initial mobile phase to elute BA and SA. The detector wavelength was set at 254 nm. The average test results observed for BA concentrations in fruit juice, yogurt, cheese, dried fruits, jam, and margarine were 40.26±0.02, 2.07±0.06, 0.02±0.09, 0.36±08, 265.30±0.02, and 27.34±0.08 mg/kg, respectively. Average concentrations of SA in these samples were 0.92±0.06, 1.06±0.07, 7.30±0.01, 14.14±0.08, 25.65±0.06, and 4.81±0.07 mg/kg, respectively. Thus, the average levels of BA and SA in the studied food items were lower than the KFDA-permitted limits. Moreover, the estimated daily intake of both BA and SA by a typical consumer were below the maximum recommended daily values.

Key words : benzoic acid, sorbic acid, HPLC, ADI(acceptable daily intake), EDI(estimated daily intake)

Introduction

Chemical preservatives always play a significant role in food preservation. Food additives are very useful to delay or prevent nutritional losses due to the presence of microbial toxins, pathogenic microorganisms and prevent consumer hazards(1). Being antimicrobial agents, benzoic and sorbic acid were reported to inhibit mold and yeast growth. These preservatives are also very effective to control bacterial proliferation and have inhibition power of neuraminidase from influenza virus(2). It was reported that highest sensory scores on odor, texture, taste and overall quality was observed in benzoic acid treated kimchi after 6 days of fermentation(3).

Food additives should not be toxic to humans. However, combinations of some of these chemicals and sunlight can produce photo toxicity and photo allergy, which can be demonstrated well on sub cellular levels where they involve damages to cell components such as DNA and RNA(4). Short term exposure to benzoic acid can cause eyes & respiratory tract irritation. An extensive or high does exposure of benzoic acid & its salt can cause skin sensitization and intermediary metabolism difficulties, including urea cycle, gluconeogenesis, fatty acid metabolism(5). Some study also noted weak clastogenic activity in *in vitro* assays(6). Some adverse effect

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e.g. asthma, urticaria, to benzoates in human body were also reported(7). Sorbic acid is generally recognized as safe because of its low toxicity; explain by the fact that it is rapidly metabolized by pathways similar to those of other fatty acid. However a few cases of idiosyncratic intolerance to sorbic acid in humans have been reported(8).

Recently the production of commercial and convenience foods had been increased. Consuming excessive amounts of these preservatives than permitted safety levels are harmful and cause serious hazards for consumers. Therefore using these additives must be monitored and controlled.

The most common analytical method for the determination of BA and SA in foods and beverage is high performance liquid chromatography (HPLC)(9-13). Other analytical methods such as gas chromatography and spectrophotometric methods(14), capillary zone electrophoresis (15), thermal desorption gas chromatography(16) have also been reported.

To ensure the safety of these foods, in Korea, Korea Food & Drug Administration(KFDA)(17) specifies some specific amount of these compounds for some foods. In order to assure the content of these additives in foodstuffs according to the permitted level of KFDA, quantification of additives is essential. This monitor the content of food additives and then contribute for consumers safety. Considering all these relevant information into account, the aim of this study was to investigate the quantitative profile of benzoic and sorbic acid in Korean processed food items. Accomplish this purpose, a simple HPLC technique with a time & cost saving extraction procedure was used in this study. Furthermore, average daily intake and estimated daily intake was evaluated to make out the safety of consumers.

Materials and Methods

Chemicals and instruments

The standard benzoic acid(>99%) and sorbic acid(>99%) were obtained from Sigma(St.Louis, MO, U.S.A.). Methanol, acetic acid and ammonium acetate employed for the mobile phase of HPLC were supplied by Merck(Darmstadt, Germany). Ortho phosphoric acid, zinc acetate, potassium hexacyanoferrate and other chemicals were analytical grade.

