Original Article

Effect of storage on the levels of sodium benzoate in soft drinks sold in some Nigerian market with exposure and health risk assessment

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Abstract

Soft drinks consumption is still a controversial issue for public health and public policy, as the influence storage condition impacts taste, color and shelf life. In all, fifty (50) soft drinks samples, acquired from Enugu, Aba, Asaba, Onitsha and Owerri markets in Nigeria, were subjected to four different storage conditions namely: room temperature (RT), refrigerated (RF), 40 °C and 60 °C for 15 days after which they were analyzed for sodium benzoate concentration using HPLC – UV/Vis detector. The results showed on the average that at RT and RF, soft drinks from Aba had the highest concentration of sodium benzoate (98.7 mg/L and 112.9 mg/L) respectively while samples from Asaba had least concentration of 39.9 mg/L and 38.1 mg/L. At increased temperature of 40 °C, the concentration of sodium benzoate increased generally across the sample, while at 60 °C, the levels in all the samples analyzed were either reduced to less than 50% or below detection level, which suggest that degradation of sodium benzoate at this elevated temperature could result in benzene formation, which is a known carcinogen. Carcinogenic and non-carcinogenic risk assessment showed that children are at risk compared to adults due to higher sodium benzoate daily intake leading to high rate of hyperactivity in correlation to malaise.

Keywords: soft drinks, sodium benzoates, storage conditions, high pressure liquid chromatography, risk assessment

Introduction

Artificial sweeteners, preservatives and dyes are widely added to soft drinks either to enhance the flavor and appearance or to extend their shelf life by preventing biological degradation [1]. Soft drinks have become part of the global lifestyle since the 19th century and many of the soft drinks being consumed at present are the same as those first enjoyed in centuries ago. The ingredients used in soft drink beverages are approved and closely regulated by bodies such as the US Food and Drug Administrator or the food standard agencies in respective countries [2]. Since several of these additives are prepared by chemical synthesis, their presence in food and drinks is the cause of extensive consumer's mistrust, in that they may exhibit adverse effects on their health over a long period [3,4]. Consequently, many governments' entities have set threshold value for the Acceptable Daily Intake (ADI) varying from country to country [5,6].

Packaged food and drinks are one of the fastest growing industries in the present era. Numerous forms of preservation techniques such as pasteurization, freezing, drying and application of chemicals have been designed to extend the which is aimed at extending the shelf life and quality of these products, reduce microbial contamination and also maintain antioxidant potential to serve customers need [6,7].

Several food and drink preservatives have been developed via physical, chemical, or biological methods or combined to maintain the shelf life such as acidification, pH control water and air removal, temperature control, freezing, irradiation, fermentation, sterilization or pasteurization [8,9]. Usually, diverse additives and preservatives such as sweeteners, carbon dioxide, acidulants, flavorings, coloring, chemical preservatives, antioxidant, and/or foaming agents are added to soft drinks to maintain taste, color and shelf life of such products [10].

Beverage manufacturers recommend that soft drinks products should be stored in room temperature (20-30 °C). This may not be realized in Nigerian weather as temperature often overshoots to 36-40 °C across varying locations [11,12]. Due to such temperature, physiochemical and biological reactions take place producing harmful products. For example, claims have been made that if Sodium benzoate and vitamin C is exposed to elevated temperature or sunlight, it forms benzene which is a Group 1 cancer causing chemical as labelled by International Agency for Research on Cancer, IARC [13,14]. Preliminary examination of various storage spaces where soft drinks are stored indicated that in most cases, soft

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drinks are left in open places usually under the sun thereby increasing the possibility for high benzene formation over a period of time. The aim of the study is to assess the effect of storage condition and risk assessment of sodium benzoate concentration in different soft drinks brands sold in Nigeria.

Materials and Methods

Sodium benzoate reference standard was purchased from Merck, assay 99%. Acetonitrile HPLC grade (Merck, assay 99%). Deionized water concentrated phosphoric acid (Buffer pH adjustment) and Potassium dihydrogen orthophosphate (AR grade), which were all sourced and supplied by Merck Nigeria. Elite Lachrome High Pressure Liquid Chromatography equipped with Ultraviolet/Visible spectrophotometer detector was used for calibration and quantitative measurement, Seven Excellence Metler Toledo pH meter, Metler Toledo analytical balance, Refrigerator, Ultrasonic bath and Carbolite oven were all used for the research work.

Sample collection

Fifty different types of soft drinks were purchased in dozen (12) from Enugu, Aba, Asaba, Onitsha and Owerri market in Nigeria as shown in Table 1. The samples were well labelled for proper identification and taken to National Agency for Food and Drug Administration and Control, NAFDAC Agulu Laboratory Services, Agulu, Anambra State, Nigeria for analysis. Triplicate samples were stored in different preservation condition such as room temperature (RT), refrigerated (RF), oven (40 °C and 60 °C) for 15 days.

