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RESEARCH ARTICLE

SPECTROPHOTOMETRIC DETERMINATION OF BROMATE IN BREADS USING PROMETHAZINE

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ABSTRACT

The levels of bromate in twenty bread samples obtained from some locations in Katsina metropolis were determined using spectrophotometric method. It was based on the redox reaction between bromate and promethazine in an acidic medium. This reaction produced a red-pink product with maximum absorption at 515nm. Prior to the quantitative determination; a qualitative test was carried out on a portion of each bread sample using 2cm³ of 0.01M promethazine and 0.6cm³ of 12M hydrochloric acid. The change in colour observed in each bread sample indicates the presence of bromate. The results obtained show bromate levels which ranged between 2.18 to 8.25µg/g in bread samples. Bromate levels in all the samples examined were found to exceed the recommended permissible level by US Food and Drug Agency and National Agency for Food and Drug Administration and Control of 0.02µg/g. All bread samples examined were considered unsafe for human consumption. Bread bakers should explore other alternative means of flour improvers instead of using bromate because of its deleterious and carcinogenic effect in humans.

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INTRODUCTION

Bromate is recognised as one of the best dough improvers in the bakery industry. Under controlled conditions, bromate is converted into bromide, which is considered harmless to the consumer. Though scientific evidence has implicated bromate as a possible carcinogen and it has been removed from the list of acceptable additives for flour treatment (Kujore, A and Serret, J M, 2010). Potassium bromate is a colourless, odourless and tasteless white crystal/powder that is used as a food additive (Joint FAO/WHO, 1992) and is commonly used as flour enhancing agent in Nigeria (Emeje *et al.*, 2010). This could be due to its efficient oxidizing properties (Gandikota *et al.*, 2005). It acts as a maturing agent and dough conditioner by oxidizing the sulfhydryl groups of the gluten protein in flour into disulphide linkages, thus strengthening the protein network, making it less extensible and more elastic; this will make the dough visco-elastic such that it can retain the carbon dioxide gas produced by the yeast. The overall effect is to make bread rise in the oven, increase loaf volume and texture (Nakamura *et al.*, 2006). Over the years several improvers have been used but studies have shown some to be deleterious to health, thereby necessitating their ban. The National Agency for Food and Drug Administration and Control (NAFDAC) announced the dangers associated with the use of bromate and banned its further use in bread in 2002 (Alli *et al.*, 2013). Despite the ban on the use of bromate in bread by NAFDAC, and the fact that there are other nontoxic, flour-enhancing alternatives such as ascorbates (Ayo *et al.*, 2002), many bakers are still using bromate to enhance their bread thereby putting the life of the consuming public at detrimental health risk. Bromate has many adverse effects on the nutritional quality of bread and the health of consumers (Fisher *et al.*, 1979; Fujii *et al.*, 1984). It degrades vitamins A2, B1, B2, E and niacin which are the main vitamins available in bread (IARC, 1999). In humans,

bromate can cause cough and sore throat when inhaled. Some other noncancer health problems associated with ingestion of bromate include: abdominal pain, diarrhoea, nausea, vomiting, kidney failure, hearing loss, bronchial and ocular disorders (Atkins, 1993). However, numerous studies have revealed the potential toxicity of bromate to cause cancer in experimental animals and in humans (Watson, 2000). Thus, the present study is aimed at determining the levels of bromate in some selected bread samples consumed in some areas of Katsina metropolis with the view of ascertaining the level of dietary exposure of consumers of bread of such areas. This investigation will also assess the bread safety and the level of compliance with the NAFDAC's directive of non-inclusion of bromate in bread in Nigeria.

MATERIALS AND METHODS

Materials

Chemicals of analytical grade purity and distilled deionized water were used throughout the experimental work. All glass wares and plastic containers used were washed with detergent solution followed by (20%v/v) nitric acid and then rinsed with tap water and finally with distilled deionized water. Promethazine hydrochloride and potassium bromate (BDH chemical, England), were used for preparation of standard solution. Also, promethazine and hydrochloric acid (BDH chemical, England), were used in the analysis.

Study area

The study area covered in this research was Katsina, a city in North Western Nigeria. It is the capital of Katsina state, one of Nigeria's 36 states. It is also the headquarters of Katsina Local Government Area. The city is located on the latitude 12° 59' N and longitude 7° 36' E with an average area of 142km² and a population of 318,459 as of 2006 (Figure 1).