Food samples

The samples were categorized as: 10 different brands of fruit juice each containing different ingredients e.g. Mango, Orange, Apple, Pineapple, Tomato, Peach, Alveoli, and Grape. 10 brands of yogurt, 5 different brands of cheese, 7 brands of dried fruits e.g. peanut, almond, banana, mixed dried fruit, 15 brands of jam and 3 brands of margarines, were collected for this analysis. Each fresh sample was analyzed in duplicate. These 6 food items were selected for studied based on susception of containing BA and SA(8-12).

Preparation of fruit juice & yogurt samples

10 mL of juice was centrifuged in 2,000 rpm for 20 min and then supernatant(1 mL) was passed through a Sep-Pak C18 disposable cartridge(waters) which was previously activated with 4 mL of methanol and 4 mL of water, following 4 mL of hexane passed through and eluted with 3 mL of methanol. The extract was concentrated and filtered through a 0.45 μm filter membrane and the extract was kept in a 1 mL glass vial prior to the HPLC injection. For yogurt samples, 15 mL of 2% H_3PO_4(ortho phosphoric acid) was added with 5 g sample and centrifuged for 10 min. 5 mL zinc acetate and potassium hexacyanoferrate($K_3[Fe(CN)_6]$) was added after that. Then followed the same extracting procedure described above.

Preparation of jam samples

Quince jam or jelly(~2 g) was thoroughly mixed with 25 mL of methanol. The extract was centrifuged(2000 x g) for 10 min and the supernatant was filtered through a 0.45 μm filter. Clear filtrate was injected into the HPLC.

Preparation of dried fruit, cheese & margarine samples

Solid food samples were finely ground prior to the extraction. About (~2 g) of samples was accurately weighed in a screw capped test tube. 25 mL of methanol was added and the test tube was next subjected to vortex mixing for few min. After that, test tubes were placed in a sonicator(JAC ULTRA sonic Model 2010) that was maintained at 50°C for 40 min. The extract was centrifuged(2000 x g) for 10 min. The contents were filtered through a 0.45 μm filter(Whatman, U.K.) and the clear filtrate was injected into the HPLC column.

HPLC condition

The high performance liquid chromatography(HPLC) for analytical separation was conducted by using a UV - visible detector at the wavelength of 254 nm. Phenomenex(250 × 4.60 mm, 4 μm) synergi 4 μm Fusion RP 80 C_{18} column was used. Acetate buffer was prepared by dissolving 3.8 g

ammonium acetate in 1 L water. Finally, this solution was adjusted by pouring acetic acid to maintain its pH 4.4. Elution solvent consisted of methanol: acetate buffer(pH 4.4)(35:65, v/v) for 30 min. The flow rate was set at 1 mL/min and the volume of sample injected was 20 μL. For HPLC chromatogram analysis, Agilent Chem. Station Rev. A.10.02(1757) for LC, LC/MS software was used.

Standard curves and percentage recovery

The calibration curves were used for quantification of sorbic & benzoic acid. To construct linear regression lines, standard solutions prepared with methanol ranging from 1~500 μg of BA/mL & 1~500 μg of SA/mL were used. The correlation coefficient (r^2) for each benzoic and sorbic acid standard curve exceeded 0.99. The limit of detection(LOD) is defined as the smallest peak detected with a signal height three times that of the baseline while the limit of quantization(LOQ) refers to the lowest level of analyst which can be determined with an acceptable degree of confidence. LOD was determined 0.4 mg L^{-1} for BA and 0.1 mg L^{-1} for SA whereas LOQ was determined 1.6 mg L^{-1} for BA and 0.3 mg L^{-1} for SA.

In order to bear out the accuracy of extracting procedure, recovery studies were carried out. The mean percentage recoveries and standard deviations of both standards in different concentrations are shown in Table 1. Mean percentage recoveries was carried out by adding 10, 50, 100 mg/kg or litter(known amount) of BA & SA into two samples of each food items. Mean percentage recoveries for fruit drinks, yogurt, cheese, dried fruit, jam/jelly, margarine were found as 91.9±0.06, 90.7±0.08, 91.5±0.03, 95.7±0.01, 93.3±0.02 and 88.7±0.05 for benzoic acid and 93.03±0.06, 92.8±0.04, 93.1±0.06, 95.5±0.07, 92.3±0.06 and 85.4± 0.03 for sorbic acid, respectively.

