

Estimation of Potassium Bromate- an Alarming Carcinogen in Commercial Bread Samples Around Dhaka City

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Abstract

The primary goal of this study was to determine the presence of potassium bromate in bread samples both qualitatively and quantitatively, as well as to investigate the composition of the bread (moisture, ash, and sugar contents). Thirty different commercial bread samples from different areas of Dhaka city were collected for analysis. The ultraviolet-visible spectrophotometric technique was employed in this study to estimate potassium bromate in bread samples. It was based on the redox interaction of bromate and promethazine hydrochloride in an acidic medium which generate a red-pink coloration with maximum absorbance at 515nm. The amount of potassium bromate was found in the range of 1.24 to 24.91 μ g/g. Moisture and ash content were found to be 15.37 to 29.8% and 1.75 to 2.94%, respectively. The total sugar content was determined by the ultraviolet-visible spectrophotometric method by the modified phenol-sulfuric acid test and the maximum absorbance was obtained at 489 nm. Total sugar content was found in different types of white bread samples ranging from 3.33 to 11.31g per 100g. The average recovery (n=5) of potassium bromate was $91.58 \pm 5.61\%$ at a dosage of 10 μ g/mL.

Keywords: Potassium bromate, carcinogen, redox interaction, promethazine hydrochloride.

I. Introduction

Bread is a common food in large parts of the world and is one of the oldest man-made foods, having been of significant importance since the dawn of agriculture. The main ingredient of bread is flour and water. Various types of other ingredients such as yeast, salt, sugar, milk, eggs, etc. are also used in bread making. For making the bread more attractive different types of additives for example oxidants/reductants, emulsifiers, hydrocolloids, and preservatives are also used. Bread consumption is increasing globally due to its low cost, ready-to-eat form, and high nutritional value.¹ Fast foods are very popular among young people. Bread is one of the main ingredients for making fast foods such as burger, hotdog, sandwich or pizza etc. Potassium bromated (KBrO_3) is mainly used as flour improvers to prevent degradation and to make them taste delicious and appealing.² It is a regularly used flour enhancing agent,³ because of its high oxidizing properties⁴. It is a colorless, odorless, and tasteless white crystal/powder.⁵ It acts as a dough conditioner and developing agent by converting the sulfhydryl groups in gluten protein into disulphide bridges, resulting in less extensible and more elastic dough. This increases the viscoelasticity of the dough, allowing it to hold the carbon dioxide gas formed by the yeast. The overall effect is that the bread rises in the oven, increasing its volume and texture.⁶

KBrO_3 has been shown to be nephrotoxic and poisonous in biological organisms and humans. Kurokawa et al.⁷

discovered that it is a genotoxic carcinogen that can cause renal, mesothelioma, and thyroid follicular cell tumors in animals. Many countries have forbidden the use of KBrO_3 because of health concerns. When potassium bromate is inhaled, it causes a cough and sinus infection^[8]. Other noncancerous health problems associated with potassium bromate consumption include abdominal pain, diarrhea, nausea, vomiting, kidney failure, hearing loss, bronchial and ocular problems.⁸

Bread has become a household staple in many households, and its popularity is growing day by day. Bread fits well with tea or coffee. Legislation should therefore govern the composition of breads. The main purpose of this study is to analyze the compositions of bread and to determine the harmful food additives in the bread qualitatively and quantitatively. Moreover, the moisture, ash and sugar content of these breads will be evaluated as well to estimate longevity and quality of breads present in various sites throughout Dhaka, Bangladesh.

II. Materials and Methods

Sampling

A total of 30 varieties of breads (bun, white bread and sliced breads) were collected randomly from different parts of Dhaka city in between October 2021 to April 2022. Samples were marked as B1-B10 for white bread, B11-B20 for sliced bread and B21-B30 for burger bun and preserved in refrigerator at 0° C.

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Reagents

All chemicals and reagents used in the study were of analytical grade. KBrO_3 (SMART LAB, Tangerang, Indonesia), promethazine hydrochloride (Harika Drugs Private Limited, Hyderabad, India), 12M hydrochloric acid (BDH, UK), Concentrated sulfuric acid (98%, w/w, RCL Labscan Limited, Bangkok, Thailand), phenol (Merk, Mumbai, India), extra pure D Glucose (Aldrich Chemical Co. Ltd) and distilled deionized water (Milli Q water) were used.

