



Research article

Health risk assessment of potassium bromate in bread in Ghana



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ABSTRACT

Introduction: Potassium bromate (KBrO_3) is an oxidizing agent added to flour to improve bread quality. However, KBrO_3 is nephrotoxic, and a class B carcinogen banned in most countries, including Ghana.

Aim: This study aimed to determine the residual KBrO_3 concentration in bread and to estimate the chemical and carcinogenic risk that is associated with the consumption of these breads in Ghana.

Methods: A total of 285 samples of bread, consisting of 97 tea breads, 95 sugar breads, and 93 butter breads were randomly sampled from bakeries across ten regions of Ghana for this study. The bromate contents were qualitatively and quantitatively determined spectrophotometrically at 515 nm, based on the redox reaction between KBrO_3 and promethazine. The change in colour from colourless to pink indicated the presence of KBrO_3 in the sample. The data were analyzed with Microsoft Excel 365 Apps for Enterprise and GraphPad Prism and the map was plotted with QGIS.

Results: Out of the 285 bread samples analyzed, 12.28% (35/285) tested positive for KBrO_3 with concentrations ranging from 0.46 to 26.67 $\mu\text{g/g}$ (mean 6.13 $\mu\text{g/g}$). This exceeded the permissible dose (0.02 $\mu\text{g/g}$) by 23–1334 times, respectively. Except Ashanti and Volta regions, all the other 8 regions had at least one bread type testing positive for KBrO_3 . There was no statistically significant difference in the concentration of KBrO_3 between the bread types analyzed at $p < 0.05$. The hazard ratio ranged from 0.166 to 0.435 and the chronic hazard quotient ranged from 0.0037 to 0.00141.

Conclusion: Breads from 8 regions of Ghana tested positive for KBrO_3 , but their concentrations may not pose chemical or carcinogenic risks after regular consumption.

1. Introduction

In Ghana, bread is a staple food among all ethnic groups and socioeconomic classes of people. Bread is a readily available foodstuff in homes, hotels, and restaurants. It is eaten as part of breakfast in almost every home or as a snack. It is a cheap commodity to buy though wheat is not locally cultivated in Ghana for climatic reasons [1]. Wheat dough mixed with yeast is baked to produce bread. A major challenge faced by bakeries and flour millers is the baking of quality flour, which is a measure of the ability of the prepared dough to retain gas [2]. Food additives added to bread help improve the flavour and prolong preservation. Potassium bromate (KBrO_3)

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is the most used additive because of its efficient oxidizing properties and its ability to preserve the taste, flavour, and appearance of bread. Besides that, it also enhances the uniformity and even rise of bread during baking [3,4]. It is preferred because it is a far cheaper commodity than ascorbic acid and glucose oxidase [5]. By converting flour's gluten protein's sulfhydryl groups into disulfide bridges through oxidation, it works as a dough conditioner and maturation agent [6], increasing the elastic properties and decreasing the extensibility of the dough. The dough thus becomes visco-elastic and accumulates the carbon (IV) dioxide yeast produces. The overall effect is to make the dough rise and increase the volume, texture, and strength of the loaf produced [4,7,8].

However, the International Agency for Research on Cancer (IARC) has classified KBrO_3 as a poisonous and carcinogenic compound (Group 2B compound) [9,10]. Korea reported the first case of acute renal failure in humans due to KBrO_3 poisoning in 1980 [11]. In Japan, young women, especially hairdressers used KBrO_3 as a suicidal agent [12]. In the acute phase of poisoning, KBrO_3 causes diarrhoea, vomiting, and abdominal pain [12,13]. Subsequently, it can aggravate deafness, anuria, oliguria, depression of the central nervous system, hypotension, kidney failure, cancer, and thrombocytopenia [2,6,14,15].

Currently, there is no data on the carcinogenicity of KBrO_3 in humans but in rodents. Thus, there is a possibility that it can cause cancer in humans [10,12,14]. KBrO_3 is known to cause kidney, thyroid, and mesothelium cancer in rats, as well as renal cancer in male B6C3F1 mice [16]. As low as 0.02 g/L concentration of KBrO_3 was carcinogenic in rodents [16].

KBrO_3 exerts genotoxic and nephrotoxic effects in animals' models as well as in humans [17]. [18] reported that KBrO_3 is hepatotoxic, and causes distortion in the architecture of liver tissues, central vein congestion, sinusoidal dilation, and cellular necrosis in Wistar rats. Studies by Refs. [19–21] reported that KBrO_3 induces haemorrhage, liver congestion, pneumonia, and demyelination of the brain, revealing that KBrO_3 has a neurotoxic effect in Wistar albino rats. They also reported hepatocellular injury and elevated serum urea and creatinine, indicating the nephrotoxic effect of KBrO_3 . Oral administration of KBrO_3 to rats induced oxidative stress [9, 22].

In reproductive health, [23] reported that exposure to KBrO_3 by prepubertal rats caused growth retardation, testicular hypoplasia, and impaired spermatogenesis, which is a determinant of future sterility and infertility. [24] reported a case of accidental KBrO_3 poisoning resulting in acute renal failure in a 17-month-old girl in Obafemi Awolowo University Teaching Hospital, Nigeria. They recommended that public education on the dangers of KBrO_3 should be carried out in child welfare clinics and parents and pharmaceutical companies should package KBrO_3 in child-resistant packages. Certainly, this should reduce the likelihood of serious mortality and morbidity following KBrO_3 exposure.

Besides its health hazards, KBrO_3 adversely affects the bread's nutritional value. It degrades the main vitamins in bread: Niacin, E, A1, B1, and B2 [3].

Bearing these health hazards in mind, in 1997, a bill was passed in Ghana banning the use of KBrO_3 for baking bread, and ascorbic acid was recommended as a substitute [25]. However, the milling industry and bakeries still smuggle and use it secretly. Apart from Ghana, KBrO_3 use is banned in many countries in the world as flour conditioning agent (Table 1) [5].