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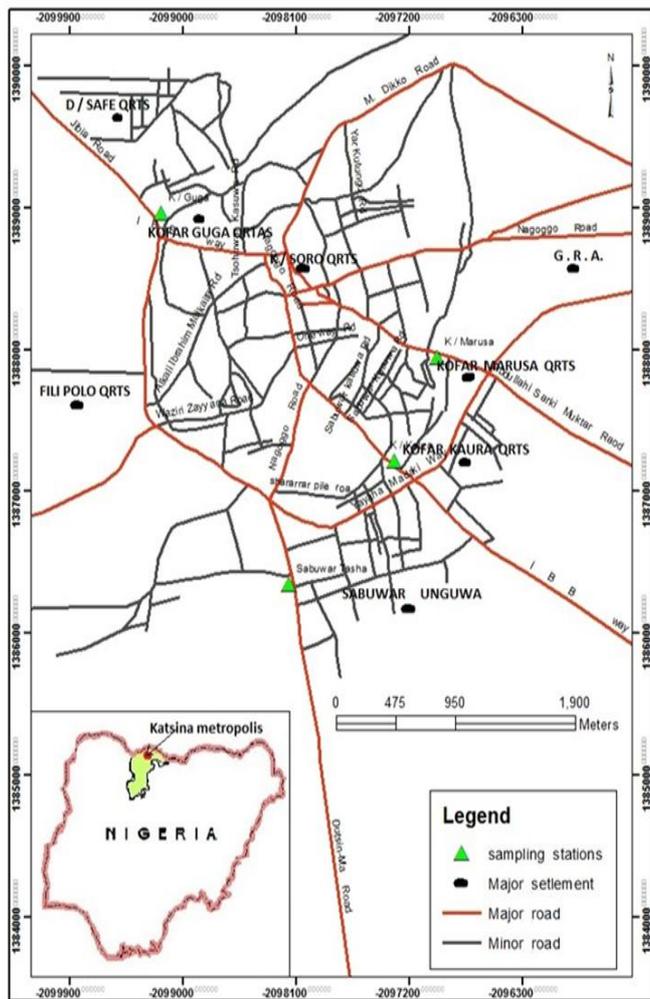


Fig. 1. Map of Katsina Metropolis Showing the Sampling Stations (retail outlets).

Source:- Cartography Geo. Dept. UMYU 2013.

Sampling

Five bread samples were purchased from each retail outlet at different periods of sampling at four designated locations in Katsina metropolis, making a total of twenty bread samples. The samples examined were the most commonly consumed in the respective locations.

Standards preparation

50mg of potassium bromate was dissolved in distilled deionized water and diluted to 1 Litre. 3210mg of promethazine hydrochloride (PTZ) was dissolved in distilled deionized water and diluted to 1 Litre to obtain stock solution of 0.01M of PTZ. Also, 2840mg of promethazine was similarly dissolved in 1 Litre of distilled deionized water to obtain stock solution of 0.01M promethazine.

Working standards preparation

Aliquots of 0.1cm³, 0.2cm³, 0.4cm³, 0.6cm³ and 0.8cm³ from the primary stock solution of potassium bromate were placed in 20cm³ capacity tubes, and 1cm³ of 0.01M promethazine hydrochloride were added. Mixtures were diluted with distilled deionized water up to 10cm³ and 0.2cm³ of 12M hydrochloric acid were added to obtain final concentration of bromate in the range of 0.5µg/ml to 5µg/ml (Alli *et al.*, 2013).

Sample preparation

A quantity of 10g was taken from the centre of each loaf of bread and dried in oven for about an hour at 75°C. The dried crust was

pulverized and 1g of each powdered sample was weighed into a clean 250cm³ beaker and 20cm³ of distilled deionized water was added. The mixture was stirred thoroughly using a spatula and filtered using a Whatman no 1 filter paper. 8cm³ of the filtrate solution was transferred into a 20cm³ volumetric tube and mixed with 1cm³ of 0.01M promethazine. 0.2cm³ of 12M hydrochloric acid was added; the mixture was shaken for 1minute and used for analysis (Alli *et al.*, 2013).

Sample analysis

Prior to the quantitative determination of bromate contents of the bread samples, qualitative tests were performed directly on a portion of each bread sample with 2cm³ of 0.01M promethazine and 0.6cm³ of 12M hydrochloric acid. Quantitative determination of bromate content in the bread samples was carried out following the spectrophotometric method described by El Harti *et al.* (2011). Absorbance of the coloured solution obtained was measured using a spectrophotometer (CAM-spec. M330) at 515nm. The concentration of bromate was calculated from the linear regression curve obtained from the working standards.

