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GC-MS Analysis of Benzoate and Sorbate in Saudi Dairy and Food Products with Estimation of Daily Exposure

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Abstract: Benzoic and sorbic acids and their salts are extensively used in the preservation of foods. Their levels of use in different food products are regulated by national and international regulatory agencies. A GC-MS according to AOAC procedure was applied for the determination of the content levels of benzoic and sorbic acids in a wide range food samples commercially distributed in Riyadh local markets. The results obtained from this study indicated that the levels of benzoic acid were between 2.23 mg kg^{-1} in cream caramel to 921 mg kg^{-1} in yogurt rice dressing and the levels of sorbic acid were between 0.67 mg kg^{-1} in fresh yoghurt to 2275 mg kg^{-1} in processed cheese. The sample of processed cheese had sorbic acid in a quantity above the maximum allowable level whereas, none of the rest analyzed samples was found to violate the legal limits. The samples were tested to determined if they were produce in accordance with their label claims. Out of 50 examined samples only 8 samples deviated from their label claims. In addition, the concentration of these food preservatives have been converted into the daily intakes doses based on food consumption date. In except of yogurt rice dressing, it was estimated that the maximum daily intakes of benzoic acid and sorbic acid in analyzed food samples were less than acceptable daily intakes.

Key words: Food additives, benzoic acid, sorbic acid, daily intake, dairy products

INTRODUCTION

The potential of food contamination makes it necessary to add preservatives like benzoates and sorbates to foods to control microbial growth and extend the shelf-life. Benzoates have a long history of safe use as preservatives in foods (Chichester and Tanner, 1972; Kelley, 1975; Furia, 1978; Jones, 1978; Philips and Woodroof, 1981; Lindsay, 1985). Benzoic acid and its salts, mainly sodium benzoate, remain widely applicable as preservatives in a number of products consumed by humans (Chipley, 1993; Baldwin *et al*., 1995; Ishida, 1996). Benzoic acid (E210, benzene carboxylic acid, $C_7H_6O_2$) and its sodium (E211), potassium (E212) and calcium (E213) salts further, referred to as benzoates are a group of food additives important to preserve foods and to protect the consumer from microbiological risks of various bacteria, yeasts and fungi that can be involved in food poisoning, such as *Esherichia coli*, *Listeria monocytogenes*, *Aspergillus* sp. and *Pencillium* sp. (Sieber *et al*., 1995). Benzoic acid is least active in neutral, medium and its preservative effect is increased considerably with decreasing pH. This is due to the fact that more undissociated benzoic acid exists at lower pH (Phillips and Woodroof, 1981). The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1973) has evaluated benzoic acid and its salts several times and found them to be acceptable for use in foods (FAO/WHO, 1996). JECFA established an Acceptable Daily Intake (ADI) of 0.5 mg kg^{-1} bwt/day based on a four-generation feeding study in rats. The referee board of consulting scientific experts of the US Department of Agriculture concluded that sodium benzoate was not injurious to health even in large doses (up to 4,000 mg day^{-1}) mixed with the food (Remsen, 1909; US Department of Agriculture, 1909).

Sorbic acid as well as potassium and calcium sorbate (E202 and E203) is legally used as preservatives in numerous processed foods (Ferrand *et al*., 2000a). Sorbic acid and sorbates were first used for their activity as inhibitors of mould and yeast growth and they have been proved to have the same role on a wide range of bacteria. The use of sorbic acid or its salts is authorized in a large variety of foods to lengthen their shelf-life (margarine, fruit, deserts, other drinks, cheeses, etc.). The levels at which they are used cover the range 100-2000 mg L^{-1}. The authorized daily intake has been fixed for many years at 25 mg kg^{-1} bwt (JECFA, 1973).

Under the provision set forth by the US Food and Drug Administration (FDA) in the Code of Federal Regulations (1999), food additives can be used if they are

Generally Recognized as Safe (GRAS) and declared on the label. The EU scientific committee on food has recommended that special attention should be given to intake by children, since there is evidence suggesting that the intake of some additives expressed on a body weight basis, may be markedly higher than that of adults (Ostergaard and Knudsen, 1998; ILSI, 2003).