Table 1. Recovery of Benzoic acid and Sorbic acid from different products

Food Items	Added amount (mg/kg or liter)	Mean percentage recovery[1)]±SD	
		Benzoic Acid	Sorbic Acid
Fruit juice	10	91.4±0.01	93.6±0.08
	50	91.9±0.05	92.4±0.01
	100	92.3±0.01	93.1±0.09
	Mean	91.9±0.06	93.0±0.06
Yogurt	10	90.9±0.05	92.6±0.09
	50	90.3±0.01	93.1±0.09
	100	91.1±0.07	92.8±0.04
	Mean	90.7±0.08	92.8±0.04
Cheese	10	92.3±0.05	92.4±0.08
	50	90.7±0.02	93.8±0.01
	100	91.4±0.02	93.2±0.08
	Mean	91.5±0.03	93.1±0.06
Dried fruit	10	95.2±0.06	96.3±0.07
	50	96.1±0.04	96.1±0.08
	100	95.8±0.04	94.1±0.06
	Mean	95.7±0.01	95.5±0.07
Jam	10	92.4±0.06	93.4±0.07
	50	94.0±0.08	92.8±0.01
	100	93.5±0.02	90.8±0.01
	Mean	93.3±0.02	92.3±0.06
Margarine	10	88.7±0.04	84.4±0.01
	50	88.1±0.06	86.1±0.02
	100	88.3±0.06	85.6±0.07
	Mean	88.7±0.05	85.4±0.03

[1)]Average of two different analysis.

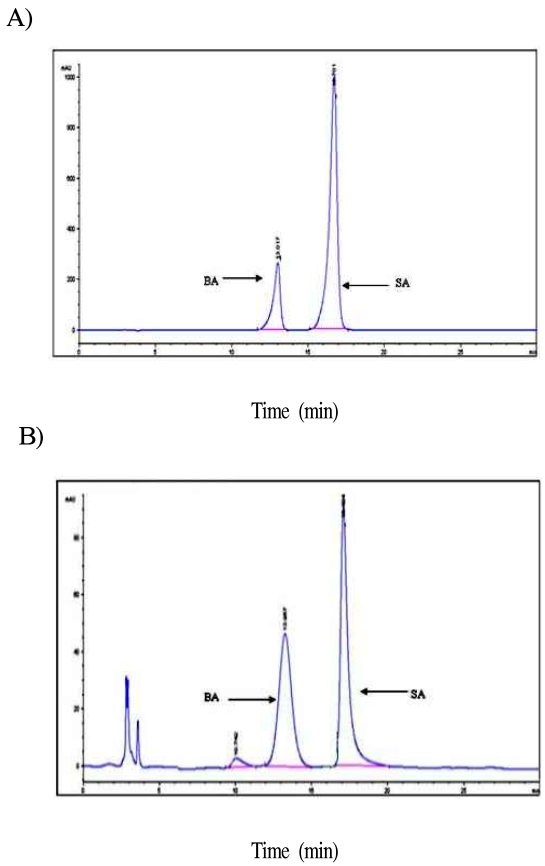

Figure 1. HPLC chromatogram of BA and SA in Korean some processed foods.

A) Typical HPLC chromatogram of standard mixture of preservatives. The retention times for BA (300 μg/L) and SA (100 μg/L) were 13.03 min and 17.70 min respectively. B) Chromatogram of BA & SA positive samples. The retention times for BA and SA were almost similar according to standards preservatives(13.96 and 18.10 min for BA and SA respectively).

Peak identification of the preservatives in various food stuffs was determined by comparing the retention time of standard compounds with the pike of the sample. Fig. 1A shows a typical chromatogram of a standard mixture of preservatives, BA(300 μg L^{-1}) & SA(100 μg L^{-1}) respectively. Under the stated experimental conditions, baseline resolutions of the two components were achieved. The retention times for BA and SA were 13.0 and 16.7 min, respectively. Chromatogram of one preservative - positive sample is shown in Fig. 1B. Peaks of BA & SA were identified according to the retention time of these two components.