Instruments

The experimental work was carried out using oven and furnace (GSM 11/8 Hope Valley, S336RB, England), analytical balance (AL 104 Mettler Toledo, US), double beam ultraviolet visible spectrophotometer (UV-1800, Shimadzu), and vortex machine (Cat/Art No 444-1372 (EU), Made in Germany) and centrifuge machine (Cowbell).

Standard solutions preparation

To prepare a primary standard solution of potassium bromate, a stock solution of 1000 mg/L was prepared by dissolving 0.1000 g of KBrO_3 in a 100 mL volumetric flask and topping it up with distilled water to the required concentration. Dilution with distilled water yielded 1, 2, 3, 4, 6, and 8 mg/L solutions from the primary solution. The absorbance was measured against the wavelength corresponding to the absorption maximum (max) at 515 nm after adding 2.0 mL of 0.01 M promethazine hydrochloride and 0.2 mL of 12 M HCl to test tubes and shaking well for one minute (Fig. 3). A standard concentration vs. absorbance curve was plotted (Figure 4).

For the preparation of 200 mg/L primary standard solution of D-glucose, approximately 2.0 mg of certified standard D-glucose was placed in a 10.0 mL volumetric flask with 3.0 mL concentrated H_2SO_4 D-glucose standard solution and vortexed for 1 minute before being diluted up to the mark with concentrated H_2SO_4 . The primary standard solution was diluted in distilled deionized water and 150, 100, 60 and 12.5 mg/L working standard solutions were prepared. Total carbohydrates in the primary standard solutions were determined by modified phenol sulfuric acid method.⁹ At

489 nm, the maximum of the working standard solutions was identified (figure 1). UV-visible spectroscopy was used to measure the total sugar content of the burger bun samples. A typical D-(+)-Glucose calibration curve (fig. 2) was plotted.

Sample preparation

For bromate analysis, 1 g of powdered bread sample was placed in a clean centrifuge tube with 20 mL of distilled water. After filtering, the mixture was vortexed for 2 minutes. The filtered bread solution was then put in a 10 mL volumetric flask with 2.0 mL of 0.01M promethazine hydrochloride and 0.2 mL of 12M HCl, agitated for 1 minute, and the pink-colored solution's absorbance was measured spectrophotometrically. The concentration of bromate in the sample solutions was determined using a standard calibration curve.

For sugar content determination, 40 mg of each sample were placed in a 10 mL volumetric flask, along with 3 mL of concentrated sulfuric acid, and vortexed for one minute. Then concentrated H_2SO_4 was used to dilute it to the desired concentration. The total sugar content of each solution was evaluated using the modified phenol-sulfuric acid test⁹ and a standard glucose calibration curve (three replicates for each company of bread).

Moisture and ash content determination

The moisture content of the samples was tested using the air-oven drying method¹⁰. In a previously weighed porcelain crucible, about 2.0 g of finely ground samples were filled. The crucible was placed in a 105°C oven for 4 hours or until it reached a consistent weight. Three times the analysis was carried out.

The dry ashing method was utilized to determine the ash content of the samples¹¹. In glass crucibles, about 2.0 g of the sample was weighed. The crucibles were heated to roughly 700°C in a muffle furnace. The ignition took roughly 6-8 hours to complete. The crucibles were then placed in a desiccator to cool before being weighed.

