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RESEARCH PAPER

Dietary exposure assessment of Filipinos to sodium benzoate in water-based beverages

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Abstract

Sodium benzoate (SB) is a common preservative in food used to inhibit the growth of mold, yeast, and bacteria. One of the common sources of SB in the diet is processed beverages. However, excess intake of SB can be detrimental to health. Thus, the present study assessed the exposure of Filipinos to SB through the consumption of water-based beverages. The actual SB content of water-based beverages available in the Philippine market was determined using a validated reversed-phase high-performance liquid chromatography (HPLC) method. A total of seventeen samples were collected from four districts in Metro Manila for SB analysis. The SB concentration data were then combined with the consumption data from the 2008 Philippine National Nutrition Survey (NNS) to derive a dietary exposure estimate of Filipinos to SB in beverages. The dietary exposure estimates were then compared with the upper bound ADI for SB set by the Joint FAO/WHO Committee on Food Additives (JECFA) which is 20 $mg.kg^{-1}$ day^{-1}. Results have shown that the analyzed sample beverages contained SB from 1 to 286 $mg.kg^{-1}$. Filipinos were found to be exposed to SB at levels below its ADI considering average (2 to 6% ADI) and high consumption (4 to 18% ADI) of commonly consumed water-based beverages. Children ages 1.0-5.9 years had the highest risk of exposure to SB. In general, exposure of Filipinos to SB through the consumption of water-based beverages does not constitute a significant health risk. However, to better validate the level of risk of children to SB, it is recommended that the scope of the dietary exposure assessment be extended to include other food groups known to contain SB and conduct a probabilistic approach to exposure assessment using the latest consumption data from the NNS.

1. Introduction

Additives in food can be used in regulated and limited quantities provided that they have a technological need for their usage, do not mislead and present no health risk to consumers (Grembecka *et al.*, 2014) since excessive intake of additives can cause adverse physical and physiological effects in humans (Inetianbor *et al.*, 2015). Different kinds of additives are used to perform technological functions including preservation, coloring, and sweetening (Inetianbor *et al.*, 2015).

Benzoic acid and its salts are one of the commonly used food preservatives to inhibit the growth of mold, yeast, and other bacteria (Amirpour *et al.*, 2015; Azuma *et al.*, 2020). Preservatives are added to delay the onset of food degradation due to microbiological, enzymatic, and/or chemical changes, with the goal of prolonging the shelf-life and quality of food (Amirpour *et al.*, 2015; Inetianbor *et al.*, 2015).

Benzoates are commonly added in beers, fruit-based fillings, fruit juices, jam, salad cream, marinated fish, soft drinks, and soy sauces (Shahmohammadi *et al.*, 2016; Bruna *et al.,* 2018). Excess intake of benzoate has been found to provoke urticaria, angioedema, rashes, metabolic acidosis, hypernoea, and asthma (Reddy *et al.*, 2015; Shahmohammadi *et al.*, 2016) and have been linked with childhood hyperactivity (Bruna *et al.*, 2018). Similarly, benzoate in combination with ascorbic acid may induce formation of benzene which is a known carcinogen (Azuma *et al.,* 2020). The Joint FAO/WHO Committee on Food Additives (JECFA), therefore, has designated an acceptable daily intake (ADI) of 0 to 20 mg per kilogram of body weight to benzoate based on its recent meeting (FAO/WHO, 2022). ADI is defined as the amount of substance present in foods and drinks which

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can be ingested daily without risk to humans and are established for food additives that may present health risks for the consumers (Mancini *et al.*, 2015).

Dietary exposure assessments have been widely conducted as a means of analyzing and quantifying safety concerns, especially for vulnerable population groups. The results of these activities may range from crude to a more refined estimation based on the quality of occurrence and food consumption data used. Determining the actual concentration of food additives is important for both quality assurance and safety. There are different methods for analyzing the actual concentration of SB in food samples including UV spectrophotometer, reverse-phase high-performance liquid chromatography (HPLC), capillary electrophoresis, and gas chromatography-mass spectrometry (Reddy *et al.*, 2015). However, the most common analytical technique for SB analysis is HPLC (Grembecka *et al.*, 2014; Amirpour *et al.*, 2015; Aşçi *et al.*, 2016; Dinç Zor *et al.*, 2016; Akter *et al.*, 2017; Shaikh *et al.*, 2019; Mam Rashid *et al.*, 2021).