	Table 1. Soft drinks according	g to pla	ce of purchase	brand identity and	packaging quantity.
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S/N	Enugu	Owerri	Asaba	Aba	Onitsha
1	FFD (500 mL)	1FPD (350 mL)	STW (500 mL)	CD (330 mL)	FPGF (500 mL)
2	MPF (500 mL)	RA (500 mL)	SCS (500 mL)	MCD (750 mL)	PSFD (250 mL)
3	NBBLE (500 mL)	LCAD (500 mL)	VSBRFD (500 mL)	FCNAGFD (750 mL)	PCFD (250 mL)
4	2CTMF (500 mL)	NHFD (750 mL)	BTF (500 mL)	PDMFD (750 mL)	HH (180 mL)
5	GJOFD (3000 mL)	ESCD (500 mL)	SBL (500 mL)	CAFD (750 mL)	POFD (250 mL)
6	RP (500 mL)	FCGFD (750 mL)	FED (500 mL)	DSRGFJ (750 mL)	PPFD (250 mL)
7	RO (500 mL)	BBD (1000 mL)	SFD (500 mL)	MBHFD (500 mL)	MFHPFD (230 mL)
8	SD (350 mL)	BLC (1000 mL)	TT (500 mL)		
9	FD (350 mL)	BOD (1000 mL)	SLD (500 mL)		
10		JOFD (3000 mL)	SU (500 mL)		
11		FFN (250 mL)	PC (500 mL)		
12		CSFD (200 mL)	CC (350 mL)		
13			SCNAD (500 mL)		
14			BBL (500 mL)		
15			Hoh (500 mL)		

Preparation of standards

Preparation of buffer pH 3.5 solution

1.70 g of potassium dihydrogen orthophosphate were weighed into 1000 mL volumetric flask, 750 mL of deionized water were added and shake for dissolution. pH was checked and adjusted to 3.5 with phosphoric acid and later made up to mark with deionized water. The mobile phase was prepared by mixing equal volume of 500 mL of acetonitrile and buffer pH 3.5 solutions into a 1000 mL volumetric flask and sonicated for 30 minutes for degassing.

Preparation of stock solution

50 mg of sodium benzoate was weighed into 50 mL volumetric flask, about 20 mL of deionized water added, sonicated for 10 minutes and later made up to mark with deionized water to get 1 mg/mL (1000 ppm) concentration accordingly. High Pressure Liquid chromatography (HPLC) calibration was done by pipetting 0.1, 0.3, 0.4, 0.5 and 0.6 mL of stock solution into different 10 mL flask with deionized water to obtain 10, 30, 40, 50 and 60 ppm. These samples were filtered through $0.45 \mu m$ pore size membrane filter and filled into auto sampler vial for injection. Calibration curve of concentration was plotted against area to get slope.

Determination of sodium benzoate

The samples (soft drinks) were aspirated into HPLC equipped with Ultraviolet/Visible spectrophotometer detector for qualitative and quantitative analysis of sodium benzoate at different storage condition [15]. Each sample was

shaken for proper homogenization and the gas allowed to settle. 20 mL of sample was measure into 100 mL volumetric flask, 50 mL deionized water added and sonicated for 5 minutes, and was later made to 100 mL mark. The resultant solution was filtered using membrane filter with pore size of 0.45 μ m and transferred to sample vial for injection. The samples were analyzed using HPLC with wavelength of 220 nm, flow rate of 0.8 mL/minutes, injection volume 20 μ L, column temperature set at ambient, diluent being deionized water, mobile phase was 500 mL acetonitrile with 500 mL buffer 3.5 solution (50%: 50%) [16]. The concentration of sodium benzoate, which produced a regression of 0.9997 were calculated using the equation below:

$$Concentration (mg/L) = mg/mL (from calibration curve) \times 1000$$
(1)

Human exposure and health risk assessment

Risk assessment is a systematic toolkit used to estimate carcinogenic and non-carcinogenic health effect across diverse exposure medium [17]. Estimated exposure dose (EED) was considered to assess sodium benzoate content across different soft drinks sold in selected markets in Nigeria using the equation (2):

$$EED = \left(\frac{(C_x \times IR)}{BW} \right)$$
(2)

where, EED is predictable exposure dose of soft drinks (mg/kg/day); Cx is sodium benzoate concentration (mg L⁻¹) in soft drinks; IR is ingestion rate (1.2 L/day for adults and 0.6 L/day for children); BW is body weight (70 kg for adults and 15 kg for children).

The EED results were analyzed to assess non-carcinogenic health effect (HQ) and carcinogenic health effect (ECR) using the formula [18,20,21]:

$$HQ = (EED/_{RfD})$$
(3)
$$ECR = (BMDL/_{EED})$$
(4)

where, HQ is hazard quotient, a non-carcinogenic risk that expresses probable health effect (unit-less); RfD is reference dose of sodium benzoate (4.00 mg/kg/day); ECR is excess cancer risk used to characterize carcinogenic effect to oral exposure of sodium benzoate; BMDL is bench mark dose lower limit (17.60 mg/kg/day).

Results and Discussion

Sodium benzoate concentration

In this study, HPLC separation techniques were used for determination of sodium benzoate in selected soft drinks samples across different storage condition and locations.