There is no data on the analysis of KBrO_3 content and the assessment of human health risks associated with bread consumption in Ghana.

Thus, this study was aimed at determining the residual KBrO_3 concentration in bread and estimating the chemical and carcinogenic risk that is associated with the consumption of these breads in Ghana.

2. Materials and methods

2.1. Materials

Promethazine (M.W. 320.88 g mol⁻¹, purity, 99.9%) (Biobmei, China), potassium bromate (M.W. 167.00 g mol⁻¹, purity, 99.7%) (Qualikems Fine Chemical Pvt Ltd, India), starch (Central Drugs House, India), Cecil CE-72000 Double Beam Spectrophotometer (Cecil Instruments Ltd, UK), hydrochloric acid (purity, 35–38%, [HCl] = 11.81 M, M.W. 36.46 g mol⁻¹, Research Lab, India), distilled water, tea bread, sugar bread, and butter bread purchased across Ghana. All reagents used for the study were from standard suppliers and of analytical grade.

Table 1
The status of KBrO_3 regulation as a bread dough conditioner.

Country	Status and year of banned
European Union	Banned (1990)
United Kingdom	Banned (1990)
Nigeria	Banned (1993)
Canada	Banned (1994)
Sri Lanka	Banned (2001)
Peru	Banned (2002)
Columbia	Banned (2002)
China	Banned (2005)
Ghana	Banned (1997)
United States	Not Banned
Tunisia	Not Banned
Ethiopia	Not Banned

(Source: CSE Study, 2016)

2.2. Study design

This was an experimental study. It was a qualitative and quantitative assessment of the level of $KBrO_3$ in three types of bread randomly sampled across ten (10) regions of Ghana using a spectrophotometric method. The sample analysis was done in triplicate.

2.3. Study area

The study was conducted across ten (10) out of the sixteen (16) regions of Ghana. Ghana is a sub-Saharan African country in the western part of Africa. Togo, Burkina Faso, Cote d'Ivoire, and the Gulf of Guinea form its eastern, northern, western, and southern borders, respectively. It has an estimated population of 30.8 million people according to the Ghana Statistical Service report for the 2021 population and housing census [26]. Ghana is located at latitude 7.9465° N and longitude 1.0232° W of the equator. The bread samples were purchased from the following regions: the Upper East, Upper West, Northern, Brono, Ashanti, Western, Eastern, Central, Greater Accra, and the Volta regions of Ghana. Fig. 1 is a map of Ghana showing the regions where the bread samples were sampled for the study.

2.4. Sampling procedure

With an estimated bakeries of 1000 population in the study area, the sample size computation was done using the Slovin' formula as described in equation (1) [27]. With a margin of error of 5%, bread samples of 285 were arrived at.

$$n = \frac{N}{1 + Ne^2} \tag{1}$$

where n = sample size.

N = Population

e = margin of error.

In August and September 2022, two hundred and eighty-five (285) bread samples consisting of 95 tea bread, 97 sugar bread, and 93 butter bread were randomly and conveniently purchased from different bakeries in ten (10) regions of Ghana and analyzed. The choice of bread was dependent on its popularity and rate of consumption in Ghana. The samples were appropriately coded using alpha-numeric codes, which depict the type of bread and the region it was purchased from. The bread samples were brought to the Science Laboratory Technology Department of Accra Technical University in sealable sampling bags for residual bromate concentration estimation using the promethazine spectrophotometric method.

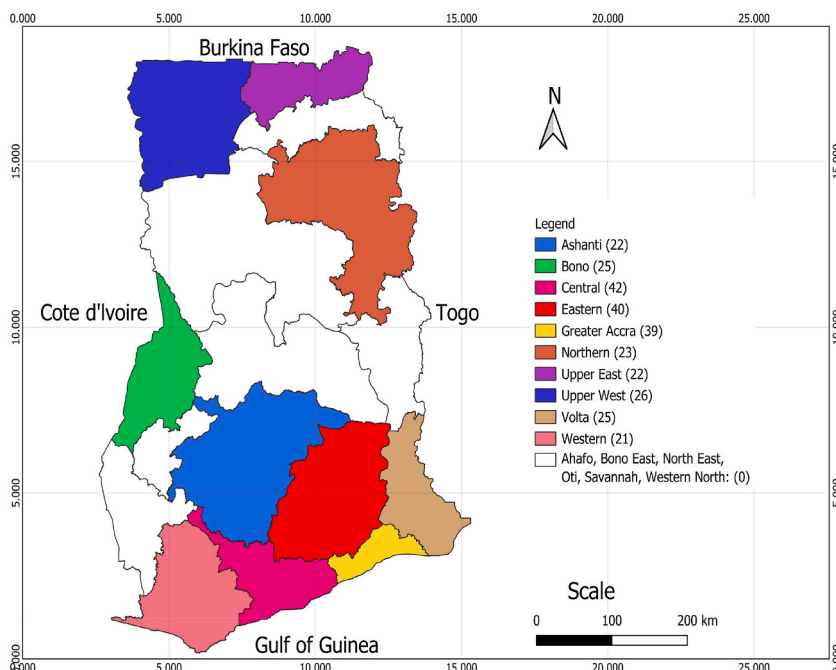


Fig. 1. Map of Ghana showing the geo-distribution of bread sampled for the study. Map plotted with QGIS software.

2.5. Sample pretreatment

The pre-treatment of the sample was done according to [7,28] with slight modifications. Ten (10) g of sliced bread from the centre of each loaf was dried in an oven at 85 °C for 1 h. The dried sample was pulverized, and 1.0 g of each powdered bread sample was weighed into labeled test tubes containing 10 mL of distilled water. The mixtures were agitated and left to stand for 20 min at room temperature. The mixtures were centrifuged at 15,000 rpm for 5 min, and the supernatant was decanted. The supernatant was then filtered using Whatman No. 1 filter paper with a diameter of 110 mm. The filtrates were aliquoted for the analysis. Sample pre-treatment was done in triplicates.