These additives do not accumulate in the body with ingested sorbic acid being catabolized through β-oxidation to CO_2 and H_2O while benzoic acid is transformed into more water-soluble derivatives through conjugation with glycine or glucuronic acid and eliminated in the urine (Lindsay, 1996). Benzoic acid has been implicated in allergic reactions in humans (Luck and Jager, 1997). The development of allergic reactions to benzoates in humans, such as urticaria, non-immunological contact urticaria and asthma, has been reported in some studies (Michaelsson and Juhlin, 1973; Juhlin, 1981; Hannuksela and Haahtela, 1987; Lahti *et al*., 1987; Rademaker and Forsyth, 1989; Safford *et al*., 1990). Insensitive persons, even lower doses than 5 mg kg^{-1} bwt/day can cause non-immunological contact reactions (pseudo allergy) (WHO, 2000). Recent study shown that Artificial colours or a sodium benzoate preservative (or both) in the diet result in increased hyperactivity in 3-years-old and 8/9-years-old children in the general population. The study did not try to link specific consumption with specific behaviors. The study's authors noted that other research suggested that the hyperactivity could increase in as little as an hour after artificial additives were consumed (McCann *et al*., 2007). Working with the US Food and Drug Administration (FDA), the industry found that when ascorbic acid (Vitamin C) was used as an ingredient along with sodium benzoate (a preservative), benzene formation could occur. This formation was exacerbated when the beverage was stored for extended periods at elevated temperatures (ICBA, 2006).

Sorbic acid has been reported to act as a nucleophile and to form complexes with amino compounds under normal food processing conditions with the resulting adducts being devoid of genotoxic activity (Ferrand *et al*., 2000b). Sorbic acid has low toxicity, explained by the fact that is rapidly metabolized by pathways similar to those of other fatty acids. In humans, a few cases of idiosyncratic intolerance to sorbic acid have been reported (non-immunological contact urticaria and pseudo-allergy) (Deuel *et al*., 1954; Juhlin, 1981; Hannuksela and Haahtela, 1987; Safford *et al*., 1990; Walker, 1990).

It is not applicable to extrapolate results of dietary exposure studies from a country to another because both eating behavior and manufacturing practices vary significantly across countries and regions (HMSO, 1993). Therefore, for a country like Saudi Arabia where no food consumption data or food additive level data exist, it was decided to evaluate the presence and levels of benzoic and sorbic acids in certain dairy and food categories in Saudi market then to assess the dietary exposure to benzoates and sorbate for a subgroup of the Saudi population, i.e., teenagers who are considered to be at higher risk of exceeding the ADIs of food additives.

MATERIALS AND METHODS

Samples: Fifty samples of various foodstuffs; classified to 7 categories, were obtained from local food stores. Sample sizes ranged from 10-50 g. The selected food categories were sweets (gelatin dessert and jam), beverages (blue berry, cola, energy drink, fruit drink, malt beverage), tomato products (natural tomato, tomato paste and tomato ketchup), milk and dairy products (muhalabia, cream caramel, milk fruit drink, flavored milk, flavored cream, triangles and block processed cheese, fruit yoghurt, yoghurt rice dressing, ice cream, orange yoghurt and fresh yoghurt), cereals and bread (mini rolls cake, date bar, croissant and cake caramel), snacks (potato chips, crispy corn chips and chicken flavored chips), others (mixed pickles, French fries, margarine and mayonnaise). All the collected samples were tested for the two preservatives, benzoic acid and sorbic acid.

Standards and chemicals: Commercial standards of benzoic and sorbic acids were used (Sigma, St. Louis, MO). Other reagents (analytical grade) were purchased from Merck (Merck, Darmstadt, Germany). Standards were diluted in Millipore Milli-Q water (Millipore, Billerica, MA).

Sample preparation: In general, 50 mL of water were added to 15 g sample and then stirred by glass rod to suspend test portion. The samples shacked mechanically for 5 min with 5 mL H_2SO_4 (20%) and 75 mL ether and then centrifuged for 10 min at 2000 rpm. The ether layer was transferred to separating flask and the extraction was repeated twice with 50 mL ether. The combined ether layers were extracted twice with 50 mL of 0.5M NaOH and 30 mL saturated NaCl, then the extracts were acidify to pH <1 by HCL (15%). An excessive portion of dichloromethane (CH_2Cl_2) was added to the extract and drain through 1.5 g anhydrous Na_2SO_4 in 250 mL round bottle flask and then the extract was evaporated to dryness at 40°C (AOAC, 1993).

