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Bread sold at stores in Ile-Ife City, in southwestern Nigeria, was analyzed for bromate and trace metal content

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Abstract:

Ile-Ife bread from nine different stores and bakeries was tested for bromate and trace metal levels to ensure it was safe for human consumption. Trace metals in the digested bread samples were profiled using a Flame Atomic Absorption Spectrophotometer, and bromate concentrations were determined using a spectrophotometric approach. Trace metal values were as follows: 0.03-0.10 g/g Co = 0.23-0.46 g/g Cu 2.23-6.63 g/g Zn 25.83-75.53 g/g MN, whereas bromate levels varied from 2.051 0.011 g/g to 66.224 0.014 g/g throughout the bread samples tested. Despite claims to the contrary on their packaging, several Ile-Ife bread bakeries were not in complete compliance with NAFDAC's bromate-free norm, despite the fact that their bread was labelled as such. Essential and dangerous trace metals were present in the bread samples at concentrations that might be harmful over the long term.

Introduction

Bread is a staple meal in many parts of the globe, notably in the southern and eastern parts of Asia and Africa [1, 2]. Bread is a staple in the diets of over 70% of Nigeria's over 150 million people—the poor [3] and the young. Wheat flour, which is what bread is produced from, is low in protein. In addition to flour, you'll need things like salt, sugar, flavouring, and (at the very least) a flour improver like potassium bicarbonate. Bromate [4, 5] For over eighty years [5,7,8] people have used potassium bromate as a means to better their flour. Because of its low cost and high efficiency as an oxidizing agent, potassium bromate is widely used by flour millers and bakers across the globe [9]. Its primary role is as a maturing agent, giving the dough strength in the last stages of the rising process before baking [5]. Throughout the fermentation, proving, and baking processes, it serves as a gradual oxidizing agent, altering the dough's structure and rheological qualities. Thiele groups are thought to be oxidized to disulphide links, which subsequently reinforce the protein network [10]. It aids in the bread's rise and development of a pleasant texture when baking. A lot of Nigerian bakers have taken advantage of this situation to increase their earnings. In terms of human health, toxicological studies have shown that consuming potassium bromate can lead to non-cancerous effects such as the degradation of vitamins A1, A2, B1, B2, E, and niacin, which are the main vitamins available in bread [11,12]; causing significant differences in the essential fatty acid content of flour treated with bromate [13]; and causing the development of symptoms like cough, sore throat, abdominal pain, diarrhoea, nausea, vomiting, However, several investigations [16–19] have shown that potassium bromate may induce oxidative stress in tissues [12,20–24], which can lead to cancer in both experimental animals and people. Since there is substantial evidence that potassium bromate caused cancer in experimental animals [25,26], the International Agency for Research on Cancer (IARC) has categorized potassium bromate as a class 2B carcinogen (a probable human carcinogen). As a result, its prohibition was proposed in the United States, the United Kingdom, Canada, Sri Lanka, and China, among other places, between 1990 and 2005. Bread with up to 50 milligrams of potassium bromate per kilogram of flour mass is legal in the United States and China, whereas only 10 milligrams is allowed in Japan [27]. In California, bromated flour must include a warning label, and it is widely acknowledged that potassium bromate should not be used in any product or manufacturing technique that cannot be created without residues below the threshold of 20 parts per billion (ppb; 0.020 mg/kg; 0.020 g/g) [28]. Because long-term toxicity and carcinogenicity tests in vitro and in vivo showed kidney cell tumours in hamsters, the joint FAO/WHO [11] committee withdrew its earlier recommendation of an acceptable level of 0-60 mg $KBrO3$/kg flour. In 2003, the National Agency for Food and Drug Administration and Control