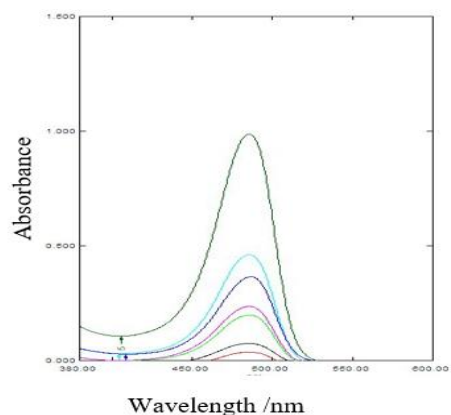


Fig. 1. UV-visible overlain spectrum of standard D-glucose

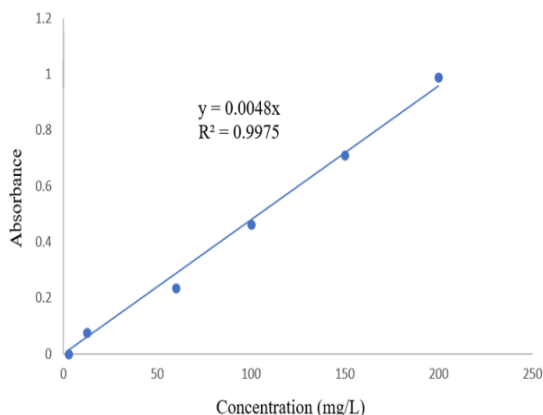


Fig. 2. Calibration curve for standard D-glucose

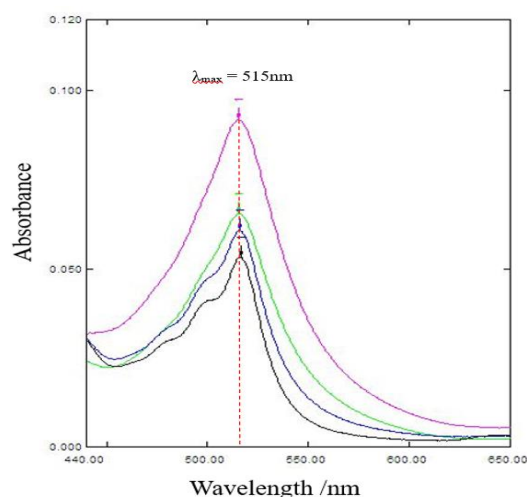


Fig. 3. Overlain spectra for different concentration of standard $KBrO_3$ solutions

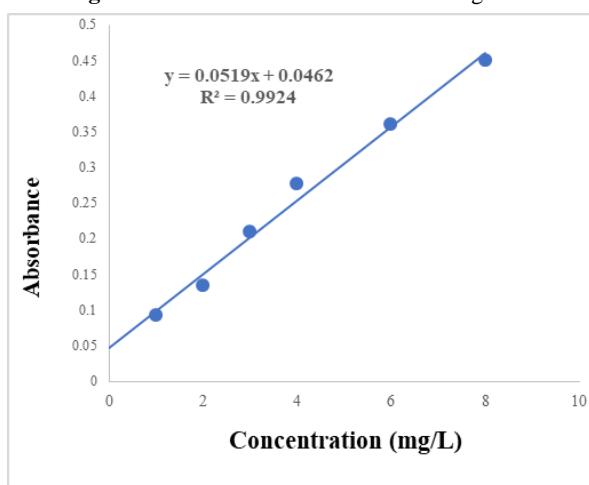


Fig. 4. Calibration curve for standard $KBrO_3$ solutions

Recovery Experiment for potassium Bromate

Traditional white bread sample was prepared by baking with wheat flour, yeast, sugar, sodium chloride, oil, milk and water. For the recovery experiment the blank sample was spiked with potassium bromate at a dosage of 10 $\mu\text{g/mL}$. Five replicate analyses were performed. The sample was prepared for the analysis of potassium bromate by following the same procedure as the commercial samples.

III. Results and Discussion

The moisture level is a crucial parameter in the food industry due to its role in determining a product's shelf life and overall stability. Excessive moisture in bread indicates an increase in the rate of microbial growth, which can not only cause it to perish before it reaches the shelves but also shorten its shelf life.

The average moisture content showed (Fig. 5) variations among the different samples ranging from 15.37-

29.48g/100g of the bread samples, which matches the literature value- varied from 15-30%¹². The highest moisture content value was observed in sample B22 (29.80%) and the lowest content was observed in sample B24 (15.37%).

The average ash content of the breads was in the range of 2.0275- 2.7808 g/100 g. Low amount of ash in breads indicates the presence of mineral elements to be very low. The amount of minerals in breads can influence their physiochemical characteristics as well as their ability to inhibit the growth of microorganisms.

Potassium Bromate and total sugar content

The results of the analysis are shown in Table 2. Out of 30 bread samples potassium bromated was found to be present in 22 samples. Bread sample B19 recorded the least amount of residual bromate ($1.24 \pm 0.0873 \mu\text{g/g}$). Highest level of bromate ($24.51 \pm 0.47 \mu\text{g/g}$) was recorded in bread sample

B14. The colour change ranged from light to dark pink with increase in concentration.