Derivatization: Five milliliters of Chloroform ($CHCl_3$) was added to test tube with Teflon-lined screw cap to the residue and shacked for 2 min and then 1 mL of the extract was transferred to tube. Add 0.20 mL silylating agent MSTFA (N-methyl-N-trimethylsilytrifluoroacetamide) to the test tube. Screw the cap and let it stand for 15 min in an oven at 60°C (AOAC, 1993).

Identification and quantification: GC-MS QP5050 system (Shimadzu Co., Kyoto, Japan) was used, equipped as follows: gas chromatograph, masses spectrum-detector, auto ampler and vacuum pump. The used column was Rtx-5MS (crossbond 5:95% dimethyl polysiloxane), 30 m, 0.25 mm internal diameter, 0.25 μm df (Resteck capillary column Co., USA). Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was maintained at 80°C for 5 min and then increased to 230°C at rate of 2°C min^{-1} and then held for 10 min. The temperature of the injector was 250°C and of the flame ionization detector, 280°C. The split ratio used was 1:60 and a volume of 1 μL of extracts was injected for each run. The mass spectra ranged from 40-550 m e^{-1} and the ionizing voltage was 70 eV.

Detected components were identified by comparing the spectra obtained with a mass spectrum library (Wiley NBS 139, Labsolution software, drug library, Nist library) and by comparing GC retention indices versus those of using known standards. In chemical ionization, the multiple-ion monitor is set to the quasi-molecular ion, that is detected sorbic acid at m/z 113 and benzoic acid at m/z 123.

Food consumption data for evaluation of benzoic and sorbic acids intake: Food consumption data were based on a quantitative frequency questionnaire answered by 100 student subjects aged 18-25 years who recorded their food consumption during a week. All estimated intakes were adjusted for the individual's self-reported body weight (bwt = 60 kg, WHO, 1983) and expressed as daily.

RESULTS AND DISCUSSION

Levels of benzoic and sorbic acids: A method for the simultaneous determination of sorbic acid and benzoic acid using gas chromatography chemical ionization mass spectrometry is described. The two components were separated and eluted in the order of sorbic acid and then benzoic acid where the retention times were 9.15 and 12.5 min for both compounds, respectively (Fig. 1). The minimum detectable amounts of benzoic and sorbic acids were found to be 200-500 pg.

A total of 50 samples (liquid products, viscous products and solid products) were tested in this study. Table 1 and 2 shown only the samples that were found to contain (n = 18) benzoic acid or sorbic acid or both (36%).

Figure 2 was shown the chromatograms of mixed pickles, margarine and yogurt rice dressing samples containing benzoic acid, sorbic acid and both additives respectively.

The samples selected for this study were chosen to full under 7 categories. Some of these products declared the preservatives benzoic acid and/or sorbic acid on their labels and some products tested declared no preservatives on the label (Table 1 and 2). These products were tested to determine if the products were in accordance with their label claims. Most of the products tested 84% were in compliance with their labels, while 16% of the products tested deviated from their label claims. Pylypiw and Grether (2000) reported that most of the products tested were in compliance with labels, <0.1% (<1000 mg benzoate or sorbate/l), while only 4.6% of the samples deviated from their label claims.

Mean concentrations of benzoic and sorbic acids in different samples are given in Table 1 and 2. As can be shown in Table 1, the concentration levels of benzoic acid were found to vary from 3.23 mg kg^{-1} in cream caramel to 921 mg kg^{-1} in yogurt rice dressing. All the analyzed samples contained benzoic acid at percentage levels below the maximum permitted by the food legislations. Benzoic acid percentage of yogurt rice dressing was not exceeded 0.09% (Table 1). In the only available study on fruit juices in Saudi market, Alghamdi *et al.* (2005) shown that the benzoic acid mean content was found to be 16.14 mg kg^{-1}.