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

W= Cc × V/ m × 1/ R

Where,

W= amount of preservatives in sample(mg/kg or mg/L);

Cc= amount of preservative read on device(mg/L);

V= total volume of sample solution(mL);

m = amount of sample(g or mL);

R= recovery

Results and discussion

The mean concentrations of benzoic acid, mean concentrations of all BA positive samples and the range of BA concentration in comparison to KFDA permitted level are shown in Table 2. From ten analyzed fruit juice samples, BA was detected in 80% samples(Fig. 2A). Mean concentration for fruit juice sample was assessed 40.26±0.02 mg/kg. Concentration of benzoic acid was found in yogurt samples from not detected to 3.15±0.03 mg/kg. BA contents in different kinds of cheese samples(Fig. 2 C) were evaluated for instantaneous verification among the analyzed samples. The range for benzoic acid concentration was from ND to 0.06±0.08 mg/kg. Dried fruit(Fig. 2 D) samples concentration of benzoic acid was found in the range from not detected to 2.05±0.08 mg/kg. Fig. 2E showed the concentration of BA assayed in the fifteen analyzed jam samples. Among the analyzed samples, concentration of BA was in a range of not detected to 1161.94±0.07 mg/kg. The mean concentration was detected 265.30±0.02 mg/kg. Among the analyzed samples only one sample contained very high concentration of BA(1161±0.07 mg/kg) which exceeded the KFDA permitted limit of 1000 mg/kg. Mean concentration of BA was 27.34±0.08 mg/kg for margarine samples.

Table 2. Benzoic acid levels in different food categories

Food Items	N[1)]	KFDA[2)]	Benzoic acid level (mg/kg or liter)		
			Mean[3)]±SD[5)]	Mean[*4)]±SD	Range
Fruit juice	10	600	40.26±0.02	50.32±0.03	ND[6)]~101.50±0.08
Yogurt	10	50	2.07±0.06	2.29±0.02	ND~3.15±0.03
Cheese	5	NR[7)]	0.02±0.09	0.03±0.03	ND~0.06±0.08
Dried fruit	7	NR	0.36±0.08	0.50±0.04	ND~2.05±0.08
Jam	15	1000	265.30±0.02	356.28±0.02	ND~1161.94±0.07
Margarine	3	1000	27.34±0.08	41.01±0.02	ND~49.57±0.09

[1)]N = number of analyzed samples.
[2)]KFDA= Permitted level mg/kg or liter.
[3)]Mean= Mean of all analyzed samples.
[4)]Mean*=Mean of all BA positive samples.
[5)]SD= Standard deviation.
[6)]ND= Not detected. (All not detected samples assumed as zero for statistical analysis).
[7)]NR= Permitted value are not reported by KFDA.

The mean concentration of SA, mean concentrations of all SA positive samples and the range of SA concentration comparing with KFDA permissible limit was shown in Table 3. The mean concentration of SA in fruit juice and yogurts were 0.92±0.06 and 1.06±0.07 mg/kg, respectively. 50% of analyzed fruit juice samples contained SA(Fig. 3 A), although permitted concentration of SA for fruit juice was not published by KFDA yet. SA was observed in eight samples of yogurts(Fig. 3 B). The range was from not detected to 2.36±0.09 mg/kg.

SA was detected in four samples among five analyzed cheese samples(Fig.3 C). Mean concentration of SA was detected 7.30±0.01 mg/kg and 14.14±0.08 mg/kg for cheese and dried fruit samples, respectively(Table 3). SA was found as common preservatives in analyzed jam(Fig. 3 E) and margarine(Fig. 3 F) samples. The mean concentrations of SA for jam and margarine were 25.65±0.06 and 4.81±0.07 mg/kg, respectively.

BA and SA are generally used to inhibit yeast and mold growth; these are also effective against a wide range of bacteria. It was reported that most yeasts and molds are inhibited by 0.001~0.1% SA and 0.002~0.07% BA(8). According to the results of this study, most of the analyzed samples contained necessary amount of BA or SA to control microbial growth. Only one margarine sample which was labeled as “No preservatives”, did not detect any of this preservatives.