(NAFDAC) outlawed the use of potassium bromate in flour milling and baking in Nigeria due to violations of NAFDAC Decree 15 of 1993 and NAFDAC Decree 20 of 1999, both of which regulate the registration of drugs and other related products [29]. However, it is unclear how strictly Nigerian bakeries have adhered to the prohibition on potassium bromate in bread production set by NAFDAC. In addition to bromate, other trace metal contaminations may be found in the ingredients used to make bread and the locations in which these bakeries are situated. There are a number of potential causes of trace metal contamination in bread: the raw materials used, unsanitary conditions in the baking settings, and a lack of effective precautionary steps to prevent cross-contaminations from other environmental sources. High concentrations of a number of trace metals have been linked to the development of a wide variety of health problems [30,31]. Cd is linked to a variety of health problems, including respiratory damage, cancer of the lungs, and impaired kidney function. Humans exposed to lead are at risk for developing neurological and behavioural abnormalities. Overexposure to Zn may cause stomach cramps and skin irritations, while low-dose exposure can cause loss of appetite, diminished sense of taste and smell, poor wound healing, and impaired sense of taste and smell. Toxic levels of Cu lead to anaemia, renal and liver failure, and stomach and intestinal discomfort. Eye infections such conjunctivitis, thyroiditis, retinitis, and so on have been linked to excessive Fe levels. Long-term exposure to even low levels of Cr may cause severe health problems, including kidney and liver failure. Ni exposure is linked to a wide range of health problems, including lung cancer, respiratory failure, congenital abnormalities, cardiovascular disease, and asthma. Excessive exposure to aluminium is associated with amnesia, severe tremors, and nervous system impairment. In high doses, Co may lead to asthma, pneumonia, heart difficulties, thyroid damage, vomiting, and nausea; manganese, on the other hand, is linked to manganese toxicity, obesity, neurological symptoms, and birth abnormalities [32]. The purpose of this research was to assess bromate and trace metal content in commercially available breads in the Ile-Ife area. Using this method, we could determine whether the bread's bromate and trace metal levels complied with government standards for safe consumption.

Experimental

Sampling

Nine samples of commercially available bread were acquired from various stores and bakeries in and around Ile-Ife. These samples were sent directly from the store to the lab for initial testing.

Sterilization of apparatus

All of the vials and glasses were meticulously cleaned by soaking in detergent solution in a wash basin overnight. With a nylon brush, we washed the insides of the glasses and vials, and then we rinsed them well with hot distilled water to remove any trace of soap. After a thorough cleaning with acetone and cool distilled water, they were submerged in 10% HNO3 for 48 hours. We dried them in an oven at 105 degrees Fahrenheit. The vials and other glassware were kept in clean, sealed polythene bags before use to avoid contamination from dust and other particles floating in the air of the laboratory.

Sample preparation and pre-treatment

Four slices of each kind of bread were cooked at 55 degrees Celsius for 24 hours for the bread drying experiment. The ensuing crust was ground into fine powder using a mortar and pestle carved from agate. In a 250 mL beaker, 5 g of powder was weighed out for each sample, then 2 x 10 mL of distilled water and a good stir were added. The mixture was filtered into a 25 mL flask and brought to the correct volume by adding distilled water.

Bromate determination

The procedure described by Ojai et al. [33] was used, with few changes, in the present investigation. Each of the nine bread samples was weighed and a 4 mL aliquot was measured using a calibrated pipette into one of three 25 mL calibrated flask. In addition, either 5 mL of a Congo Red solution (5 104M) or 5 mL of a Crystal Violet solution (5 104M) was added, followed by 10 mL of a 2 M Hall solution. Before performing a colorimetric analysis, the sample was diluted with distilled water to the 25 mL mark and gently yet thoroughly shaken. Crystal Violet samples were measured at max= 580 nm using a Fenway 6051 Colorimeter, whereas Congo Red samples were measured at max= 520 nm. All readings were taken using pure water at room temperature as a standard. Dye oxidation with bromate was performed in a hydrochloric acid media.

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$$BrO_3^- + 6H^+ + 6e \rightleftharpoons Br^- + 3H_2O$$

For indicators that undergo irreversible oxidation, the amount of bromate solution consumed by the dyestuff indicator is negligible, and the indicator is bleached in the presence of 2 M Hall:

$$10Cl^- + 2BrO_3^- + 12HCl \rightleftharpoons 11Cl_2 + Br_2 + 6H_2O + 12e \quad (2)$$

For indicators that undergo irreversible oxidation, the amount of bromate solution consumed by the dyestuff indicator is negligible, and the indicator is bleached in the presence of 2 M Hall: Congo Red went from being red to blue. Crystal Violet appeared purple in a dilute acid solution, green in a concentrated one, and lastly yellow in neutral water. Low levels of sulphuric acid (SO3H) groups in Congo Red and dimethyl amino (-CH3) groups in Crystal Violet made both dyes soluble in water.