Sodium benzoate at a general optimum concentration of 0.1% could be used for preservation of such products as soft drinks, margarine and certain fish products (Bladwin, 1995; Srour, 1998). The upper limits of benzoate allowable in foods is about 0.1% for United States of

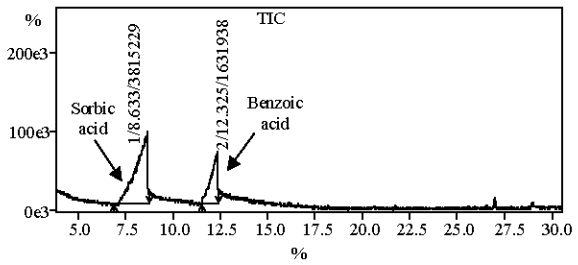

Fig. 1: Typical GC chromatogram of benzoic acid and sorbic acid standards

Table 1: Benzoic acid levels in different food categories*

Food categories	Product	Type	Maximum permitted level (mg kg^{-1} L)**	Benzoic acid declared	Benzoic acid (mg kg^{-1})	Benzoic acid (%)
Beverages	Cola	Liquid	500	Yes	3.83	0.001
	Cola light	Liquid	500	Yes	160.00	0.016
	Energy drink	Liquid	1000	Yes	129.00	0.013
	Fruit drink	Liquid	1000	Yes	150.00	0.015
	Red grape juice	Liquid	1000	No	93.00	0.010
Cereals and bread	Cake caramel	Solid	1000	No	8.84	0.001
Milk and dairy products	Cream caramel	Viscous	1000	No	2.23	0.001
	Processed cheese (block)	Semi-solid	2000	No	12.19	0.001
	Yoghurt rice dressing	Viscous	1000	Yes	921.00	0.090
Sweets	Mixed fruit jam	Viscous	1000	No	13.50	0.001
Others	Mixed pickles	Solid	1000	No	755.00	0.080

Table 2: Sorbic acid levels in different food categories*

Food categories	Product	Type	Maximum permitted level (mg kg^{-1} or L)**	Sorbic acid declared	Sorbic acid (mg kg^{-1})	Sorbic acid (%)
Beverage	Cola light	Liquid	100	Yes	6.52	0.001
Cereals and bread	Cake caramel	Solid	1000	Yes	942	0.09
	Croissant	Solid	1000	Yes	828	0.08
Milk and dairy products	Cream caramel	Solid	1000	Yes	828	0.08
	Fresh yoghurt	Viscous	600	No	0.67	<0.001
	Orange yoghurt	Viscous	600	No	0.19	<0.001
	Processed cheese (Block)	Semi-solid	1000	Yes	2275	0.23
	Processed cheese (Triangle)	Semi-solid	1000	Yes	827	0.08
	Yoghurt rice dressing	Viscous	1000	Yes	988	0.1
Sweets	Mixed fruit jam	Viscous	2000	No	2.12	0.001
Others	Margarine	Solid	1000	Yes	476	0.05
	Mayonnaise	Viscous	2000	Yes	987	0.1

*A total of 50 dairy and food products were tested, table lists only samples that contained sorbic acid; ** JECFA (1973)

America, while a range of 0.15-0.25% has been reported for other countries of the world (Chipley, 1993). For European countries, the limit report range is from 0.015-0.5% (EC, 1995). According to the food regulation issued by the Saudi Arabian standard organization, the content of benzoic acid in beverages should not exceed 0.1% of the product total weight (Alghamdi *et al*., 2005).

The levels of sorbic acid were between 0.67 mg kg^{-1} in fresh yogurt and 2275 mg kg^{-1} in processed cheese (Table 2). Sorbic acid percentage of yogurt rice dressing and mayonnaise was 0.1%, whereas in processed cheese the percentage level of sorbic acid was 0.23%. Of all the products, only the sample of processed cheese had sorbic acid in a quantity above the maximum level allowed by JECFA (1973). Potassium sorbate used at levels of 0.1-0.2% (Jones, 1992). According to the results of this study the use of both benzoic and sorbic acid was noted in different type of food, whereas, cola light, processed cheese, cake caramel, mixed fruit jam and yogurt rice dressing presented both preservatives in a wide range of concentration (Table 1 and 2).

Some of the samples were presented levels of benzoic acid or sorbic acid below the range of antimicrobial activity. For instance, cream caramel, cola, jam, processed cheese and cake caramel the detected concentration of benzoic reached 0.001%. While in case of sorbic the concentration was below 0.001% for orange yoghurt and fresh yoghurt. The minimum concentrations of benzoic and sorbic acids required to inhibit microbial growth may vary due to species, pH of the substrate and other factors. Most yeasts and molds are inhibited by 0.001-0.1% sorbic acid and by 0.002-0.07% benzoic acid (Luck, 1977).