According to various dietary exposure assessment studies, beverages are one of the top contributors to benzoate exposure in different population groups (Yoon *et al.*, 2003; Soubra *et al.*, 2007; Cressey and Jones, 2009). In the Philippines, the mean consumption of beverages among Filipino households is 8 g per day with 'soft drink, cola' having the highest consumption of about 13.5% of the total number of respondents according to the 8th Philippine National Nutrition Survey (DOST-FNRI, 2015b). The study aimed to analyze SB in some of the commonly-consumed beverages available in the market using a validated HPLC method and use the generated data in deriving an exposure estimate of the Filipino population to SB.

2. Materials and methods

The study was divided into three phases as illustrated in Figure 1: sample selection; sample analysis; and conduct of exposure assessment.

2.1 Phase 1: sample selection

2.1.1 Food categorization

Philippine food items were mapped under the Codex General Standard for Food Additives (GSFA) food group 14.1.4 'Water-based flavored beverages' (WHO/FAO, 2018). The Philippine food items included were regular and non-caloric carbonated soft drinks, 'fruit-juice' flavored drink, ready-to-drink tea, and energy and sports drink.

2.1.2 Market surveys

Subsequently, market surveys were conducted in at least three supermarkets and or groceries across the four districts in Metro Manila to identify water-based beverages commonly available in the market. Food items available in these locations were assumed to be nationally distributed to different parts of the country. Per food item, nutrition information panels (NIPS) of specific brands were surveyed for incorporation of benzoate in the product formulation. A total of 90 products were surveyed for water-based beverages and 31 were found to declare sodium benzoate in the labels. However, only seventeen beverage samples surveyed were available in all of the surveyed retail markets. Almost all the samples containing benzoate were in SB form, with only one beverage containing potassium benzoate.

The final list of samples was then prepared based on the results of the market survey wherein samples with or without declared SB were included. The final list of samples collected per food product classification is summarized in Table 1.

2.2 Phase 2: analysis of sodium benzoate in beverages

2.2.1 Sampling

Samples were collected in the northern, eastern, southern, and capital districts of Metro Manila, each district with three major collection points. Around 500 - 1000 g of the food samples per collection point or 1500 - 3000 g of food samples per district were procured and collected.

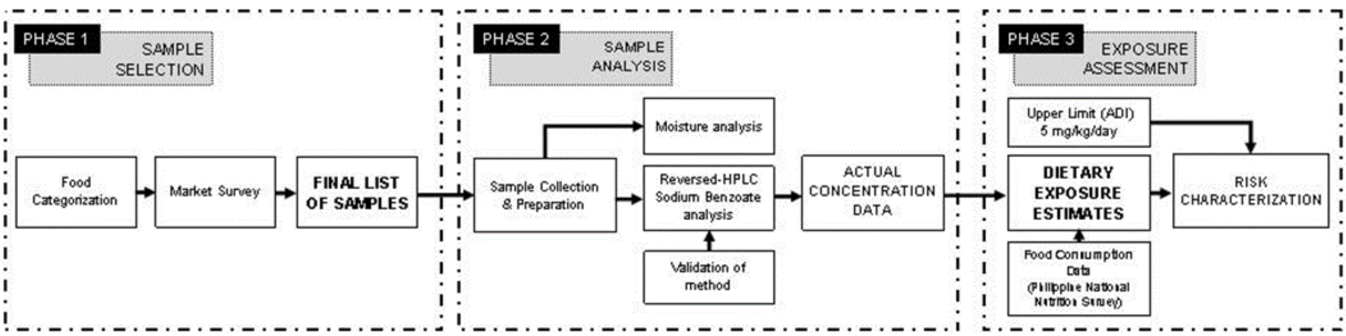

Figure 1. Conceptual framework of the study.

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2.2.1 Sample preparation

Primary samples were taken from the collected lot per district and were weighed for the net content. After weighing, samples were combined as a composite sample. The composite liquid samples per district were manually mixed in a stainless steel or glass bowl for 45 s. Analytical and contingency samples were taken from the composite sample by transferring them into labeled bottles for necessary analysis.

The bottled laboratory samples were sealed and stored in a freezer maintained at < -14°C prior to analysis.

2.2.1 Sodium benzoate analysis

The chromatographic analysis was carried out using Waters HPLC Alliance equipped as follows: automated sample injector, Deuterium Lamp Waters 2487 Detector, pump, and front panel board. The HPLC operating mode was isocratic with an injection volume of 20 µL. The chromatography column was Waters Atlantis column 4.6 × 150 mm, dC 18, 5 microns. Sample data collection was optimized to 10 mins per sample with UV detection at a wavelength of maximum absorption compounds, 254 nm for SB. The optimal mobile phase flow rate was determined at 1.0 mL/min.