Sample digestion

A solid powdered sample of each of the nine bread types was weighed and put into a 100 mL Teflon beaker. Ten mL of concentrated HNO3was added, and the mixture was gently mixed before being placed on a thermostatically controlled heating mantle maintained at 120-150C for roughly 1 hour. After adding 2 mL of HClO4, the mixture was digested for another 30 minutes. Following removal from the heating mantle, the digested sample was quantitatively transferred into a 25 mL volumetric flask and filled to the mark with doubly distilled water in preparation for Atomic Absorption Spectroscopy examination.

Trace metal quantification

Trace metal concentrations were determined using a Flame Atomic Absorption Spectrophotometer (FAAS, Buck Model 205) and the digested sample solution.

Quality control measures adopted

Blank determination

The analyses of interest in the materials and reagents used for analysis were determined by performing a blank determination. To accomplish this, an independent determination was performed using the same experimental setup as the primary study, but without the material itself. The colorimeter was used to detect the absorbance of a dye solution in an acidic medium, allowing for a precise assessment of bromate concentration. Bread concentration values were deducted from the relevant values. To determine trace metal concentrations when no samples of bread were available, we performed the digestion procedure using the same reagents but without the bread. When calculating analyse concentrations, blank determination values were deducted to ensure accuracy.

Calibration of FAAS

In order to assess the reaction of the analytical technique with regard to known amounts of the standards of the trace elements of interest and to properly predict the response to unknown quantities in the samples, FAAS calibration was required. Each metal solution used in the FAAS's assessment of metal concentration in samples was newly created by serial dilution to provide final concentrations of 20, 18, 15, 12, 10, 7.5, 5, 3, and 1 g/milk The FAAS was then used to run these solutions in order to generate a calibration graph that could be used to automatically extrapolate trace metal concentrations from sample data.

Calibration of colorimeter

According to the experimental conditions, calibration curves were obtained by running the serially diluted bromate concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm. This was done for both the Crystal Violet dye method (at max= 580 nm) and the Congo Red dye method (at max= 520 nm). Concentrations were compared to the absorbance readings. In order to extrapolate the bromate levels in a bread sample for both Crystal Violet and Congo Red dyes, we utilized the produced working graph. The resulting empirically established connections for Crystal Violet and Congo Red are given in Aqsa. (3) and (4):

where x = concentration of bromate, and y = absorbance

$$y = 0.003x + 0.600 \quad (r^2 = 0.987)$$

$$y = 0.001x + 1.396 \quad (r^2 = 0.978)$$

Recovery experiment for trace metals

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The recovery analysis followed the procedure outlined by Oyekunle et al. [34]. This recovery study compared two 1.0 g pieces of bread from the same bakery. The standard combination of the trace metal solution (25 g/mL) was added to one part (A), while the other (A; the control) was kept unaltered. In order to digest the samples, both halves were taken in a comparable yet independent manner. FAAS analysis was then performed on the generated solutions. By comparing the concentration levels of each metal between the spiked and unsliced sample data, the %R of trace metals was calculated:

$$\%R = \frac{A - A'}{B} \times 100$$

A = the quantity of trace metal used to spike the bread sample, B = the amount of trace metal found naturally in the bread, and A = the concentration of trace metals in the spiked bread sample.

Recovery experiment for bromate

We performed a recovery study using two 5 g samples from the same loaf of bread, labelled A and B. For Part A, 4 mL of a known concentration of bromate was added, but for Part B, no additional bromate was included. After exposing the samples to bromate,

Table 1

Description of bread loaves used for analysis.