Among the ten analyzed fruit juice samples, BA was not detected in two samples. Whereas, in five samples SA was not detected. Since fruit juice contain very low pH value and a high sucrose concentration, these two conditions jointly slow down the growth of microbial organisms devoid of any

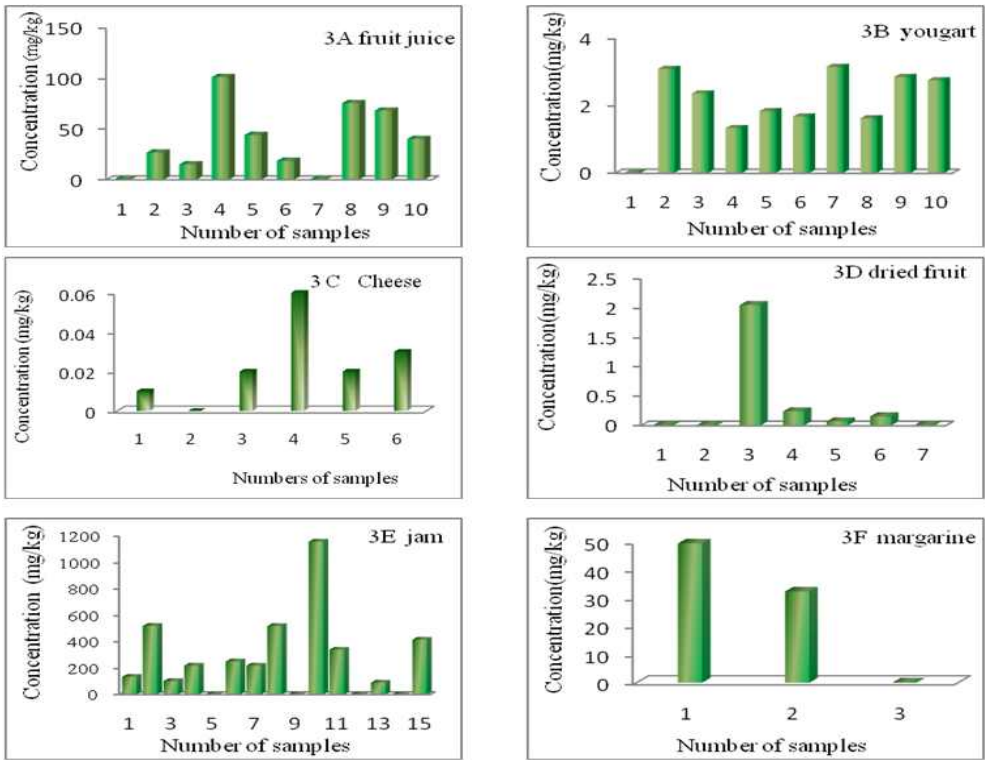

Figure 2. Concentration of benzoic acid(BA) in different kind of food items.

(A) fruit juice(n=10), (B) yogurt (n=10), (C) cheese(n=5), (D) dried fruit(n=7), (E) jam(n=15), (F) margarine(n=3).