Soft drinks in Enugu, Nigeria

The result of analysis of nine soft drinks for level of sodium benzoate in Enugu, Nigeria is presented in Table 2. Considering nine (9) different types of soft drinks, at room temperature, RT, NBBLE has the highest concentration of sodium benzoate (179 mg L⁻¹) with RO having the least (26 mg L⁻¹). At refrigeration, RF preservation condition, NBBLE also has the highest concentration of 112 mg L⁻¹ with RP and SD having the lowest of 28 mg L⁻¹. At 40 °C preservation condition, GJOFD has the highest concentration of 128 mg L⁻¹, with RP having the least (32 mg L⁻¹). At 60 °C preservation condition, the concentration of sodium benzoate in the samples all reduced drastically (with the exemption of SD with 102 mg L⁻¹), with the least being RP (15 mg L⁻¹). In all the preservation conditions, none of the samples has quantity of sodium benzoate higher than approved limit of 250 mg L⁻¹. This is in tandem with the result presented by Badamasi et al. [22] in Jigawa State, Nigeria.

 Table 2. Result of sodium benzoate in soft drinks samples obtained from Enugu, Nigeria.

S/N	Sample code	RT (mg L ⁻¹)	RF (mg L ⁻¹)	40 °C (mg L-1)	60 °C (mg L-1)
1	FFD (500 mL)	64	58	67	25
2	MPF (500 mL)	90.2	85	65	25
3	NBBLE (500 mL)	179	112	53	19
4	2CTMF (500 mL)	62.5	61.2	70	24
5	GJOFD (3000 mL)	81.8	62.1	128	53
6	RP (500 mL)	40	28	32	15
7	RO (500 mL)	26	40	48	21
8	SD (350 mL)	30	28	67	102
9	FD (350 mL)	32	37	72	45

RT=Room Temperature @29.6°C, RF=Refrigeration @8.2 °C

Soft drinks in Owerri, Nigeria

The result of analysis of twelve soft drinks for level of sodium benzoate in Owerri, Nigeria is presented in Table 3. This shows that ESCD has the highest concentration of sodium benzoate (242 mg L^{-1}) at RT with 1FPD and RA having the lowest (18 mg L⁻¹). ESCD has the highest concentration at all preservation conditions. JOFD has the lowest concentrations of sodium benzoate in all preservation conditions (17 mg L^{-1} in RF, 23 mg L⁻¹ in 40 °C and 14 mg L⁻¹ in 60 °C) with exemption of RT where it has second lowest concentration of 25 mg L⁻¹. It is noticeable that there was general increase in the concentration of sodium benzoate in all the samples at 40 °C (with ESCD having the highest of 584 mg L⁻¹), while sharp decrease was observed at 60 °C, showing there was degradation of sodium benzoate at that temperature.

S/N	Name of product	RT (mg L-1)	RF (mg L-1)	40 °C (mg L-1)	60 °C (mg L-1)
1	1FPD (350 mL)	18	31	47	19
2	RA (500 mL)	18	20	49	17
3	LCAD (500 mL)	26	25	50	14
4	NHFD (750 mL)	93	95	310	81
5	ESCD (500 mL)	242	275	584	112
6	FCGFD (750 mL)	102	115	290	83
7	BBD (1000 mL)	70	90	203	73
8	BLC (1000 mL)	193	171	267	111
9	BOD (1000 mL)	131	69	197	84
10	JOFD (3000 mL)	25	17	23	14
11	FFN (250 mL)	42	42	81	34
12	CSFD (200 mL)	30	30	80	25

Table 3. Result of sodium benzoate in soft drinks samples obtained from Owerri, Nigeria.

RT=Room Temperature @29.6 °C, RF=Refrigeration @8.2 °C.

Soft drinks in Asaba, Nigeria

The result of analysis of fifteen soft drinks for levels of sodium benzoate in Asaba, Nigeria is presented in Table 4. Sodium benzoate concentration was not detected in PC and CC at RT, RF and 60 °C. However, negligible concentrations of 17 mg L⁻¹ and 2 mg L⁻¹ respectively were detected at 40 °C. SCS has the highest concentrations of 69 mg L⁻¹ at RT, 78 mg L⁻¹ at RF, 115 mg L⁻¹ at 40 °C and 51 mg L⁻¹ at 60 °C. The excessive high level at 40 °C can be attributed to formation of more sodium benzoate because of the presence of soda in SCS drink. General decrease in the amount of sodium benzoate is noticed at 60 °C, this complies with the trend, on the assumption that sodium benzoate degrades to benzene at high temperature like 60 °C.

Table 4. Result of sodium benzoate in soft drinks samples obtained from Asaba, Nigeria.