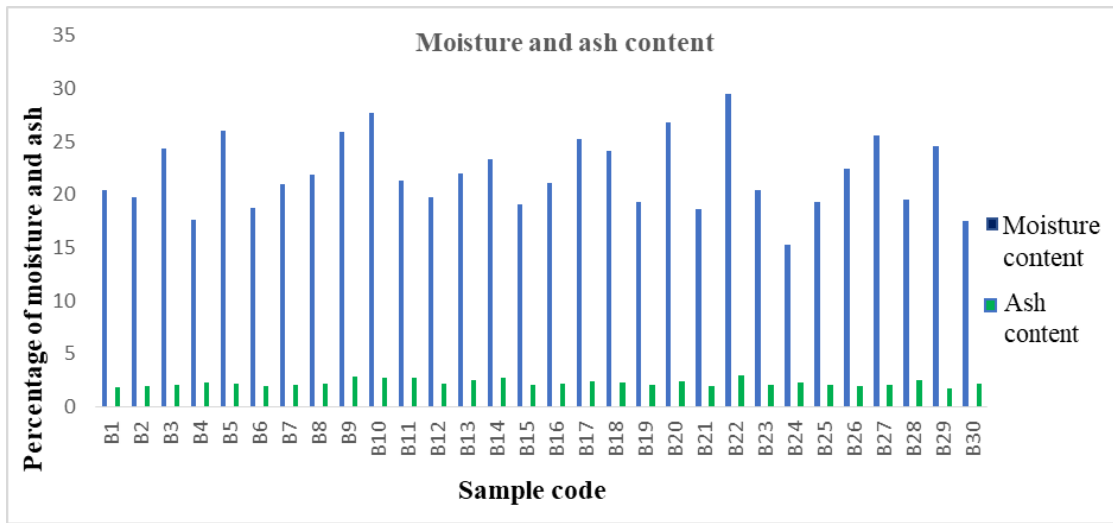


Fig. 5. Percentage of moisture and ash contents in different bread samples

Table 1. Determination of amount of potassium bromate in different bread samples collected from different locations of Dhaka city

	Sample code	Date of collection	Area of collection	Total potassium bromate content (µg/g), n= 3	Relative standard deviation (%)	Total Sugar Content (g/100g) n= 3	Relative standard deviation (%)
White Bread	B1	8-11-2021	Dhaka university area	8.88±0.37	4.22	6.57±0.50	7.59
	B2	8-11-2021	Bangobazar	ND			
	B3	8-11-2021	Dhaka Medical area	10.30±0.46	4.45	10.60±0.78	7.37
	B4	24-11-2021	Azimpur	11.53±0.48	4.20	10.02±0.30	3.01
	B5	24-11-2021	Chankharpul	6.36±0.18	2.88	10.17±0.48	4.73
	B6	03-4-2022	Chankharpul	18.57±0.31	1.70	5.12±0.54	10.56
	B7	03-4-2022	Azimpur	6.90±0.32	4.61	5.02±0.25	5.02
	B8	08-4-2022	Mouchak	ND			
	B9	09-4-2022	Lalbag	ND			
	B10	03-4-2022	Neelkhet	18.47±0.81	4.40	4.68±0.12	2.69
Sliced Bread	B11	22-11-2021	Azimpur	11.24±0.24	2.12	6.51± 0.32	4.94
	B12	24-11-2021	Neelkhet	6.94±0.22	3.12	6.46± 0.22	3.41
	B13	24-11-2021	Neelkhet	ND			
	B14	24-11-2021	Chankharpul	24.51±0.47	1.93	11.31± 0.45	3.99
	B15	06-4-2021	Puran Dhaka	17.49±0.48	2.75	9.83± 0.23	2.39
	B16	06-4-2021	Malibag	21.96±0.49	2.23	7.37± 0.22	2.97
	B17	27-11-2021	Sylhet	7.77±0.42	5.39	4.69± 0.14	3.06
	B18	09-4-2022	Farmgate	ND			
	B19	09-4-2022	Mouchak	1.24±0.09	7.02	5.44± 0.29	5.44
	B20	24-11-2021	Neelkhet	9.01±0.11	1.22	8.60± 0.44	5.08
Burger Bun	B21	10-10-2021	Dhaka university area	10.27±0.26	2.59	5.34±0.23	4.32
	B22	10-10-2021	Dhaka university area	7.21±0.13	1.77	9.28±0.39	4.20
	B23	28-10-2021	Dhanmondi	ND			
	B24	03-11-2021	Segunbagicha	17.06±0.07	0.39	3.30±0.23	7.06
	B25	07-11-2021	Neelkhet	21.71±0.59	2.73	6.44±0.34	5.31
	B26	09-12-2021	Segunbagicha	22.98±0.57	2.5	9.83±0.27	2.79
	B27	21-03-2022	Neelkhet	ND			
	B28	27-03-2022	Dhaka university area	5.30±0.16	3.04	5.87±0.11	1.88
	B29	06-04-2022	Dhanmondi	3.96±0.05	1.19	10.99±0.01	0.01