2.6. Preparation of reagent and solution

2.6.1. Preparation of potassium bromate standard solution

A 0.25 g of powdered KBrO_3 was dissolved in 250 mL of distilled water to prepare a 1000 ppm stock solution [29]. A concentration range of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 $\mu\text{g/mL}$ respectively were prepared from the stock solution as working concentrations for standard curve calibration.

2.6.2. Promethazine standard solution

A stock solution of 0.01 M of promethazine (MW: 320.88 g mol^{-1} , purity: 99.9%) (Biobmei, China), was prepared by dissolving 0.6418 g of powdered promethazine in 200 mL of distilled water in a volumetric flask [30].

2.7. Promethazine spectrophotometric method procedure

The concentration of KBrO_3 in bread in Ghana was quantitatively determined according to previously reported methods with slight modification [30,31]. To a 4.4 mL aliquot of each of the prepared bread filtrates, 0.5 mL of freshly prepared 0.01 M promethazine solution and 100 μl of 11.81 M hydrochloric acid (HCl) were added. A change in colour from colourless to pink showed the presence of KBrO_3 in the sample. The promethazine spectrum showing the maximum absorption was scanned using a Cecil CE-72000 Double Beam Spectrophotometer (Cecil Instruments Ltd, UK) and the maximum absorption (λ_{max}) was recorded at a wavelength of 515 nm (Fig. 2). The mixtures were then shaken, and the absorbances of the mixtures were read at 515 nm. A KBrO_3 calibration curve was prepared at different concentrations (0, 5, 10, 15, 20, 25, 30, 35, 40, and 45) $\mu\text{g/mL}$ by serial dilution from the stock solution (Fig. 3). A 0.5 mL quantity of freshly prepared 0.01 M promethazine solution and 100 μl of 11.81 M HCl was pipetted to each pure sample, and the absorbances were read at 515 nm. The absorbances measured were plotted against the concentrations of the solutions. The concentration of KBrO_3 in each of the samples was determined using the line equation of the calibration curve: $y = 0.0083x + 0.0093$ with $R^2 = 0.9956$ (Fig. 3). The results were reported as the mean \pm STD of the triplicate determination for each bread sample. Quality control was ensured by the analytical method verification. Similarly, bread samples with spectral interfering additives such as preservatives (methylparaben) and colorants were avoided.

2.8. Assessment of KBrO_3 health risk

This study assessed both the carcinogenic and non-carcinogenic risks of KBrO_3 in bread samples. To determine the short-term or acute (acute hazard quotient, aHQ) and the long-term or chronic health risk of consumer (chronic hazard quotient, cHQ) associated with KBrO_3 in bread sold in Ghana, the estimated daily intake (EDI) or human dietary exposure, and the estimated short-term intake (ESTI) were computed. The carcinogenic risk assessment was predicted using the hazard ratio (HR).

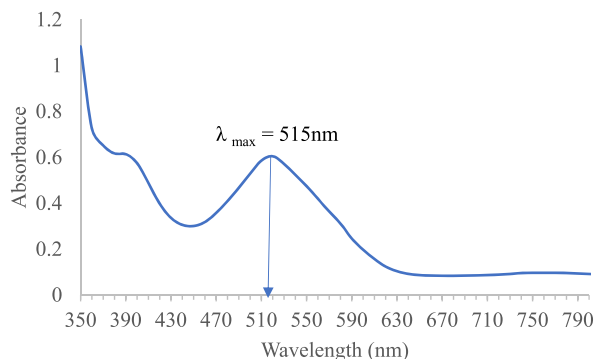


Fig. 2. UV-vis spectrum of oxidized product between promethazine and KBrO_3 (1000 ppm) solution in distilled water. The wavelength corresponding to the maximum absorption λ_{max} is 515 nm.

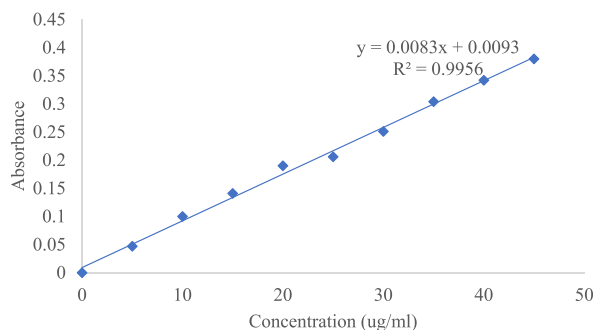


Fig. 3. KBrO₃ calibration curve for spectrophotometric determination of bread samples.

2.8.1. Approximate daily intake of KBrO₃

The concentration of KBrO₃ in the body depends on the daily intake and the daily food consumption. The tolerance of KBrO₃ contamination is dependent on the average adult body weight. These assumptions underpin the EDI calculation using equation (2) [32, 33].

$$EDI\ ingestion = \frac{K \times B}{Bw.t} \tag{2}$$

whence, EDI ingestion represents the estimated oral daily intake (mg/kg BW per day).

K – average KBrO₃ concentration in the analyzed bread samples (mg).

B – bread daily intake (kg).

Bw.t– the average human adult body weight (kg).

The average amount of bread consumed during each meal is estimated as 0.6 kg. The Ghanaian adult’s average body weight is 71.35 kg [34], which is approximately the average human adult body weight (70 kg). The estimated daily intake (EDI) of KBrO₃ over a long period of bread consumption was calculated using the average KBrO₃ concentration (in mg).