S/N	Sample code	RT (mg L-1)	RF (mg L-1)	40°C (mg L-1)	60°C (mg L-1)
1	STW (500 mL)	52	54	48	21
2	SCS (500 mL)	69	78	115	51
3	VSBRFD (500 mL)	28	36	29	25
4	BTF (500 mL)	45	25	67	25
5	SBL (500 mL)	23	30	26	09
6	FED (500 mL)	50	34	68	28
7	SFD (500 mL)	28	30	58	18
8	TT (500 mL)	42	47	62	25
9	SLD (500 mL)	21	24	29	14
10	SU (500 mL)	34	38	41	19
11	PC (500 mL)	-	-	17	-
12	CC (350 mL)	-	-	02	-
13	SCNAD (500 mL)	38	24	57	22
14	BBL (500 mL)	58	43	59	23
15	Hoh (500 mL)	31	32	57	17

Soft drinks in Aba, Nigeria

The result of analysis of seven soft drinks for levels of sodium benzoate in Aba, Nigeria is presented in Table 5. There was general increase in the concentrations of sodium benzoate in all the samples at 40 °C. At 60 °C, the concentrations

dropped generally, with MBHFD having the least (14 mg L⁻¹). PDMFD the highest concentrations on 161 mg L⁻¹ at RT, 193 mg L⁻¹ at RF, 475 mg L⁻¹ at 40 °C and 131 mg L⁻¹ at 60 °C. At RT, DSRGFJ has the least concentration of 46 mg L⁻¹ while CD has the least of 36 mg L⁻¹ at RF condition.

S/N	Sample code	RT (mg L-1)	RF (mg L-1)	40 °C (mg L-1)	60 °C (mg L-1)
1	CD (330 mL)	70	36	139	39
2	MCD (750 mL)	111	133	296	112
3	FCNAGFD (750 mL)	70	52	233	68
4	PDMFD (750 mL)	161	193	475	131
5	CAFD (750 mL)	122	189	418	112
6	DSRGFJ (750 mL)	46	68	126	41
7	MBHFD (500 mL)	111	119	295	14

Table 5. Result of sodium benzoate in soft drinks samples obtained from Aba, Nigeria.

RT=Room Temperature @29.6 °C, RF=Refrigeration @8.2 °C

Soft drinks in Onitsha, Nigeria.

The result of analysis of seven soft drinks for levels of sodium benzoate in Onitsha, Nigeria is presented in Table 6. Upon examination of the table, at RT, it was observed that FPGF has the highest concentration of sodium benzoate (116 mg L⁻¹), MFHPFD has the least (27 mg L⁻¹). There was general steady reduction (with exemption of MFHPFD, which has 42 mg L⁻¹) in the concentrations at RF preservation condition when compared with RT preservation condition. General increment (except FPGF and HH) in the concentration was observed at 40 °C. In consonance with the trend, general decrease in concentration of sodium benzoate was observed when the samples were subjected to preservation condition of 60 °C.

S/N	Sample code	RT (mg L-1)	RF (mg L-1)	40 °C (mg L-1)	60 °C (mg L-1)
1	FPGF (500 mL)	116	64	60	12
2	PSFD (250 mL)	37	33	46	25
3	PCFD (250 mL)	42	24	83	27
4	HH (180 mL)	43	43	01	00
5	POFD (250 mL)	42	33	88	24
6	PPFD (250 mL)	45	29	58	24
7	MFHPFD (230 mL)	27	42	63	24

Table 6. Result of sodium benzoate in soft drinks samples obtained from Onitsha, Nigeria.

RT=Room Temperature @29.6 °C, RF=Refrigeration @8.2 °C.

Effect of storage condition on sodium benzoate

Sodium benzoate was found to vary across different storage conditions. The World Health Organization, WHO limit for benzoate (benzoic acid) is 250 mg L^{-1} [23]. Our findings showed that some samples had extremely high concentration of sodium benzoates. This increases the possibility of benzoic acid formation in the presence of light or heat, which further produces benzene as shown in the equations below:

	Heat or Light	
Sodium Benzoate + acids additives	\longleftrightarrow Benzoic acid + sodium acid salts	(5)

Benzoic acid $\xrightarrow{\text{Heat or Light}}$ Benzene + carbondioxide

Several studies by Onwordi et al. [24] confirmed high level of benzoic acid of about 71.4%, in agreement to Venu and Austin. [25], Amponsah [26] and Mazdeh et al. [27] assessments, which implies that high storage conditions above room temperature have potential to increase benzoic acid concentration that is detrimental to human health and wellbeing in addition to benzene formation, a known carcinogen over a period of time. As seen in equation (5) and (6), food grade acidulants such as dilute citric acid and sodium citrate are added for preservatives and taste can be impacted by temperature change. So therefore, when high to extreme temperature influences the soft drink beverages, it can lead to decrease in sodium benzoate, as seen in Table 2-6 implying that further reaction to benzene is formed [12,48].

Benzoates are categorized into E210-E219 used as food labels according to international numbering system (INS) [28,29]. Benzoates are used as preservative for antimicrobial mitigation with antioxidant properties in soft drinks by increasing acidity, when dissolved in water [30,31].

Sodium benzoates is used for treatment of urinary cycle disorder (UCD) in newborn [32], it is also used in treatment and/or reduction of hyperammonemia in humans, that is breakdown of amino acid to ammonia is rapidly

(6)

converted to urea, which is excreted as urine, as ammonia is highly toxic to nerve cells [33,34]. Several studies have indicated that sodium benzoates have anti-inflammatory properties and neurological protection to humans [35-37].