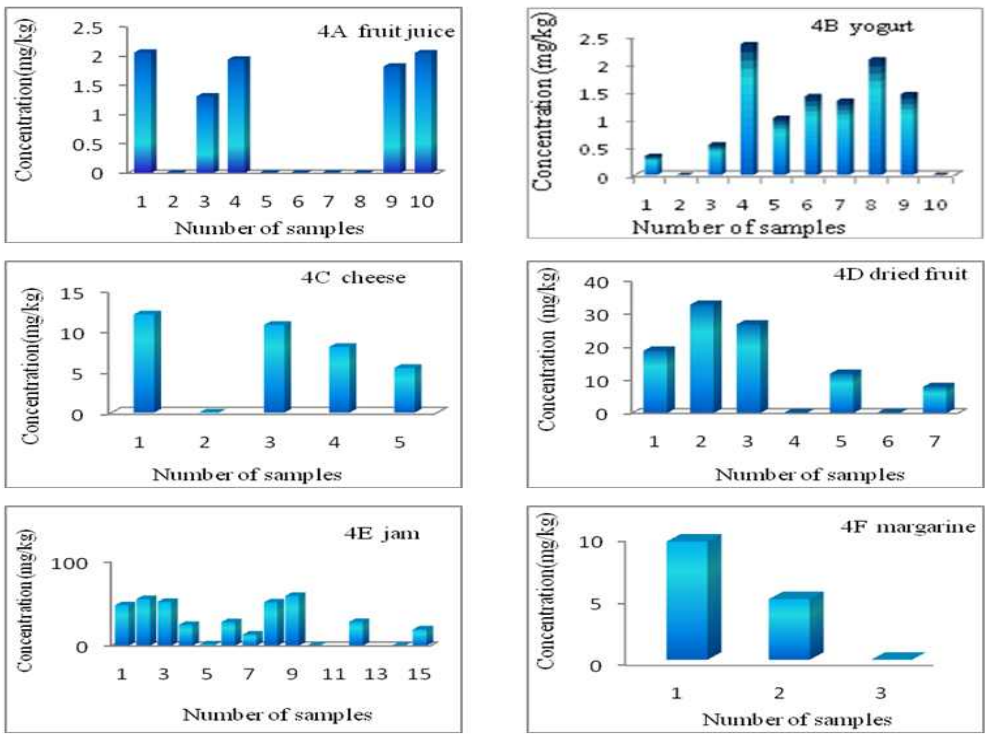

Figure 3. Concentration of sorbic acid(SA) in different kind of food items.

(A) fruit juice(n=10), (B) yogurt(n=10), (C) cheese(n=5), (D) dried fruit(n=7), (E) jam(n=15), (F) margarine(n=3).

other preservatives(8). In addition, industrial products contain a large number of fruits mixtures and prepared in a highly controlled procedure. As a result, it might be anticipated that, for fruit juice samples, a very low concentration or not having any preservative is not at a risk for microbial growth.

BA and SA contents in milk and milk based products may be influenced by different factor, such as fermentation, contamination and storage condition. Several studies(5,9) reported that high concentration of BA or SA in milk based product might be resulted from the action of lactic acid bacteria on the hippuric acid of milk during the fermentation or the storage conditions.

Table 3. Sorbic acid levels in different food categories

Food Items	N[1)]	KFDA[2)]	Sorbic acid level (mg/kg or liter)		
			Mean[3)]±SD[5)]	Mean[*4)]±SD	Range
Fruit juice	10	NR[7)]	0.92±0.06	1.85±0.02	ND[6)]~2.07±0.02
Yogurt	10	50	1.06±0.07	1.32±0.01	ND~ 2.36±0.09
Cheese	5	3000	7.30±0.01	9.13±0.02	ND~12.09±0.08
Dried fruit	7	500	14.14±0.08	19.80±0.08	ND~33.11±0.08
Jam	15	1000	25.65±0.06	34.98±0.06	ND~59.55±0.01
Margarine	3	1000	4.81±0.07	7.21±0.07	ND~ 9.53±0.01

[1)]N = number of analyzed samples.
[2)]KFDA= Permitted level mg/kg or liter.
[3)]Mean= Mean of all analyzed samples.
[4)]Mean*=Mean of all BA positive samples.
[5)]SD= Standard deviation.
[6)]ND=Not detected.(All not detected samples assumed as zero for statistical analysis).
[7)]NR= Permitted value are not reported by KFDA.

According to our study, the mean contents of BA(265.30 mg/kg) and SA(25.65 mg/kg) in jam were in safe range compared to KFDA(BA & SA both 1000 mg/kg). Only in one jam sample, it was showed exceeded BA content(1161.94 mg/kg) than the KFDA permitted limits(1000 mg/kg). Among the analyzed samples, presences of BA and SA was determined in common and these preservatives were widely used in commercially processed foods like jam. Microbial growth may vary due to species, storage condition of the substrate and other factors(8,10). Since jam had low pH, heat sterilization and high level of sweetener, it could be relatively safe from microbial growth and no need of much additives for preservation. Even though, manufactures use additive like BA and SA in jam preparations because by using these additives they can avoid deferent kinds of pre-treatment procedure(like add SO2) and able to use much amount of fruit pulp to produce jam(18).