Acute exposure to KBrO₃ was also determined. Equation (3) was used to compute the estimated short-term intake (mg/kg BW per day) as follows:

$$ESTI = \frac{K\ highest \times B}{Bw.t} \tag{3}$$

The parameters in the equation are defined as follows:

ESTI represents estimated short-term intake (mg/kg BW per day).

K highest– highest concentration of KBrO₃ in the analyzed bread samples (mg).

B – bread daily intake (kg).

Bw.t– the average human adult body weight (kg). The ESTI (acute) was computed using the highest KBrO₃ concentrations in the different bread samples analyzed.

The toxicity due to daily inhalation of KBrO₃ was not calculated in this study because the amount of KBrO₃ inhaled during baking was not estimated.

2.8.2. Estimation of dietary carcinogenic risk of potassium bromate

The evaluation of a person’s lifetime risk of developing cancer based on exposure to KBrO₃ from bread is known as a carcinogenic risk assessment. The risk of cancer was predicted using the hazard ratio (HR), which was computed using equation (4) as follows: [33]:

$$HR = \frac{EDI\ Ingestion}{CBC} \tag{4}$$

where CBC represents cancer benchmark concentration and EDI ingestion stands for expected daily intake through ingestion. The CBC for the carcinogenic potential of KBrO₃ was estimated using the risk of one in a million chances of lifetime exposure.

The CBC value was estimated using equation (5) as follows:

$$CBC = \frac{RL \times Bw.t}{CR \times OSF} \tag{5}$$

The parameters in the equation are defined as follows:

RL-maximum allowable risk level (1 × 10⁻⁶), which represents the lifetime risk of acquiring cancer because of exposure to KBrO₃

and Bw.t is the average human adult body weight (70 kg).

OSF stands for oral slope factor (mg/kg/day), and CR stands for food consumption rate (g/day) [35]. The KBrO₃ oral slope factor is 7×10^{-1} per mg/kg/day [36]. Potential health risk to human health is recorded if the Hazard ratio (HR) > 1.

2.8.3. Estimation of non-carcinogenic dietary health risk

In addition to the carcinogenic risk, the non-carcinogenic risk associated with eating each type of bread containing KBrO₃ was calculated utilizing the non-hazard quotient (HQ) value.

The chronic hazard quotient was estimated using equation (6) (cHQ) [36];

$$cHQ = \frac{EDI \text{ ingestion}}{ADI} \tag{6}$$

where cHQ represents the oral chronic hazard quotient (no unit).

EDI ingestion represents the estimated oral daily intake (mg/kg per day).

ADI represents acceptable daily intake (mg/kg per day).

In the calculation of the estimated chronic risk of KBrO₃ in the various types of bread, the recommended ADI of 0.02 µg/g (0.02 mg/kg) KBrO₃ in bread by the US Food and Drug Administration (FDA) was used [15].

The acute hazard quotient (aHQ) was estimated using equation (7) as follows:

$$aHQ = \frac{ESTI}{RfD} \tag{7}$$

aHQ represents the acute hazard quotient.

ESTI represents estimated short-term intake (mg/kg BW per day).

RfD represents the reference dosage of KBrO₃ (mg/kg per day) with a reference limit of 0.004 mg/kg/day [36]. RfD is a calculation of the human population's daily exposure to KBrO₃ including vulnerable groups that are most likely to pose a significant lifetime risk of

Table 2
Regional distribution of bread types analyzed.

Region	Bread Type	Percentage of Each Bread Type on Total Bread in Each Region (%)	Total Number of Bread per Region	Percentage of Total Number of Bread per Each Region on Total Bread Analyzed (%)	Percentage of Each Type of Bread on Total Bread Analyzed (%)
Greater Accra	Tea	15/39 (38.46%)	39	13.68	15/285 (5.26%)
	Sugar	14/39 (35.90%)			14/285 (4.91%)
	Butter	10/39 (25.64%)			10/285 (3.51%)
Western	Tea	5/21 (23.81%)	21	7.37	5/285 (1.75%)
	Sugar	8/21 (38.10%)			8/285 (2.81%)
	Butter	8/21 (38.10%)			8/285 (2.81%)
Volta	Tea	10/25 (40.00%)	25	8.77	10/285 (3.51%)
	Sugar	10/25 (40.00%)			10/285 (3.51%)
	Butter	5/21 (20.00%)			5/285 (1.75%)
Upper West	Tea	6/26 (23.08%)	26	9.12	6/285 (2.11%)
	Sugar	10/26 (38.46%)			10/285 (3.51%)
	Butter	10/26 (38.46%)			10/285 (3.51%)
Northern	Tea	10/23 (43.48%)	23	8.07	10/285 (3.51%)
	Sugar	8/23 (34.78%)			8/285 (2.81%)
	Butter	5/23 (21.74%)			5/285 (1.75%)
Bono	Tea	8/25 (32.00%)	25	8.77	8/285 (2.81%)
	Sugar	7/25 (28.00%)			7/285 (2.46%)
	Butter	10/25 (40.00%)			10/285 (3.51%)
Central	Tea	15/42 (35.71%)	42	14.74	15/285 (5.26%)
	Sugar	13/42 (30.95%)			13/285 (4.56%)
	Butter	14/42 (33.33%)			14/285 (4.91%)
Ashanti	Tea	7/22 (31.82%)	22	7.72	7/285 (2.46%)
	Sugar	6/22 (27.27%)			6/285 (2.11%)
	Butter	9/22 (40.91%)			9/285 (3.16%)
Eastern	Tea	12/40 (30.00%)	40	14.04	12/285 (4.21%)
	Sugar	13/40 (32.50%)			13/285 (4.56%)
	Butter	15/40 (37.50%)			15/285 (5.26%)
Upper East	Tea	7/22 (31.82%)	22	7.72	7/285 (2.46%)
	Sugar	8/22 (36.36%)			8/285 (2.81%)
	Butter	7/22 (31.82%)			7/285 (2.46%)
Total			285		

Table 3
The proportions of bread types testing positive for KBrO₃ in the analyzed breads in Ghana.