Sodium benzoates has negative adverse effects to human health in high concentration, when consumed, it has negative effect on asthmatic patients causing rhinitis, chronic urticarial and as benzoates can cause brain damage [13,38,39]. Also, high benzene in the body is carcinogenic, which is formed during storage condition influenced by high temperature through decarboxylation of benzoates by hydroxyl radicals as seen in equation 5 and 6 [40]. Elevated temperature and ultraviolet light initiates or accelerate sugar degradation and hydroxyl radical formation by metallic ions, as recommendation by WHO/FDA and other international agencies has recommended use of potassium benzoates that is least reactive compared to sodium benzoates [12,41].

Non-carcinogenic risk assessment

Non-carcinogenic assessment was conducted for oral exposure to sodium benzoates in adults and children at different storage condition as shown in Table 7. It shows the estimated exposure dose and hazard quotient calculated from sodium benzoate concentration values. The values were analyzed for adults and children at different storage condition that is room temperature (RT), refrigeration and 40 °C, as temperature above 60 °C is not possible except direct exposure to external heat source (boiling).

Preliminary assessment shows that Estimated Exposure dose, EED values for children were higher than adults across all locations as higher values are attributed to sodium benzoate detected at different storage conditions. The hazard quotient analyzed depicts that values above one (1) shows probable health effect due to consumption of sodium benzoate, as we can see, children are at risk compared to adults across exposed population over a period of time [19,42].

Recipients	Locations	Estimated expo	osure dose (EED)	(mg kg-1 day-1)	Haz	ard Quotient (HQ)
		RT	RF	40 °C	RT	RF	40 °C
	Enugu	0.45-3.07	0.48-1.92	0.55-2.19	0.11-0.77	0.12-0.48	0.14-0.55
Adults	Owerri	0.31-4.15	0.29-4.71	0.39-10.00	0.08-1.04	0.07-1.18	0.10-2.50
Adults	Asaba	0.36-1.18	0.41-1.34	0.03-1.97	0.00-0.30	0.00-0.33	0.01-0.49
	Aba	0.79-2.76	0.62-3.31	2.16-8.14	0.20-0.69	0.15-0.83	0.54-2.04
	Onitsha	0.46-1.99	0.41-1.10	0.02-1.51	0.12-0.50	0.10-0.27	0.00-0.38
	Enugu	1.04-7.16	1.12-4.48	1.28-5.12	0.26-1.79	0.28-1.12	0.32-1.28
Children	Owerri	0.72-9.68	0.68-11.00	0.92-23.36	0.18-2.42	0.17-2.75	0.23-5.84
Children	Asaba	0.84-2.76	0.96-3.12	0.08-4.60	0.21-0.69	0.24-0.78	0.02-1.15
	Aba	1.84-6.44	1.44-7.72	5.04-19.00	0.46-1.61	0.36-1.93	1.26-4.75
	Onitsha	1.08-4.64	0.96-2.56	0.04-3.52	0.27-1.16	0.24-0.64	0.01-0.88

Table 7. Range of estimated exposure dose and hazard quotient of sodium benzoate across different soft drinks.

Values presented as: minimum-maximum

Carcinogenic risk assessment

Carcinogenic risk assessment was conducted using excess cancer risk (ECR), which deals with carcinogenic and genotoxic influence. Table 8 shows the margin of exposure calculated from estimated exposure dose for different soft drinks across different locations. In terms of locations, consumption of soft drinks from Owerri and Aba is very likely compared to other locations. The ECR shows carcinogenic and genotoxic influence of benzene from consumption of soft drinks with sodium benzoate concentration [20], that is within USEPA standard of 10,000-1,000,000 for exposed population [43]. Although, ECR values were okay for the population size, that is ECR<10,000, it shows that adults are more at risk compared to children for Asaba and Onitsha. According to USEPA carcinogenic range (10,000-1,000,000) is desirable to prevent carcinogenic health effect over a population; so therefore, adults are at risk compared to children as ECR values were less than 10,000 raise health concerns to consumption of sodium benzoate [18,20,44,45].

Expert has correlated that food and drinks causes adverse health effect over a lifetime leading to cancer related issues. Chemical precursors of benzene such as benzoates and benzoic acid triggers hyperactivity in children, as other related health effect includes malaise (vomiting, headaches, body weakness, esophagus irritation), pseudo-allergy, urticarial and DNA changes [23,46-48]. World health organization (WHO) review shows that sodium benzoate symptoms are relatively mild and fade-off within few hours but can be adverse health effect over prolong consumption of soft drinks [23, 47-50].

Recipient	Locations		Excess cancer risk (E0	CR)
		RT	RF	40 °C
	Enugu	5.74-39.49	9.17-36.67	8.02-32.08
Adults	Owerri	4.24-57.04	3.73-60.39	1.76-44.64
Adults	Asaba	14.9-48.89	13.20-42.78	8.93-513.33
	Aba	6.38-22.32	5.32-28.52	2.16-8.15
	Onitsha	8.85-38.02	16.00-42.78	11.70-1026.67
	Enugu	2.46-16.92	3.93-15.71	3.44-13.75
Children	Owerri	1.82-24.44	1.60-25.88	0.75-19.13
Children	Asaba	6.38-20.95	5.64-18.33	3.83-220
	Aba	2.73-9.57	2.28-12.22	0.93-3.49
	Onitsha	3.79-16.30	6.88-18.33	5.00-440

Table 8. Range of margin of exposure of sodium benzoate across different soft drinks.