Code	Loaf size[a]	Loaf type	Manufacturer's specification
A	Big	Sliced	Bromate free
B	Big	Sliced	Bromate free
C	Big	Sliced	Bromate free
D	Big	Unsliced	Bromate free
E	Big	Unsliced	Bromate free
F	Big	Unsliced	Bromate free
G	Small	Unsliced	Bromate free
H	Small	Unsliced	Bromate free
I	Small	Unsliced	Bromate free

Table 2

Calibration curve (r2) and percentage recovery (%R) of bromate using the two methods.

Analytical method	Slope (m)	r^2	%R
Crystal Violet method	0.003	0.987	50.24 ± 5.11
Congo Red method	0.001	0.978	89.93 ± 2.46

analysis in the manner described. Triple samples were run to calculate the %R of bromate by comparing the concentration levels of bromate in the spiked and unsliced sample results using the following relationship:

$$\%R = \frac{A - B}{C} \times 100$$

The quantity of bromate used to spike the bread is denoted by C, while the concentration of bromate in the spiked sample is denoted by A.

Results and discussion

Levels of bromate in bread samples

Table 1 details the characteristics of the bread utilized in the experiment. For this analysis, researchers looked at nine unique samples total, including three large sliced loaves, three large unsliced loaves, and three tiny unsliced loaves. The producers labelled all of them as bromate-free. Under the experimental settings shown in Table 2, the r2 values for the Crystal Violet and Congo Red techniques were 0.987 and 0.978, respectively. Accordingly, there was a significant degree of linearity between the two procedures, allowing for accurate comparison of bromate amounts in bread samples. However, the values of 50.24 5.11 % and found in the recovery analysis.

Table 3

Sample code	Manufacturer's specification	$[BrO_3^-]$ by Crystal Violet oxidation	$[BrO_3^-]$ by Congo Red oxidation
A	Bromate free	28.025 ± 0.005	36.012 ± 0.007
B	Bromate free	3.129 ± 0.024	10.029 ± 0.007
C	Bromate free	41.336 ± 0.009	66.224 ± 0.014
D	Bromate free	2.051 ± 0.011	22.356 ± 0.008
E	Bromate free	7.667 ± 0.012	21.397 ± 0.017
F	Bromate free	4.205 ± 0.012	24.461 ± 0.004
G	Bromate free	10.313 ± 0.012	26.258 ± 0.043
H	Bromate free	6.333 ± 0.023	23.326 ± 0.011
I	Bromate free	20.296 ± 0.022	40.231 ± 0.012

Bromate levels (g/g)ain the bread samples.

Table 4

Trace metal	Calibration curve, r^2	(%R)
Cu	0.9834	90.25 ± 3.65
Co	0.9956	78.92 ± 4.15
Mn	0.9696	85.35 ± 2.76
Pb	0.9919	93.22 ± 5.23
Zn	0.9752	95.91 ± 3.56

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Calibration curve (r2) and percentage recovery (%R) of metals in bread sample.

Code	Bread type	Co	Cu	Mn	Pb	Zn
A	Sliced	0.08±0.01	0.38±0.03	75.53±1.02	0.08±0.01	6.63±0.25
B	Sliced	0.09±0.02	0.30±0.06	73.90±0.56	0.05±0.01	4.98±0.12
C	Sliced	0.06±0.02	0.38±0.03	72.23±1.21	0.09±0.03	5.04±0.31
D	Unsliced	0.08±0.01	0.46±0.12	43.90±1.06	0.06±0.02	5.38±0.11
E	Unsliced	0.10±0.03	0.35±0.04	64.10±3.11	0.10±0.02	4.99±0.12
F	Unsliced	0.07±0.01	0.35±0.05	52.75±0.29	0.07±0.02	3.84±0.04
G	Unsliced	0.08±0.02	0.38±0.02	49.68±2.13	0.09±0.03	3.88±0.08
H	Unsliced	0.09±0.02	0.23±0.06	47.40±1.35	0.06±0.02	5.03±0.03
I	Unsliced	0.03±0.01	0.39±0.05	25.83±0.59	0.03±0.00	2.23±0.16
Mean±s.d.		0.08±0.02	0.36±0.05	56.15±1.26	0.07±0.02	4.67±0.14
CV		25.0	13.9	2.24	28.6	3.0