Region	Bread Type	Total Number of Bread Type	Total Bread	Number of Bread Type with KBrO ₃	Percentage of Each Bread Type with KBrO ₃ per Each Bread Type in Each Region (%)	Percentage of Each Bread Type with KBrO ₃ Per Total of Each Bread Type (%)	Percentage of Each Bread Type with KBrO ₃ Per Total Bread in Each Region (%)	Percentage of Total Bread with KBrO ₃ in Each Region Per Total Bread in Each Region (%)	Percentage of Total Bread with KBrO ₃ in Each Region Per Total Number of Bread (%)
Greater Accra	Tea	15	39	2	2/15 (13.33%)	2/95 (2.11%)	2/39 (5.13%)	5/39 (12.82%)	5/285 (1.75%)
	Sugar	14		3	3/14 (21.43%)	3/97 (3.09%)	3/39 (7.69%)		
	Butter	10		0	0/10 (0.00%)	0/93 (0.00%)	0/39 (0.00%)		
Western	Tea	5	21	0	0/5 (0.00%)	0/95 (0.00%)	0/21 (0.00%)	2/21 (9.52%)	2/285 (0.70%)
	Sugar	8		1	1/8 (12.50%)	1/97 (1.03%)	1/21 (4.76%)		
	Butter	8		1	1/8 (12.50%)	1/93 (1.08%)	1/21 (4.76%)		
Volta	Tea	10	25	0	0/10 (0.00%)	0/95 (0.00%)	0/25 (0.00%)	0/25 (0.00%)	0/285 (0.00%)
	Sugar	10		0	0/10 (0.00%)	0/97 (0.00%)	0/25 (0.00%)		
	Butter	5		0	0/5 (0.00%)	0/93 (0.005)	0/25 (0.00%)		
Upper West	Tea	6	26	0	0/6 (0.00%)	0/95 (0.00%)	0/26 (0.00%)	2/26 (7.69%)	2/285 (0.70%)
	Sugar	10		0	0/10 (0.00%)	0/97 (0.00%)	0/26 (0.00%)		
	Butter	10		2	2/10 (20.00%)	2/93 (2.15%)	2/26 (7.69%)		
Northern	Tea	10	23	0	0/10 (0.00%)	0/95 (0.00%)	0/23 (0.00%)	1/23 (4.35%)	1/285 (0.35%)
	Sugar	8		0	0/8 (0.00%)	0/97 (0.00%)	0/23 (0.00%)		
	Butter	5		1	1/5 (20.00%)	1/93 (1.08%)	1/23 (4.35%)		
Bono	Tea	8	25	3	3/8 (37.50%)	3/95 (3.16%)	3/25 (12.00%)	5/25 (20.00%)	5/285 (1.75%)
	Sugar	7		0	0/7 (0.00%)	0/97 (0.00%)	0/25 (0.00%)		
	Butter	10		2	2/10 (20.00%)	2/93 (2.15%)	2/25 (8.00%)		
Central	Tea	15	42	4	4/15 (26.67%)	4/95 (4.21%)	4/42 (9.52%)	7/42 (16.67%)	7/285 (2.46%)
	Sugar	13		2	2/13 (15.38%)	2/97 (2.06%)	2/42 (4.76%)		
	Butter	14		1	1/14 (7.14%)	1/93 (1.08%)	1/42 (2.38%)		
Ashanti	Tea	7	22	0	0/7 (0.00%)	0/95 (0.00%)	0/22 (0.00%)	0/22 (0.00%)	0/285 (0.00%)
	Sugar	6		0	0/6 (0.00%)	0/97 (0.00%)	0/22 (0.00%)		
	Butter	9		0	0/9 (0.00%)	0/93 (0.00%)	0/22 (0.00%)		
Eastern	Tea	12	40	2	2/12 (16.67%)	2/95 (2.11%)	2/40 (5.00%)	8/40 (20.00%)	8/285 (2.81%)
	Sugar	13		1	1/13 (7.69%)	1/97 (1.03%)	1/40 (2.50%)		
	Butter	15		5	5/15 (33.33%)	5/93 (5.38%)	5/40 (12.50%)		
Upper East	Tea	7	22	0	0/7 (0.00%)	0/95 (0.00%)	0/22 (0.00%)	5/22 (22.72%)	5/285 (1.75%)
	Sugar	8		3	3/8 (37.50%)	3/97 (3.09%)	3/22 (13.64%)		
	Butter	7		2	2/7 (28.57%)	2/93 (2.15%)	2/22 (9.09%)		
Total		285	285	35					35/285(12.28%)

adverse effects [36].

The acceptable KBrO_3 risk is $\text{HQ} < 1$. However, when $\text{HQ} > 1$ it indicates a high risk to the human body [36]. The hazard index was not estimated in this study because only the oral route of exposure and one chemical substance (KBrO_3) were considered.

2.9. Statistical analysis

The data was inputted into Microsoft Excel 365 Apps for enterprise analysis. The results obtained from the analysis were reported as the mean \pm standard deviation. The results were presented as charts and tables. A significant difference between the means at a 5% confidence interval was determined by One-way analysis of variance (ANOVA) using GraphPad Prism version 8.4.2. The map was plotted with QGIS version 3.34.1 software.

3. Results and discussion

3.1. Regional distribution of bread types

This study sought to determine the levels of KBrO_3 in Ghanaian bread using the promethazine spectrophotometric method and to estimate the carcinogenic and chemical risks of consuming bread baked in Ghana.

Three types of bread; tea, sugar, and butter breads were randomly sampled across different bakeries in 62.5% (10/16) regions of Ghana (Fig. 1). Overall, a total of 285 bread samples were analyzed in this study. This was a relatively large sample size when compared to the reported range of bread, sample size (10–40) [3,37,38]. From Table 2, the majority of the analyzed bread samples (42) came from the Central region, accounting for 14.74% of the total number of breads analyzed, followed by the Eastern region (40), accounting for 14.04% of the total number of breads analyzed, and the Western region (21) accounting for 7.37% of the total number of breads analyzed.