Conclusions

Having assessed the influence of storage condition, we observed on the average that room temperature showed Aba having high level of sodium benzoates (98.7 mg L⁻¹) while Asaba has least value (39.9 mg L⁻¹). In the same trend, refrigerated soft drinks showed that Aba has high sodium benzoate (112 mg L⁻¹), while Asaba has least concentration (49 mg L⁻¹). For 40 °C and 60 °C, Aba have samples with highest sodium benzoate concentration (283.1 mg L⁻¹ and 73.9 mg L⁻¹) while Asaba and Onisha were least in concentration (49 mg L⁻¹ and 19.4 mg L⁻¹ respectively). In addition, low sodium benzoate concentration was observed across all samples at elevated temperature of 60 °C, which implies that sodium benzoate degrades, forming benzene that is toxic to human health over a long period. Non-carcinogenic risk assessment showed that children are at risk compared to adults as HQ were greater than one across all samples, while carcinogenic risk assessment depicts that adults are risk compared to children from genotoxic and carcinogen influence of benzene precursors (benzoic acid and benzoates). We recommend that all food and beverage manufacturers should look into different additives that have similar property to sodium benzoate like potassium benzoates and other relevant stakeholders look into the extreme concentration of sodium benzoates in all food and drugs products to mitigate adverse health complications to humans. Finally, packaging labels should state that food and drugs products to mitigate adverse health complications to humans. Finally, packaging labels should state that food and drugs products to mitigate adverse health complications to heat to preserve taste and color.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT author statement

PAC: Conceptualization, Supervision, Methodology; JAS: Software, Investigation, Data Curation, Writing- Original draft preparation; DOO: Visualization, Writing- Reviewing and Editing.

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References

 Seyinde DO, Ejidike IP, Ayejuyo S. HPLC Determination of Benzoic acid, Saccharin, and caffeine in carbonated soft drinks. Int J ChemTech Research. 2019;12(4):15-23. <u>http://dx.doi.org/10.20902/IJCTR.2019.120403</u>

- [2] Chaleshtoria, FS, Arianb A, Chaleshtoric RS. Assessment of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of human health risk. Food Chem Toxicol 2018;120:634-638. <u>https://doi.org/10.1016/j.fct.2018.08.010</u>
- [3] Carocho, M. Barreiro MF, Morales P, Ferreira IC. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. Comprehen. Reviews Food Sci. Food Safety 2014;13(4):377-399. <u>https://doi.org/10.1111/1541-4337.12065</u>
- [4] Pressman P, Clemens R, Hayes W, Reddy C. Food additive safety: A review of toxicological and regulatory issues. Toxicol. Res. Appl 2017;1:1-22. <u>https://doi.org/10.1177/2397847317723572</u>
- [5] World Health Organization (WHO). Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria, Geneva, Switzerland;1987. Accessed on March 25, 2020. <u>http://www.who.int/iris/handle/10665/37578</u>
- [6] Yadav P, Garg N, Kumar S. Improved shelf stability of mulberry juice by combination of preservatives. Indian J Nat Prod Res 2015;5(1):62-66. <u>https://doi.org/10.56042/ijnpr.v5i1.1268</u>
- [7] Sarkar S, Saha S. Rai C, Bhattacharyya S. Effect of storage and preservatives on antioxidant status of some refrigerated fruit juices. Int J Current Microbiol Appl Sci 2014;3(7):1007-1013.
- [8] Glevitzky M, Dumitrel GA, Perju D, Popa M. Studies regarding the use of preservatives on soft drinks stability. chemical bull. Politehnica University (Timisoara) 2009;54(68):1-12.
- [9] Silva MM, Lidon F. Food preservatives-An overview on applications and side effects. Emirates J Food Agricul 2016;28(6):366-373. <u>https://doi.org/10.9755/ejfa.2016-04-351</u>
- [10] Moritaka H, Kitade M, Sawamura SI, Takihara T, Awano I, Ono T, et al. Effect of carbon dioxide in carbonated drinks on linguapalatal swallowing. Chem Senses 2014;39(2):1-10. <u>https://doi.org/10.1093/chemse/bjt062</u>
- [11] Ashurst P, Hargitt R, Palmer F. Soft drink and fruit juice problems solved. Woodhead Publishing, Oxford 2009.
- [12] Kregiel D. Health safety of soft drinks: contents, containers, and microorganism. BioMed Research Int 2015;128:1-12. <u>https://doi.org/10.1155/2015/128697</u>
- [13] Badenhorst CPS, Erasmus E, Van der Sluis R, Nortje C, Van Dijk AA. A new perspective on the importance of glycine conjugation in the metabolism of aromatic acids. Drug Metabolism Reviews 2014;46(3):343-361. <u>https://doi.org/10.3109/03602532.2014.908903</u>
- [14] Herbst M.C. Fact sheet and position statement on sodium benzoate and vitamin C. Cancer Association of South Africa (CANSA) 2019;1:1-20. Accessed on March 12, 2022. <u>https://cansa.org.za/files/2020/11/Fact-Sheet-and-Position-Statement-on-Sodium-Benzoate-and-Vitamin-C-Nov-2020.pdf</u>
- [15] Antakli S, Alahmad A, Badinjki H. Simultaneous determination of sodium benzoate and potassium sorbate preservatives in foodstuffs using high performance liquid chromatography. Asian J Chem 2010;22(4):3275-3282.
- [16] Meyer VR. Practical high-performance liquid chromatography. Aufl Chichester WILEY 2010.
- [17] Omokpariola JO, Omokpariola DO, Chioma E, Omokpariola O. Risk assessment of polycyclic aromatic hydrocarbons and total petroleum hydrocarbons in oilfield producing water and seawater at Gulf of Guinea, Nigeria. Adv J Chem Section B 2011;3(1):68-85. <u>https://doi.org/10.1007/ajcb.2021.121909</u>
- [18] United States Environmental Protection Agency (USEPA). Provisional peer reviewed toxicity values for benzoic acid. Superfund Health Risk Technical Support Center, National Center for Environmental Assessment Office of Research and Development, Cincinnati, OH, USA 2005. Accessed on April 12, 2021. https://cfpub.epa.gov/ncea/pprtv/documents/BenzoicAcid.pdf
- [19] United States Environmental Protection Agency (USEPA). Risk assessment guidance for superfund volume 1: Human Health Evaluation Manual (Part A). Washington, DC, USA. (1989). Accessed on April 19, 2021. <u>https://www.epa.gov/sites/production/files/2015-09/documents/rags a.pdf</u>