Data analysis

Data obtained were analyzed using Microsoft Excel and results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Results

The results of the analysis are shown in Table 1. Bread sample KG2 recorded the least amount of residual bromate (2.18±0.26µg/g). Highest level of bromate (8.25±0.35µg/g) was recorded by bread sample KM5. The colour change ranged from light to dark pink with increase in concentration. The quantity of bromate in each bread sample correlates with the degree of pink colour obtained in the qualitative test (Table 1).

Table 1. Qualitative and Quantitative Determination of Bromate in Some Bread

Location	Bread	Colour change	Bromate (µg/g)
Kofar Guga	KG1	Light- pink	3.21±0.24
	KG2	Light- pink	2.18±0.26
	KG3	Pink	5.21±0.13
	KG4	Pink	4.19±0.23
	KG5	Pink	6.18±0.15
Kofar Kaura	KK1	Pink	6.16±0.00
	KK2	Pink	4.49±0.20
	KK3	Pink	5.10±0.20
	KK4	Pink	4.41±0.18
	KK5	Pink	4.70±0.49
Kofar Marusa	KM1	Pink	6.22±0.28
	KM2	Pink	6.24±0.20
	KM3	Pink	5.23±0.19
	KM4	Pink	5.24±0.17
	KM5	Dark- pink	8.25±0.35
Sabuwar Tasha	ST1	Pink	5.19±0.14
	ST2	Pink	6.20±0.13
	ST3	Pink	6.22±0.21
	ST4	Pink	5.19±0.16
	ST5	Pink	5.20±0.00

Key: KG= Kofar Guga; KK= Kofar Kaura; KM= Kofar Marusa; ST= Sabuwar Tasha. Values represent Mean±standard deviation of five determinations.

Discussion

The amount of residual bromate in each bread sample examined was higher than US FDA's permissible level of 0.02µg/g. These values also contravened the NAFDAC's permissible level, implying that,

none of the bread samples examined in this study was safe for human consumption. The bread sample KG2 with the least concentration of bromate contains > 100 times the permissible level, while the bread sample KM5 with the highest concentration contains > 400 times the permissible level of bromate. Similar values of bromate (3.6 to 9.2µg/g) in Gwagwalada area council of Abuja are reported by Alli *et al.* (2013). Satisfactory recoveries of 77 to 120% were obtained when the samples KD1, KD2, KD3, KD4 and KD5 were spiked with bromate at level of 50µg/ml (Table 2).

Table 2. Recoveries of bromate added as KBrO₃ to the mentioned sample at 50µg/ml

Bread Sample	Bromate found (µg)	Mean Recovery %
KD1	189±6	77
KD2	195±7	80
KD3	239±8	95
KD4	296±9	118
KD5	298±12	120

Values represent Mean ±standard deviation of five determinations.

This was carried out to ensure correct response of the instrument used during bromate determination. Considering the high amount of bromate found in the examined bread samples which are consumed regularly on a daily basis by residents of Katsina, we can infer that there is high dietary exposure to bromate through consumption of bread. This implies that bread bakers did not comply with NAFDAC's directive. Bromate added to bread is harmful to consumers because it has been associated with neuro- and nephrotoxicity (Kurokawa *et al.*, 1990). Sunmonu and Oloyede (2009) also reported adverse effects on liver and kidney functions of rats fed on diet formulated with bread containing bromate in Ilorin, Nigeria. Though many harmful effects of bromate had been reported in literature. Application of other alternative oxidizing agents, such as ascorbic acid, ascorbates, etc., which is nontoxic, can equally enhance the quality and value of bread in place of bromate. Basically, there are two ways by which humans may get poisoned with bromate; by ingestion when it is present in food such as bread and by inhalation. The use of bromate has been a common choice among flour millers and bakers throughout the world because it is cheap and probably the most efficient oxidizing agent (Emeje *et al.*, 2010).

Conclusion

The results obtained here confirm that the sampled breads from the retail outlets contained bromate above the permissible level and thus, bread consumers and bakers are at health risk of exposure to bromate with serious health implications. This is an important result as human health is directly affected by consumption of breads containing bromate. The monitoring of bromate in breads needs to be continued because bread bakers have continued to use the toxic substance in their products. This study suggests that surveillance and enforcement on the ban of the use of bromate by appropriate authority in Nigeria should continue in order to ensure that bakers comply with safety guidelines. Bread bakers should be enlightened to explore other alternative means of flour improvers instead of using bromate because of its deleterious and carcinogenic effect in humans.

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