The HPLC method used in the determination of SB was validated in terms of precision, specificity, accuracy, linearity, limit of detection (LOD), and limit of quantification (LOQ).

2.3 Phase 3: Estimation of dietary exposure to sodium benzoate

The dietary exposure of the various Philippine population groups to SB from commonly consumed water-based beverages was estimated by using three data components: (1) the actual concentration of SB in beverage samples from Phase 2; (2) beverage consumption data; (3) the average body weight of the Filipino population group.

The beverage consumption data was derived from the dietary component of the 7th Philippine National Nutrition Survey (NNS) conducted in 2008. The 7th NNS followed a stratified multi-stage design covering 17 regions and 79 provinces in the Philippines. The Institute's trained field interviewers were deployed to conduct a 2-day non-consecutive food recall to individuals ages 6 months and above, pregnant women, and lactating mothers. A total of 22,176 individuals were included in this round of survey (DOST-FNRI, 2008).

Equation 2 shows the relationship of the three data components used in the estimation of sodium benzoate:

$$DE_{SBi} = \frac{\sum_{wb=(1;n)} SB_{i,wb} \times FC_{wb,i}}{BW_i}$$

Where DE_{SBi} is the exposure to sodium benzoate for the population group *i,* $SB_{i,wb}$ is the actual analyzed concentration level of SB in commonly consumed water-based beverages *(wb)* by the population group *i,* $FC_{wb,i}$ is consumption level of the commonly consumed water-based beverages *(wb)* by the subject *i,* BW_i is the average body weight of the population group *i, n* is the total number of food items in a food group.

The mean SB concentration per food category was multiplied by the mean and high (95th percentile) daily food consumption of 'all subjects' and 'consumers only divided by the average body weight of the age group based on CODEX guidelines for the simple evaluation of dietary exposure to food additives (FAO/WHO, 2014). The summation of this resulted in the estimated daily exposure for 'all subjects', 'average consumers only' and 'high-level consumers only' of the Philippine population age group to SB in water-based beverages. 'All subjects' refers to all respondents in the survey which included the consuming and non-consuming population, whereas 'consumers only' included the respondents who consumed the food items of interest. The average body weights used per age group were taken from the Philippine Dietary Reference Intake (DOST-FNRI, 2015a).

Table 1. List of samples collected for sodium benzoate analysis.

Food category/product	Number of samples surveyed	Number of samples with declared benzoate in the label	Number of brands collected and tested
Fruit juice drink, orange	21	9	4
Fruit juice drink, apple	5	0	2
Lemon tea drink	14	2	2
Energy drink	8	2	2
Softdrink, cola regular	4	3	3
Softdrink, cola non-caloric (zero sugar)	3	2	1
Softdrink, orange	8	6	3

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Table 2. Linearity test, limit of detection (LOD), and limit of quantitation (LOQ) determination based on the standard deviation of the response and the slope.

n	R-Squared Value (R^2)	Correlation Coefficient (r)	Slope (B)	Intercept (A)
1	0.9999	0.9999	11182.757	1760.643
2	0.9999	0.9999	11523.848	-7041.976
3	0.9998	0.9999	11647.881	-9101.238
4	0.9999	0.9999	10813.728	1152.905
5	0.9999	0.9999	10423.838	-859.833
6	1.0000	1.000	13004.304	-208.928
7	0.9999	0.99999	11676.323	-2798.238
Mean	0.9999	0.9999	11467.526	-2422.381
LOD (µg/mL)		1.89		
LOQ (µg/mL)		7.26		

3. Results and discussion

3.1 Method validation results

Tables 2 to 5 present the obtained method validation results for the analysis of SB in water-based beverages. The parameters obtained demonstrate the fitness-for-purpose of the developed analytical method.

Table 3. Precision as within day repeatability (r).

Parameter	
Number of analyses (N replicates)	10
Mean sodium benzoate (ug/100 g)	19538.166
Standard deviation (SD)	142.165
Relative standard deviation (RSD)	0.728
Achievable coefficient of variation (ACV)	2.56
Horwitz ratio (Acceptable <2)	0.285

Table 4. Precision as with different days reproducibility (R).

Parameter	
Number of analyses (N replicates)	7
Mean sodium benzoate (ug/100 g)	19440.030
Standard deviation (SD)	179.453
Relative standard deviation (RSD)	0.908
Achievable coefficient of variation (ACV)	2.560
Horwitz ratio (Acceptable <2)	0.355

Table 5. Accuracy via recovery method.