- [20] Smith B, Cadby P, DiNovi M, Setzer RW. Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic: example: benzene, CAS: 71–43-2. Food Chem Toxicol 2010;48:S49-S56. <u>https://doi.org/10.1016/j.fct.2009.10.015</u>
- [21] Azuma SL, Quartey NA, Ofosu IW. Sodium benzoate in non-alcoholic carbonated (soft) drinks: Exposure and health risks. Sci African 2020;10:e00611. https://doi.org/10.1016/j.sciaf.2020.e00611
- [22] Badamasi H, Yaro MN, Dauda A, Yahaya M, Bashir IA. Assessment of benzoic acid in some soft drinks marketed in Dutse, Jigawa State, Nigeria. Int J Sci Res Dev2019;7(4): 801-803.
- [23] World Health Organization (WHO). Evaluation of Certain Food Additives (51th Report of the Joint FAO/WHO Expert Committee on Food Additives), heavy metal content of Ayurvedic herbal medicine products. WHO Technical Report Series No 891, Geneva, Switzerland,(2000a). Accessed on April 9, 2021. http://apps.who.int/iris/bitstream/10665/42245/1/WHO TRS 891.pdf
- [24] Onwordi CT, Olanrewaju AJ, Wusu AD, Oguntade BK. Levels of benzoic acid, sulphur (IV) oxide and sorbic acid in carbonated drinks sold in Lagos, Nigeria. American J Food Sci Technol 2017;5(2):38-44. <u>https://doi.org/10.12691/ajfst-5-2-2</u>
- [25] Venu LN, Austin A. Study and quantification of preservative (E211) in caarbonated soft drink samples. IOSR J Appl Chem 2019;12(4):17-23. <u>https://doi.org/10.9790/5736-1204011723</u>
- [26] Amponsah A. Determination of the amount of caffeine and benzoic acid in selected soft drinks. Int J Sci Eng Res 2014;5(6):112-121.
- [27] Mazdeh FZ, Moradi Z, Moghaddam G, Moradi-Khatoonabadi Z, Aftabdari FE, Badaei P, et al. Determination of synthetic food colours, caffeine, sodium benzoate and potassium sorbate in sports drinks. Trop J Pharma Res 2016;15(1):183-188.
- [28] Alimentarius C. General standard for food additives (CODEX STAN 192-1995) Rev. 7-2006. 2006. Accessed on March 21, 2021. <u>http://www.codexalimentarius.net/gsfaonline/docs/CXS-192e.pdf</u>
- [29] Abdulmumeen HA, Risikat AN, Sururah AR. Food: Its preservatives, additives and applications. International Journal of Chemical and Biochemical Sciences 2012;(1):36-47.
- [30] Elhkim MO, Héraud F, Bemrah N, Gauchard F, Lorino T et al. New considerations regarding the risk assessment on Tartrazine: an update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regulatory Toxicology and Pharmacology 2007;47(3):308-316. <u>https://doi.org/10.1016/j.vrtph.2006.11.004</u>
- [31] Anand SP, Sati N. Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int J Pharm Sci Res 2013;4(7):2496-2501. <u>https://doi.org/10.13040/IJPSR.0975-8232.4(7).2496-01</u>
- [32] Husson MC, Schiff M, Fouilhoux, A, Cano D, Dobbelaere A, et al. Efficacy and safety of i.v. sodium benzoate in urea cycle disorders: a multicentre retrospective study. Orphanet J Rare Dis 2016;11(127). <u>https://doi.org/10.1186/s13023-016-0513-0</u>
- [33] Aggarwal A, Sreedharan R, Uso TD, Perez-Protto. Acute hyperammonemic encephalopathy post liver transplant with normal graft function. Critical Care Med 2015;43(12):313. <u>https://doi.org/10.1097/01.ccm.0000475076.79422.47</u>
- [34] De Las Heras J, Aldámiz-Echevarría L, Martínez-Chantar ML, Delgado TC. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. Expert opinion on drug metabolism & toxicology 2017;13(4):439-448. <u>https://doi.org/10.1080/17425255.2017.1262843</u>
- [35] T Baltazar M, J Dinis-Oliveira R, A Duarte J, L Bastos M, Carvalho F. Antioxidant properties and associated mechanisms of salicylates. Current Medicinal Chemistry 2011;18(21):3252-3264. <u>https://doi.org/10.2174/092986711796391552</u>
- [36] Stavinoha RC, Gomada Y, Jamison B, Vattem D. In vivo neuroprotective effects of cinnamon bioactive compounds in *C. elegans* and *D. melanogaster*. FASEB J 2015;29(1):608-631. <u>https://doi.org/10.1096/fasebj.29.1_supplement.608.31</u>