3.2. Distribution and occurrence of KBrO_3 in different bread types

In an acidic medium, KBrO_3 reacts with promethazine hydrochloride to form a product with a pink colour. Qualitatively, there was no visible colour change in the samples that tested negative for KBrO_3 , while for those that tested positive for KBrO_3 , the colours of the reaction mixtures changed from colourless to light pink and dark pink as the concentration of KBrO_3 increased in the sample.

Out of a total of 285 pieces of bread analyzed (Table 2), KBrO_3 was detected in 35 of the breads, constituting 12.28% of the total breads analyzed (Table 3), suggesting that most of the breads in Ghana analyzed do not contain KBrO_3 . The majority of the bread that tested positive for KBrO_3 were from the Eastern region (8 pieces of bread), constituting 2.81% of the total number of bread analyzed and 22.86% (8/35) of the total number of positive bread, followed by 7 pieces of bread from the Central region, constituting 2.46% of the total number of bread analyzed and 20% (7/35) of the total number of positive bread, and then Greater Accra and Upper East regions had 5 pieces of bread each testing positive for KBrO_3 which constitute 1.75% each of the total number of bread tested and 14.29% (5/35) of the positive bread. No bread from the Ashanti and Volta Regions tested positive for KBrO_3 content. A total of 11 tea breads tested positive for KBrO_3 nationwide constituting 3.86% (11/285) of the total bread analyzed and 11.58% (11/95) of the total tea breads tested (Table 3). A total of 10 sugar breads tested positive for KBrO_3 nationwide constituting 3.51% (10/285) of the total breads tested and 10.31% (10/97) of the total sugar breads analyzed. On the other hand, 14 butter breads tested positive for KBrO_3 nationwide constituting 4.91% (14/285) of the total breads analyzed and 15.05% (14/93) of the total number of butter breads analyzed (Table 3). Thus, the highest number of breads containing KBrO_3 were butter breads, and the least were sugar breads.

At the regional level, most of the tea breads that tested positive for KBrO_3 were from the Central region (4), constituting 11.43% (4/35) of the total bread that tested positive and 36.36% (4/11) of the total positive tea bread. Also, most of the sugar bread that tested positive for KBrO_3 were from Greater Accra and the Upper East regions, with 3 positive breads each constituting 8.57% (3/35) of the total positive breads and 30% (3/10) of the total positive sugar breads tested. Finally, the Eastern region had the most butter bread with KBrO_3 (5), accounting for 14.29% of the total positive bread and 35.71% (5/14) of the total positive butter bread (Table 3). All types of bread from the Central and Eastern regions tested positive for KBrO_3 . The use of KBrO_3 as a dough conditioner is banned in Ghana by the Ghana Standards Authority [25]. The detection of KBrO_3 in baked Ghanaian bread implies that bakers in Ghana do not comply with the directive of the regulator, and the Central and Eastern regions may be the major culprits. It also implies that bread-baking practices may be poor in Ghana including the use of lower temperature and short time to bake bread. Consequently, since KBrO_3 is a Group 2B compound classified by the IARC and its ability to interfere with thyroid function, continual consumption of these breads puts the Ghanaian population at serious public health risk and the regulator should intensify strict monitoring.

According to the results of the analysis, the highest national concentration of KBrO_3 was 26.67 $\mu\text{g/g}$, with a national average of 6.13 $\mu\text{g/g}$ and a minimum concentration of 0.46 $\mu\text{g/g}$ recorded from all breads tested (Fig. 5). The data showed that tea bread recorded the highest concentration of KBrO_3 (26.67 $\mu\text{g/g}$) followed by sugar bread (25.83 $\mu\text{g/g}$) and butter bread had the lowest concentration of KBrO_3 (8.88 $\mu\text{g/g}$) (Fig. 5). The concentrations of KBrO_3 in the bread that tested positive far exceeded the maximum allowable KBrO_3 concentrations of (0.025 mg/kg, 0.02 mg/kg, and 10 mg/kg) in finished bakery products set by the WHO, U.S.A., and Japan, respectively [12,39]. Besides that, the highest and lowest concentrations of KBrO_3 in this study were 23–1334 times above the maximum allowable level limit of 0.02 mg/kg in bakery products set by the U.S.A Food and Drug Administration (FDA) (Fig. 3) but within allowable reference range compared to India, which set its maximum limit at 50 ppm [5]. Such high concentrations of KBrO_3 are a cause for concern because they imply a high risk of bread consumption in Ghana. The lethal dose of KBrO_3 is estimated to be in the

range of 5–500 mg/kg body weight [12]. Thus, these breads may be lethal for consumption. At the regional level, sugar bread from the Western region, tea bread from Bono region and butter bread from Central region recorded the highest KBrO₃ concentration (Fig. 4).