The standard calibration curves produced under the experimental circumstances utilized demonstrated a good linearity level, with correlation coefficients (r2) ranging from 0.9752 to 0.9956. Heavy metals were recovered at levels as low as 78.92 4.15% Co and as high as 95.91 3.56% Zn. There was a consensus that these figures were reasonable. Acquired values of 2.76–5.23% relative standard deviation (RSD) for bread samples demonstrate accuracy over the RSD10% threshold. Table 5 shows the range of trace metal concentrations found in the bread samples we tested. Co levels were between 0.03 0.01 and 0.10 0.03 g/g. The quantity of cobalt required by the human body is very low yet necessary for survival. The FAO and WHO both agree that adults may safely consume up to 0.1 g of cobalt per day in their diet, which is comparable to 2.4 g of vitamin B12 per day. Co levels were often found to be in this range for the bread samples tested. Co levels from bread alone seemed to substantially surpass the range advised by the FAO/WHO [11], suggesting that frequent consumers of bread may experience negative consequences over time. Coalmines are coenzymes whose active centre is cobalt; vitamin B12 is the most prevalent coalman. So, it's a necessary trace mineral for the diets of all kinds of animals. Cobalt, in its inorganic form, is a useful nutrient for microorganisms including bacteria, algae, and fungus. Soil with even a trace amount of cobalt significantly enhances the health of grazing animals; a daily intake of 0.20 mg/kg is advised since these animals can't naturally produce vitamin B12. Soluble cobalt salts have an LD50 estimated between 150 and 500 mg/kg [11]. Cu concentrations in the bread utilized in this analysis varied from 0.23 0.06 to 0.46 0.12 g/g. Copper is the most powerful oil-pro-oxidant, and fat and oil or their derivatives with a high concentration of Coin are the most stable.

to be less than 0.02 g/g [39,40]. The sample Cu concentrations were almost five times greater than 0.02 g/g. This suggests that the vegetable oil used as a releasing agent in bread baking will deteriorate quickly. The Cu contamination may have originated not just from the cereal raw materials used to make flour, but also from the depreciation and disintegration of certain copper alloy equipment used to make flour and bake bread. Copper, like cobalt (Co), is a mineral that can be found all throughout the body and is needed for many bodily functions. It is used to produce red blood cells and maintain healthy neural and immune cells. Collagen formation is aided as well, which is important for the development of bones and connective tissue [41,42]. Cu, in the right concentration in the human body, may also have antioxidant properties, neutralizing free radicals that may cause harm to DNA and other cellular components (DNA). The human body retains copper steadily and only needs a little amount of copper to operate well, therefore copper shortages are uncommon. However, there are populations at very high risk [42]. Due to the depleting effects of zinc, vitamin C, and fructose on Cu levels, those who need to eat excessive quantities of these nutrients may be at risk for Cu deficiency. Patients with disorders that result in poor absorption rates may also be unable to store the minute quantities of Cu their systems need. Infants who are given just cow milk formula may have Cut deficits, according to recent research [43]. Infants born prematurely, especially those with very low birth weights, are also more likely to have Cu deficiency. Some 25% of adolescents, adults, and the elderly, according to a variety of nutrition studies, do not get enough Cu in their diets. Fortunately, extremely low serum metal and ceruloplasmin concentrations in the blood may indicate Cu deficiency. Osteoporosis, osteoarthritis, rheumatoid arthritis, cardiovascular disease, colon cancer, and chronic bone, connective tissue, heart, and blood vessel disorders have all been related to Cu deficiency in the past. Copper aids in iron absorption, therefore Cu is required for human energy production [43]. Cu levels in the samples ranged from 0.23 0.06 g/g to 0.46 0.12 g/g, which is within the acceptable range of 0.008-9 g/g established for Cu levels in foodstuffs [44,45].