Analyte Level (n = 7)	Actual Concentration (µg/mL)	Mean % Recovery	% RSD
Level 1, Low	23.4	98.6	2.82
Level 2, Medium	28	111	4.11
Level 3, High	32.3	101	2.5
	Acceptance Criteria	80-110%	≤11%

3.2 Levels of sodium benzoate in selected water-based beverages

The SB of commonly consumed water-based beverages are presented in Table 6. Results showed that thirteen (13) of the seventeen (17) samples analyzed contained SB at 1 to 286 mg.kg^{-1} range with a mean concentration of 84 mg.kg^{-1}. In this study, SB was detected in all samples of orange-flavored fruit drinks, apple-flavored fruit drinks, RTD lemon tea drinks, and energy drinks, at levels ranging from 8 to 286 mg.kg^{-1}, 84 to 193 mg.kg^{-1}, 10 to 148 mg.kg^{-1}, and 1 to 142 mg.kg^{-1}, respectively. Orange flavored fruit juice drink (Brand C) had the highest sodium benzoate use level at 286 mg.kg^{-1}, which exceeded the GSFA 2018 limit of 250 mg.kg^{-1} for water-based beverages. However, the Philippine limit for benzoates based on the DOH Bureau-Circular No. 2006-016 in water-based beverages remained at 1000 mg.kg^{-1} (DOH, 2006).

Results of the study by Martyn *et al*. (2017) showed that the average benzoate use level in Brazil, Canada, Mexico, and United States ranges from 169 to 197, 37 to 115, 0 to 89, 102 to 144, 0 to 39, and 56 to 74 mg.kg^{-1} for non-caloric soft drinks, regular soft drinks, fruit-juice based drinks, energy drinks, sport drinks, and ready-to-drink teas, respectively. The majority of the population groups among the countries mentioned highly consumed low-caloric soft drinks which also contained the highest amount of benzoate among other food groups.

In other countries, the major sources of benzoates in their diet came from water-based beverages. In Lebanon, results from the study by Soubra *et al.* (2007) showed that the highest benzoate contribution came from canned juices (35%) and sodas (20%). Yoon *et al.* (2003), meanwhile assessed the estimated daily intakes (EDI) of benzoate for average and high consumers in Korea and significant sources of benzoates in their diets were from mixed beverages and soy sauces. In New Zealand, soft drinks were the main contributor to benzoate dietary exposure in all population (ages 5 years to 65+ years) groups (Cressey and Jones, 2009).

Table 6 also shows that in five (5) beverage samples, SB was not declared on the NIP, but with detectable levels of SB at 1.23 to 148 mg.kg^{-1} range. Although SB occurs in these beverage samples within the designated regulatory limit, this finding shows that much is still to be done in the implementation and monitoring of

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compliance with labeling requirements for manufacturers. Non-detects were treated with zero value in the calculation for DEA (section 3.3).

Table 6. Sodium benzoate (mg.kg^{-1}) of commonly consumed water-based beverages in the local Philippine market per district.

Food Grouping	Food Item	Sodium benzoate (mg kg^{-1})	Benzoate declared in label (Y/N)
Fruit juice drink, orange	Brand A	139.07±15.05^{a}	Y
	Brand B	149.84±12.78^{a}	Y
	Brand C	285.69±4.91^{b}	Y
	Brand D	8.39±0.06^{c}	N
Fruit juice drink, apple	Brand E	83.77±2.26^{a}	N
	Brand F	192.58±10.78^{b}	Y
Lemon tea drink	Brand G	10.69±0.20^{a}	N
	Brand H	148.09±1.69^{b}	N
Energy drink	Brand I	1.23±0.03^{a}	N
	Brand J	142.23±14.18^{b}	Y
Softdrink, cola	Brand K	ND	N
	Brand L	ND	N
	Brand M	48.88±6.00^{a}	Y
	Brand N	ND	Y
Softdrink, orange	Brand O	ND	N
	Brand P	123.29±6.62^{a}	Y
	Brand Q	93.97±8.90^{b}	Y

Values are presented as mean±SD, n = 3. Values with different superscripts are statistically significantly different (α = 0.05) according to LSD. ND: Not detected.

3.3 Dietary exposure estimates of Filipinos to sodium benzoate

The dietary exposure estimates of the Philippine population groups to SB based on the 2008 food consumption data and the actual SB levels in selected water-based beverages are shown in Table 7. Results have shown that mean daily SB intake of all subjects from the whole group was at 0.17 mg kg^{-1} bw (0.84% ADI), while the intake of the various age groups ranges from 0.07 mg.kg^{-1} bw (0.34% ADI) to 0.42 mg.kg^{-1} bw (2.12% ADI), with elderly >70.0 years old having the lowest intake and children 3.0 - 5.9 years old with the highest intake.