There have been no published studies on the analysis of KBrO₃ in Ghana, but the ranges of values obtained in this study (0.46–26.67 µg/g) are higher than those reported in Nigeria by [7] (3.6–9.2 µg/g), [37] (1.24–9.31 µg/g), [3] (1.01–11.33 µg/g), and [29] (2.46–13.60 µg/g). However, [40] reported higher values of KBrO₃ than in this study (2.051 µg/g to 66.224 µg/g). In Tunisia, [41] comparatively reported higher levels of KBrO₃ (5.91–49.31 µg/g) than in this study. This difference may be a result of the non-regulation of KBrO₃ usage in bread in Tunisia. In Ethiopia, a study by [39] reported KBrO₃ concentrations of (5.62–9.97 mg/kg) which are lower than those recorded in this study though Ethiopia has no recommended limit for KBrO₃. This study's values are in close agreement with [42], who also observed KBrO₃ concentrations of 10.72–39.73 ppm in India. However, because the permissible limit of KBrO₃ in bread in India is <50 ppm, these concentrations of KBrO₃ as reported by (40) are not alarming in their context. The findings from this study and the reported literature show that KBrO₃ content in bread varies depending on the manufacturing practices, geographical location, and regulations. In some cases, the levels may be above regulatory limits or recommendations set by international organizations. This raises concerns about the potential health risks of bread to consumers in these jurisdictions. While the findings in this study suggest that bakers of these types of bread in Ghana may be adding bromate to their products, drawing such conclusions may be inappropriate since bromate from natural sources can be introduced into flour. It is a known fact that bromine, when dissolved in water, forms bromate, and bromine naturally occurs in concentration range 1–10 mg/kg or even more in foods. Flour also has a natural bromine content of about 2.4–7.7 mg/kg [29]. This implies that the concentrations of KBrO₃ recorded in this study (0.49–26.67 µg/g) from the bread sampled may not necessarily be an indication that bakers add KBrO₃ to their products. Research has shown that KBrO₃ at concentrations of 50 mg/kg or less when present in flour will not be detected in bread made from that flour because KBrO₃ is quantitatively converted to bromide, a relatively harmless ion [5], in bread after 20–25 min of baking. However, a residual amount of KBrO₃ remains in the bread if too much of it is used or if it is not baked for a long time and at a high temperature. This is the reason why regulatory authorities in some countries do not strictly prohibit the use of KBrO₃. This could also imply that bread flour may not contain a lot of KBrO₃ or that bakers in Ghana may have baked bread at high temperatures for a long time, which is why most of the bread in this study does not contain KBrO₃. It will be relevant to consider the temperature and time at which bread is baked in Ghana in future studies to unravel the reasons why residual KBrO₃ content in Ghanaian bread is low. There was no statistically significant difference between the mean concentrations of KBrO₃ in tea breads, sugar breads, and butter breads (*p* > 0.05).

3.3. Assessment of carcinogenic health risk of KBrO₃

From Table 4, the hazard ratio (HR) of the three types of bread analyzed were all less than 1 (HR < 1), suggesting that consumption of bread in Ghana poses no definite cancer risk to the consumer. This was in sharp contrast with a study by [33] where all bread samples analyzed had HR > 1 meaning all the bread possessed carcinogenic risk to the consumers in Cameroon. The HR in the different kinds of bread types ranged from 0.166 to 0.435, with butter bread recording the least (0.166) and tea bread recording the highest (0.435). KBrO₃ is an oxidizing agent as well as a highly reactive oxide and radical that reacts with DNA to cause cancer. Several studies have reported that KBrO₃ stimulate and enhances renal cell tumours development in rats, thyroid, and mesothelium follicular tumours in male B6C3F₁ mice [12,16,43]. Since these facts are true, animal studies cannot be directly extrapolated to humans, and thus to comprehensively understand the cancer-causing effect of KBrO₃, further research is required. Renal and thyroid cancers are on the rise globally, and Ghana is not an exception. These cancers have been linked to clinical elements such as diabetes, hypertension, smoking, and endocrine disturbances. However, it is recommended that research should be directed to toxicological studies as well.

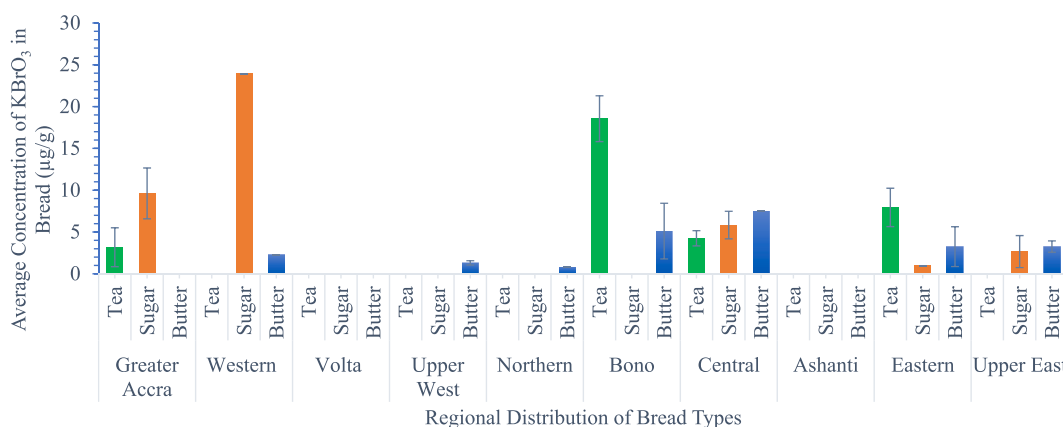


Fig. 4. Regional average concentration of KBrO₃ in bread type.

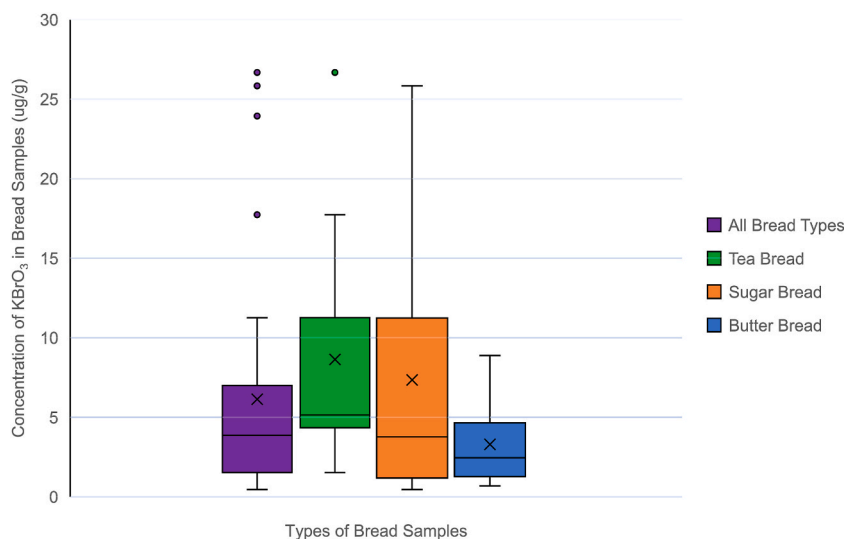


Fig. 5. Boxplot of the distribution of KBrO₃ concentration in bread types analyzed.