In the present study, wide range of dairy products were analyzed for their contents of benzoic and sorbic acids. Samples contained benzoic acid had levels ranging between 12.19 (0.001%) and 921.0 mg kg^{-1} (0.09%) whereas, samples contained sorbic acid amounted in the range of 0.19 (0.001%) and 2275 mg kg^{-1} (0.23%). For dairy products, benzoic occurs naturally in yoghurt and no reference exists as to its deliberate use in the preservation of this cultured dairy product. In cultured dairy products and cheese benzoate is produced via hippuric acid, from phenylalanine degradation and could be through the auto-oxidation of benzaldehyde (Sieber *et al*., 1995). Cultured or fermented dairy products such as yogurt, fruit yogurt, sour milk with bifidobacteria and kefir contain up to 50 mg kg^{-1} (0.41 mmol kg^{-1}) benzoic acid with most mean values

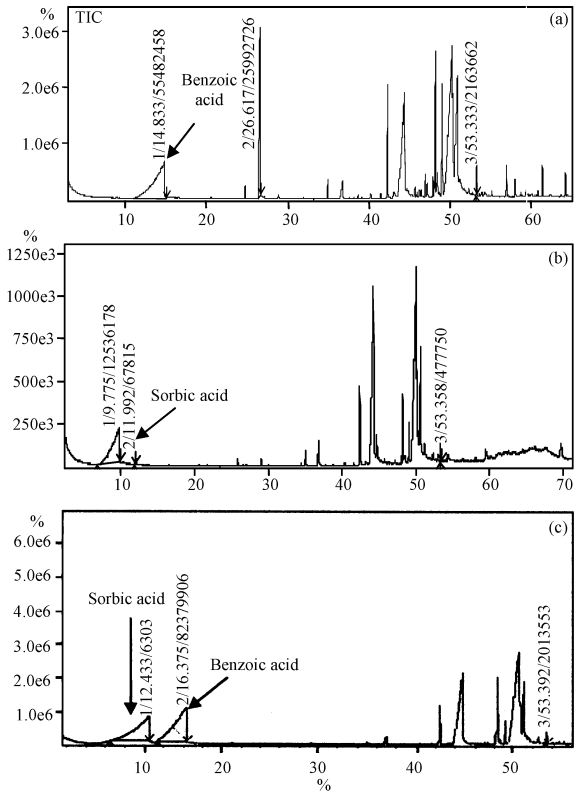

Fig. 2: Chromatograms show, a) mixed pickles contained benzoic acid, b) margarine contained sorbic acid and c) yoghurt rice dressing contained benzoic and sorbic acids

around 20 mg kg^{-1} but milk contains only a few mg/kg. Indigenous benzoic acid levels in laboratory- produced yogurt, under aseptic conditions, ranged between 5.5 and 14.7 mg kg^{-1} after storage for 4 weeks (Mrouch *et al*., 2008). References to levels of benzoic acid allowed in preservation of yogurt are difficult to locate. However, permitted levels of sorbic acid in yogurt range from 50-600-1000 mg kg^{-1} (Brazilian Standards, 1999; Turkish Standards, 1997). Sorbic acid levels up to 1000 mg kg^{-1} are allowed by the country regulations in yoghurt production presumably to ensure a reasonable commercial shelf life (Mrouch *et al*., 2008).

Estimation of the daily intakes of benzoic and sorbic acids: The purpose is to estimate the level of dietary exposure of the student population to a range of food additives; sorbate and benzoate and that can be found in the food supply. The estimated dietary exposure to each preservative from the Saudi diet was compared to international reference health standards set by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO)/Expert Committee on Food Additives (JECFA). The reference health standard for the food additives is the Acceptable Daily Intake (ADI). The ADI is the amount of a food additive that can be consumed on a daily basis over a lifetime without appreciable health risk.

The daily intakes of these investigated food additives through food consumption rely mainly on both the content levels of these food additives in the foods and the amounts consumed of these foods (Table 3 and 4).