Texture, color, flavor, test might be changed for using excess amount of BA and SA which is not desirable to the consumers.

ADI is an acceptable daily intake(mg) of food additive/ body weight(kg). ADI represents the amount of the food additives that can be consumed without health hazards. Intake of food additives over ADI limits can cause different kinds of health hazard as described previously. WHO recommended ADI of BA and SA(19) as 0~5 and 0~25 mg/kg body weight, respectively. Korean national nutrition survey(KNNS) carried out in 2001(20) showed very low ADIs of BA(0.30 mg/kg bw/day) and SA(0.88 mg/kg bw/day) compared to WHO recommendation which suggests safe amount of BA and SA intake. According to Lee et al(21), ADI of BA and SA from the survey conducted by sanitary managers working in several food processing companies in Korea, ADI of BA was 5.8 mg/kg bw/day which was over WHO recommendation (0~5 mg/kg body weight) and 19 times higher than KNNS. Also, ADI of SA(1.56 mg/kg bw/day) was in range of WHO recommendation(0~25 mg/kg body weight) and 2 times higher than KNNS.

Even though ADI of BA and SA from KNNS was in safe range of WHO recommendation, monitoring of food additives is still necessary because Lee's study result of ADI of BA and SA were much higher than KNNS data and further clarification is needed. According to KNNS(20) and Lee's study(21), EDI is the estimated daily intake of food additives/body weight. EDI for BA and SA was observed as 0.015~0.29 and 0.220~0.39 respectively.

KFDA did not specify safe amount of BA for cheese and dried fruit and SA for fruit juice. Without KFDA permissible limit for food additives in those cheese, dried fruit and fruit juice items, manufactures could use high amount of BA and SA which will results health hazard. Maximum acceptable level for SA in fruit juice is stated as 1000 mg/kg by FAO/WHO(22). In case of BA, maximum acceptable level is directed as 800 mg/kg in dried fruit and 300 mg/kg in cheese(22). So, the maximum permitted level for these food items could be recommended by following FAO/WHO level in Korea.

In conclusion, it could be say that, average concentration of BA and SA in some Korean processed food items were lower than the KFDA permitted levels moreover ADI level of these preservatives are in low range than WHO permitted level according to KNNS 2001 data. Therefore it could be predict that the use of BA and SA in food items is safe for human consumption.

요 약

본 연구에서는 소비자의 안전과 관련하여 일부 한국식품에서의 벤조산과 소르빈산의 함량과 안전소비량을 평가하고자 하였다. 몇 개 브랜드의 과일 쥬스, 요거트, 치즈, 말린 과일류, 잼, 마가린을 대전의 시장에서 구입하여 HPLC로 분석하였다. C_{18} column을 사용하여chromatographic separation을 얻었으며 35:65 비율의 methanol acetate buffer(pH 4.4)로 벤조산과 소르빈산을 초기 mobile phase에서 회수하여 254 nm에서 분석하였다. 그 결과 과일 쥬스, 요거트, 치즈, 말린 과일류, 잼, 마가린 샘플들의 벤조산 농도는 각기 평균 40.26±0.02, 2.07±0.06, 0.02±0.09, 0.36±08, 265.30±0.02와 27.34±0.08 mg/kg 였으며 소르빈산의 농도는 각기 평균 0.92±0.06, 1.06±0.07, 7.30±0.01, 14.14±0.08, 25.65±0.06와 4.81±0.07 mg/kg 였다. 따라서 조사한 식품들의 벤조산과 소르빈산의 사용량은 KFDA 허용치 이하이었으며 벤조산과 소르빈산의 평균 소비량에 대한 EDI 또한 ADI 이하의 안전한 수준이었다.

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