3.4. Assessment of non-carcinogenic health risk of KBrO₃

3.4.1. Assessment of KBrO₃ food-related acute and long-term risk

The highest KBrO₃ concentrations in the different types of bread were used to compute the acute hazard quotient (aHQ) (Table 4). The aHQ of each type of bread was less than 1 (aHQ < 1) (0.019–0.0572). Therefore, it was considered that there is no acute risk. This indicates that the highest concentration of KBrO₃ in the different types of bread analyzed does not pose any acute poisoning to consumers. Tea bread, which had the highest KBrO₃ concentration (26.67 µg/g) had the highest aHQ value (0.0572), and butter bread, which had the lowest KBrO₃ concentration (8.88 µg/g) had the lowest aHQ value (0.019) (Table 4). It is important to note that while acute toxicity studies provide information on the immediate adverse effect of exposure to a toxicant, they are not clinically relevant in some cases because the chronic and organ-specific adverse effects cannot be known immediately. Hence the chronic risk of consumption of bread containing KBrO₃ was calculated. The chronic hazard quotient (cHQ) of each type of bread was less than 1 (cHQ < 1) (0.00141–0.0037). This indicates that there is no potential long-term risk to consumers. Likewise, tea bread, which had the highest KBrO₃ concentration (26.67 µg/g) had the highest cHQ value (0.0037), and butter bread which had the lowest KBrO₃ concentration (8.88 µg/g) had the least cHQ (0.00141). This current study did not survey whether the public or bakers experience any symptoms of toxicity due to bread consumption or handling. There are currently no reported cases of bread poisoning in Ghana, which may either be because most bread does not contain KBrO₃, or the concentrations are minimal (Table 4).

4. Conclusion

In the present study, a total of 285 tea, sugar, and butter bread were purchased across 10 regions of Ghana for the study out of which 12.28% (35/285) tested positive for KBrO₃ with concentrations ranging from 0.49 to 26.67 µg/g. The use of KBrO₃ for baking is banned in Ghana, and the detection of this chemical compound in bread is not permissible. The HR and HQ of these breads were, however, less than 1 (<1). Thus, consuming the bread types analyzed in this study does not pose any carcinogenic, acute, or chronic chemical risk to the consumer.

It is recommended that regular surveillance be carried out by the Food and Drugs Authority of Ghana to crack down on suspected users of KBrO₃ in bread flour and baking to mitigate the potential health risk of KBrO₃ in bread consumption. Public health experts are encouraged to help the regulator educate the Ghanaian populace on the health hazards associated with KBrO₃ usage to help people appreciate the consequences thereof. Flour millers and bakeries are encouraged to use other alternative, non-toxic additives such as ascorbic acid and glucose oxidase to enhance the quality of their flour and bread. Future studies should include flour samples obtained directly from the millers to enable us to understand whether it is the millers or bakeries who add KBrO₃ to their bread. Also, the temperature and time for bread baking should be studied to enable us to appreciate the effect of temperature and time on residual KBrO₃ concentrations in baked bread.

4.1. Limitations of the study

In this study, only three popular and most consumed bread types; tea, sugar, and butter breads were analyzed. Bread flours used for baking bread were not analyzed for their content of KBrO₃ in this study. The temperature at which the bread is baked, and the time taken for the bread to be baked were also not assessed. Also, the study did not survey bakers or the public to find out if they had

Table 4Estimated daily intake, estimated acute intake, acute quotient, chronic hazard quotient and hazard ratio of KBrO₃ concentration in different bread types.

Bread Type	Highest KBrO ₃ Concentration (ug/g)	Average KBrO ₃ Concentration (ug/g)	Estimated Daily intake of KBrO ₃ (mg/kg/day)	Estimated short-term intake (mg/kg/day)	Cancer Benchmark Concentration (CBC)	Chronic hazard quotient (cCHQ)	Acute hazard quotient (aHQ)	Hazard Ratio (HR)
All Bread Types	26.67	6.13	5.25×10^{-5}	2.286×10^{-4}	1.7×10^{-4}	0.0026	0.0572	0.309
Tea Bread	26.67	8.62	7.39×10^{-5}	2.286×10^{-4}	1.7×10^{-4}	0.0037	0.0572	0.435
Sugar Bread	25.83	7.34	6.29×10^{-5}	2.214×10^{-4}	1.7×10^{-4}	0.0031	0.0554	0.370
Butter Bread	8.88	3.29	2.82×10^{-5}	7.611×10^{-4}	1.7×10^{-4}	0.0014	0.0190	0.166

experienced any form of toxicity due to bread consumption, handling, or baking. Besides that, this study did not experiment with the harmful effect of KBrO_3 in humans or animal models. However, a large sample size of bread was used for the study, and the bread was sampled nationwide.

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Data availability statement

All relevant data are within this published paper.

CRedit authorship contribution statement

J.A. Ayembilla: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Quarcoo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **B.K. Whyte:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Gordon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **P.N.Y. Otu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Conceptualization. **D.A. Bonah:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Formal analysis, Data curation. **W.E. Gedza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **J. Gmakame:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **I.A. Andorful:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **L. Owusu-Ansah:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation.

Declaration of competing 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.

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