The daily intake of benzoic acid via food ranged from 0.4 mg day^{-1} in block imported processed cheese to 310 mg day^{-1} in yoghurt rice dressing which violate maximum allowable daily intake (Table 3). Overall, the average benzoate daily intake value of different food items is less than the WHO maximum acceptable limit of 350 mg day^{-1} for average adult with 70 kg body weight ($\cong$5 mg kg^{-1} bwt/day, WHO, 1997). Alghamdi *et al*. (2005) shown that the average daily intake of sodium benzoate through the consumption of beverages in Riyadh was 6.46 mg day^{-1}. However, this study used less sensitive analysis method further, they did not use real food consumption data to estimate the ADI.

In the present study, the daily intake of sorbic acid from analyzed food groups ranged from 0.23 mg day^{-1} in fresh yogurt to 339.3 mg day^{-1} in yogurt rice dressing (Table 4), which is less than maximum acceptable limit set by different health agencies. The authorized daily intake of sorbic acid and potassium sorbate has been fixed for many years at 25 mg kg^{-1} bwt/day (JECFA, 1973). According to the WHO (1997), the acceptable daily intakes of benzoic and sorbic acids have been set at 0-5 and 0-25 mg kg^{-1} bwt/day, respectively. Among all food additive-containing foods, the highest contributor was yogurt rice dressing with values of 5.17 and 5.66 mg kg^{-1} bwt for benzoic and sorbic acids intake respectively.

The intake of benzoate and sorbat for average consumers in France (Verger *et al*., 1998), Norway (Bergsten, 2000), Brazil (Tfouni and Toledo, 2002) and Korea (Yoon *et al*., 2003) were well within the ADI limits. The estimates of total dietary intake of benzoate in China, Finland, France, Spain, UK and USA, using different methodologies revealed saturation of ADI in range of 9-28%. A conservative estimate of benzoate in the present study, through different food categories in student may saturate between 0.2-42.8% from daily consuming of cream caramel and mixed pickles, respectively. However, yoghurt rice dressing could totally saturate the ADI. In regard to sorbate saturation level of the daily ADI was between 0.004-22.6% corresponding to the consumption of orange yogurt and yoghurt rice dressing, respectively.

Table 3: The daily intake of benzoic acid from analyzed food groups

Food categories	Product	Consumption data (g day^{-1})	Daily intakes (mg day^{-1})	Daily intakes (mg kg^{-1} body weight)
Beverages	Cola	660	2.53	0.04
	Cola light	660	105.60	1.75
	Energy drink	500	64.50	1.08
	Fruit drink	660	99.00	1.65
	Red grape juice	500	46.50	0.78
Cereals and bread	Cake caramel	250	0.56	0.01
Milk and dairy products	Cream caramel	250	0.56	0.01
	Processed cheese (block)	32.5	0.40	0.01
	Yogurt rice dressing	340	310.00	5.17
Sweets	Mixed fruit jam	225	3.00	0.05
Others	Mixed pickles	170	128.40	2.14

Table 4: The daily intake of sorbic acid from analyzed food groups

Food categories	Product	Consumption data (g day^{-1})	Daily Intakes (mg day^{-1})	Daily Intakes (mg kg^{-1} body weight)
Beverages	Cola light	660	4.30	0.070
Cereals and bread	Cake caramel	72	67.80	1.130
	Croissant	50	41.40	0.700
Milk and dairy products	Cream caramel	250	4.15	0.070
	Fresh yoghurt	340	0.23	0.004
	Orange yoghurt	360	0.07	0.001
	Processed cheese (triangle)	40	33.10	0.550
	Processed cheese (block)	32.5	73.90	1.230
	Yoghurt rice dressing	340	339.30	5.660
Sweets	Mixed fruit jam	225	0.48	0.010
Others	Margarine	75	35.70	0.600
	Mayonnaise	100	98.70	1.650

CONCLUSION

It was shown that in all samples the amount of preservatives contained was much less than legal limitations. Incompliance with legal limits was encountered in processed cheese. Further, it is necessary in product such as yogurt rice dressing to decrease the additives level in order to lower the daily intake values. More inspection duets by food authorities and agencies are required to control the food label in the market and enforce the food production sector to declare the presence of additives whenever it added. Meanwhile, it seems necessary to perform a more precise intake estimation using a wide rang of population groups and more food items in order to ascertain that the whole population is situated below the ADI for benzoates and sorbates.

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