The bread samples had manganese (MN) concentrations ranging from 25.83 0.59 g/g to 75.53 1.02 g/g. Although manganese is an important nutrient since it is involved in many enzyme systems, it is present only in very minute quantities in human tissues and was only recognized as such in 1931 [46]. Bone mineralization, protein and energy metabolism, metabolic control, cellular protection from harmful free radical species, and glycosaminoglycan synthesis are all influenced by this nutrient [46]. A lack of MN in the diet has been linked to stunted development and trouble reproducing. The majority of the MN in the human body — about 15–20 mg — is found in the skeletal system, while the rest is dispersed across the organs responsible for hormone production and regulation. Manganese (MN) is a necessary nutrient, however exposure to high levels of MN may have neurological repercussions in the workplace [46]. Cereals (10-30 mg/kg), vegetables/fruits (0.5-5 mg/kg), and nuts (may have greater concentration) are the primary dietary donors of MN [47,48]. Over time, eating too much MN in a meal may have negative health consequences, and the amounts reported in the bread samples in this investigation much above the normal consumption of 2-3 mg/day suggested by WHO [49]. The bread had a lead (Pub) level between 0.03 0.00 and 0.10 0.02 g/g. Lead is a very toxic element that may have an adverse effect on almost every physiological system. As of [49], the Provisionally Tolerable Weekly Intake (PTWI) has been set at 0.025 mg/kg body weight by the FAO/WHO Joint Expert Committee on Food Additives. The bread's Pub concentration was obviously higher than the PTWI limit for human consumption. Possible cause of health issues in the future. The extensive use of Pub over many centuries has led to elevated levels of the element in the natural world. When lead enters the body, it may stay there for a long time in certain tissues before being reabsorbed and redistributed. The effects of Pub on the neurological system, kidneys, and blood are most notable. The United States Environmental Protection Agency (USEPA) has designated lead as a Group B2 carcinogen, meaning it is a potential human carcinogen (USEPA http://www.epa.gov/ttn/atw/hlthef/hapindex.html) [50]. A little rise in blood pressure is another effect of lead exposure (Codex Committee on Food Additives and Contaminants [51]). Samples of bread used in this investigation had Zn concentrations that varied from 2.23 0.16 to 6.63 0.25 g/g. For example, zinc is quite common and is an important trace element [52]. Zinc is essential for good taste and smell [41], assists in the operation of several metallic-enzymes [46,47], and promotes healthy growth and development throughout pregnancy, infancy, and adolescence. Because the human body lacks a dedicated Zn storage mechanism, getting enough of the mineral on a daily basis is essential for maintaining a constant level. Zinc reduces Cd and Cu toxicity [52], while zinc shortage or excess may increase carcinogenesis [47] risk. Loss of appetite, slowed development, and compromised immunity are all symptoms of a zinc shortage. Hair loss, diarrhoea, delayed sexual maturation, impotence, hypogonadism in males, eye and skin lesions, weight loss, delayed wound healing, taste anomalies, mental lethargy, and fatigue have all been linked to zinc deficiency [30,53]. Provisional Maximum Tolerable Daily Intake (PMTDI) values of 1 mg/kg body weight were set by FAO/WHOJECFA [54]. Zinc's recommended dietary allowance (RDA) was set at 0.3 milligrams per kilogram of body weight (mg/kg) by the Joint Expert Committee on Food Additives (JECFA) in 1982 [54]. The recommended daily allowance for adults is 15-22 mg [55]. Below the World Health Organization's (WHO) safe limit for human consumption, the Zn levels in the bread samples were found to be between 2.23 and 6.63 micrograms per gram. This means that a person living in this area who consumes a lot of bread may get zinc deficient illnesses.

Conclusion

The study evaluated the bromate and trace metal levels of bread commonly sold and consumed in Ile-Ife and its environs. It was revealed that the millers and bakers did not comply with the bromate free rule stipulated by both NAFDAC and WHO contrary to the “bromate free” indicated on their labels. In addition, the bromate levels in some of the breads were higher than the 60 g/g stipulated by the WHO. Results of trace metals analysis showed that bread samples contained Co, Cu, MN, Pub and Zn at levels that were at wide variance with those specified by such bodies as NAFDAC or WHO. Particularly, the Pub content of the bread was greater than the 0.025 mg/kg body weight set as the Provisionally Tolerable Weekly Intake for humans. There is the need to place a close surveillance on bakers of bread in order to ensure better compliance with health regulations.

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