- [37] Piper JD, Piper PW. Benzoate and sorbate salts: a systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Comprehensive reviews in food science and food safety 2017;16(5):868-880.
- [38] Samal D, Gouda S, Patra JK. Food preservatives and their uses: a short report. Asian J Biol 2017;4(1):1-4. https://doi.org/10.9734/AJOB/2017/36091
- [39] Sharma S. Food preservatives and their harmful effects. Int J Sci Res Publ. 2015;5(4):1-2.
- [40] Rather IA, Koh WY, Paek WK, Lim J. Sources of chemical contaminants in food and their health implications. Front Pharmacol 2017;8:830-841. <u>https://doi.org/10.3389/fphar.2017.00830</u>
- [41] National Agency for Food and Drug Administration and Control, NAFDAC. Soft Drinks Regulation. Food Regulations, Abuja, Nigeria 2019. Accessed on April 19, 2021.
 <u>https://www.nafdac.gov.ng/wp-content/uploads/Files/Resources/Regulations/FOOD______REGULATIONS/Soft-Drinks-Regulations-2019.pdf</u>
- [42] United States Environmental Protection Agency (USEPA). Health and environmental effects document for benzoic acid, office of health and environmental assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, USA. 1987. Accessed on March 12, 2021. <u>https://iris.epa.gov/static/pdfs/0355_summary.pdf</u>
- [43] Omokpariola DO, Omokpariola PL. Health and exposure risk assessment of heavy metals in rainwater samples from selected locations in Rivers State, Nigeria. Phys Sci Rev 2021;1-14. <u>https://doi.org/10.1515/psr-2020-0090</u>
- [44] United States Environmental Protection Agency (USEPA). Benzoic Acid; CASRN-65-85-0, Integrated Risk Information System (IRIS), Washington, DC, USA 1988. Accessed on April 19, 2021. <u>https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0355_summary.pdf</u>
- [45] World Health Organization (WHO). Benzoic acid and sodium benzoate. Concise International Chemical Assessment Document (CICAD), Geneva, Switzerland 2000. Accessed on April 12, 2021. <u>http://whqlibdoc.who.int/publications/2000/924153026X.pdf</u>
- [46] World Health Organization (WHO). Evaluation of certain food additives and contaminants. 80th report of the Joint FAO/WHO expert committee on food additives. Geneva, Switzerland, 2017. Accessed on March 24, 2021. <u>http://apps.who.int/iris/bitstream/handle/10665/204410/9789240695405_eng.pdf;jsessionid=1FF6E08294005EF2AE88435</u> <u>0D39C5279?sequence=1</u>
- [47] Bonaccorsi G, Perico A. Colzi A, Bavazzano P, Di-Giusto M, Lamberti I, et al. Benzene in soft drinks: a study in Florence, Italy. Igiene e sanita pubblica 2012;68(4):523-532.
- [48] Omokpariola DO. Influence on storage condition and time on properties of carbonated beverages from utilization of polyethylene terephthalate (PET) bottles: chemometric and health risk assessment. Environ Anal Health Toxicol 2022; 37(3):e2022019. <u>https://doi.org/10.5620/eaht.2022019</u>
- [49] Mmaduakor EC, Umeh CT, Morah JE, Omokpariola DO, Ekwuofu AA, Onwuegbuokwu SS. Pollution status, health risk assessment of potentially toxic elements in soil and their uptake by gongronema latifolium in peri-urban of Ora-Eri, south-eastern Nigeria. Heliyon 2022;8(8):e10362. <u>https://doi.org/10.1016/j.heliyon.2022.e10362</u>
- [50] Omokpariola DO, Nduka JK, Kelle HI. Mgbemena NM, Iduseri EO. Chemometrics, health risk assessment and probable sources of soluble total petroleum hydrocarbons in atmospheric rainwater, Rivers State, Nigeria. Sci Reports 2022;12(1): 1-19. <u>https://doi.org/10.1038/s41598-022-15677-7</u>