For the mean consumers, the daily intake of SB for the whole group was 0.51 mg.kg^{-1} bw (2.56% ADI), while the intake of the consumers among various age groups ranges from 0.38 mg.kg^{-1} bw (0.34% ADI) to 1.27 mg.kg^{-1} bw (6.36% ADI), with the same trend of elderly > 70.0 years old having the lowest intake and children 3.0 - 5.9 years old with the highest intake.

For high-level consumers (P95th), the mean daily intake of SB for the whole group was 1.40 mg.kg^{-1} bw (7.02% ADI), while the intake of the consumers among various age groups ranges from 0.88 mg.kg^{-1} bw (4.40% ADI) to 3.55 mg.kg^{-1} bw (17.75% ADI), with elderly > 70.0 years and children 1.0 - 2.9 years old with the lowest and highest intake, respectively.

Children have higher exposure to SB in water-based beverages compared with adolescents and adults due to their lower body weight of 11.0 to 36.0 kg compared with the average 55.0 kg. Meanwhile, the elderly >70.0 years have lower exposure to SB due to lower consumption of water-based beverages.

In the study by Martyn *et al.* (2017), the mean exposure to benzoates from non-alcoholic beverages of the total population or whole group from Brazil, Canada, Mexico, and the United States ranges from 0.237 to 1.240 mg.kg^{-1} bw.day^{-1} (5 to 25% ADI) using probabilistic and brand loyal model. Mean benzoate exposure of the total population among toddlers and young children (1 to 7 years old) ranges from 0.244 to 1.570 mg.kg^{-1} bw.day^{-1} (5 to 31%ADI). Similar to the results of this study, children from Brazil and Mexico

Table 7. Estimated exposure (mg.day^{-1}, mg kg^{-1} bw day^{-1}, and% ADI) of different age groups to sodium benzoates in commonly consumed and available water-based beverages in the local Philippine market.

Population Group	Dietary sodium benzoate exposure to water-based beverages								
	(mg day^{-1})			(mg kg^{-1} bw day^{-1})			(%ADI)		
	All Subjects	Consumers Only		All Subjects	Consumers Only		All Subjects	Consumers Only	
	Mean	Mean	95th	Mean	Mean	95th	Mean	Mean	95th
Whole Group	9.24	28.12	77.19	0.17	0.51	1.40	0.84	2.56	7.02
Children 1.0 - 2.9	3.00	14.45	40.82	0.26	1.26	3.55	1.30	6.28	17.75
Children 3.0 - 5.9	7.20	21.63	59.10	0.42	1.27	3.48	2.12	6.36	17.38
Children 6.0 - 9.9	7.12	21.83	62.44	0.32	0.97	2.78	1.58	4.85	13.88
Adolescents 10.0-12.9	8.11	24.91	66.80	0.23	0.69	1.86	1.13	3.46	9.28
Adolescents 13.0-15.9	10.41	28.84	71.63	0.23	0.63	1.56	1.13	3.13	7.79
Adolescents 16.0-18.9	11.86	31.60	84.80	0.23	0.61	1.65	1.15	3.07	8.23
Adults 19.0-29.9	13.91	13.91	89.94	0.26	0.64	1.71	1.32	3.20	8.57
Adults 30.0-49.9	10.56	30.72	81.55	0.20	0.59	1.55	1.01	2.93	7.77
Adults 50.0-69.9	7.16	26.63	71.63	0.14	0.51	1.36	0.68	2.54	6.82
Elderly >70.0	3.55	20.13	46.20	0.07	0.38	0.88	0.34	1.92	4.40

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have higher exposure to SB compared with adults (18+ years).

4. Conclusion

The present study was able to validate an effective method to determine the amount of SB present in commonly consumed water-based beverages. Combining the analytical data with the available food consumption data, the exposure estimates of Filipino population groups to SB were calculated. The risk of exposure was characterized by comparing the calculated estimates with the ADI of SB.

Based on the findings, Filipinos are exposed at levels below the ADI of SB for water-based beverages, therefore, indicating no safety concern. However, due to lower body weights, consumers among children ages 3.0 – 5.9 years old were identified as the most susceptible group due to the highest SB intake.

To better estimate the potential risk associated with dietary exposure to SB, it is therefore recommended that the scope of SB analysis be extended to other commonly consumed commodities known to contain the preservative like soy sauce and cakes. Furthermore, the conduct of a probabilistic and brand-loyal model approach to exposure assessment especially for children will result in more refined exposure estimates.

Conflict of interest

The authors declare that there are no competing interests to declare

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