B30	07-04-2022	Shahbagh	ND
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Note: “n” indicates number of replications, “ND” indicates not detected

In 8 bread samples (B2, B8, B9, B13, B18, B23, B27 and B30) no potassium bromate was found to be present, may be due to their better baking procedure or they actually did not use bromate. The potassium bromated content in the 24 samples analyzed is higher than 0.02 μ g/g, which is the permissible safe level of potassium bromate allowed in bread by the US Food and Drug Agency (FDA)¹³. Bangladesh Standards and Testing Institution (BSTI) set a standard for making bread of 5 mg of KBrO₃ maximum in 1 kg bread in 2016. But in 2018, the BSTI banned the use of potassium bromate in bread¹⁴. This implies that, the bread samples from Dhaka area, analyzed in this study, is not safe for human consumption as far as potassium bromate content is concerned because it has been associated with neuro- and nephro-toxicity¹⁵, ototoxicity¹⁶, and it poses additional health risk for bakery workers as potassium bromide, a decomposition product of potassium bromate, is also toxic¹⁷. The nutritional quality of bread can be reduced in presence of potassium bromate by degrading essential vitamins such as vitamin A, B and E⁵. The adverse effects potassium bromate on liver and kidney functions of rats was reported on 2009 by Oloyede and Sunmonu¹⁸. Sugar

not only imparts the sweetness in breads but also helps the fermentation of yeast. Sugar also causes the browning of the bread. Total sugar contents in buns (B21-B30) were obtained in the range 3.30-10.99g/100g. The highest sugar content value was observed in bun B29 (10.99%) and the lowest content was observed in B24 (3.30%). Sugar content of the analyzed bread samples were found slightly higher than the literature value of an ideal white bread (5g/100g)¹⁹.

Recovery

When the standard bread sample was spiked with potassium bromate at a dosage of 10 μ g/mL, satisfactory recovery of 91.58 \pm 5.61% was obtained. The relative standard deviation for the recovery of potassium bromate was 6.13%. This implies that the current work offers a straightforward, speedy, and verified spectrophotometric approach for finding bromate; as a result, it can be suggested for the routine management of bromate in bread. With the purpose of protecting consumer security, particularly in developing countries, this technology will offer an alternative for laboratories lacking pricey resources.

Table 2. Recoveries of potassium bromate added as KBrO₃ to the mentioned sample at 10 μ g/mL

Spiking concentration of potassium bromate (μ g/mL)	Absorbance at 515 nm	Concentration of potassium bromate found (μ g/mL)	Recovery (%)	Mean Recovery (%) n=5	Relative standard deviation (%)
10	0.101	8.91	89.10	91.58 \pm 5.61	6.13
	0.111	9.79	97.90		
	0.095	8.37	83.70		
	0.108	9.58	95.80		
	0.104	9.14	91.40		

Note: “n” indicates number of replications

IV. Conclusion

The results from this research provide important details regarding the sugar and ash content as well as potassium bromate contents of bread and bun samples gathered from different areas of Dhaka city. The quantitative measurement of potassium bromate levels revealed that 70% of the investigated samples have a significant amount of bromate residue. As a result, bread customers and bakers are at risk of bromate exposure, which has major health consequences. The sugar content of the analyzed bread and bun sample were found slightly higher than the literature value of an ideal white bread¹⁹. In order to protect the health of the consumers, it is necessary for the relevant regulatory bodies in Bangladesh to set stricter requirements for the production

of bread and to conduct routine inspections of bakeries to make sure that bakers do not break the law and that the environment, as well as the materials used by these bakeries, are free from any kind of contamination.

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