Acta Scientific Nutritional Health

Volume 2 Issue 8 August 2018

Research Article

Rapid Method Estimation of Bromate in Serum and Bread Consumed in Enugu, Nigeria

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Received: May 16, 2018; **Published**: July 02, 2018

Abstract

The aim of this study is to formulate rapid method for detection and estimation of bromate in serum and bread consumed in Enugu, Nigeria. All bread samples tested positive by the quantitative method, showing the presence of black spots. The black spot did not fade even after 24 hours. The filtrate of the same bread samples when applied on Whatman No 1 filter paper before dropping the potassium iodide reagent gave no black spot. Similarly, when the reagent dropped directly negative results negative results were obtained. The same samples were also tested using flooding method, described in the promethazine hydrochloride method and similar results were obtained. However, the colour for promethazine hydrochloride method was pink. The potassium iodide reagent gave consistently positive results throughout its storage period of three (3) months when applied directly on the loaf of bread. The methylene blue reagent developed for quantitative test was also tested using the quantitative procedure. The result showed the disappearance of the blue colour to the background colour almost similar to that of the bread sample. The analytical performance was measured by determining eight (8) out of ten (10) of the positive sample were reported positive while four (4) out of five (5) negative samples were reported negative. This implies a false negative of two (2) and false positive of one (1). Apart from the interference of extraneous factors, whose presence did not interfere with the positivity and sensitivity of the spot and semi-quantitative, the quantitative procedure may be applied in bromate estimation in bread with caution.

Keywords: Bromate; Estimation; Confectionaries; Formulation

Introduction

A bromate is a chemical compound (a salt or ester) containing the reactive group BrO_3 [1]. Bromate anion, (BrO_3^-) is a bromine –base oxo-anion. Examples of bromates include sodium bromate and potassium bromate. Both sodium bromate or potassium bromate are white crystalline substances that are readily soluble in water (Chemical Abstract Service Registration Number [2]. Chemical properties of the sodium salt of bromate shows that it has a molecule of $NaBrO_3$, molecular weight of 150.90g. Physical properties showed a state and appearance of odourless, white crystals with melting point of 38oC. It is slightly in organic solvent like alcohol but highly soluble in water.

For potassium bromate, its molecular formula is $KBrO_3$, while molecular weight is 167.01g. It is slightly soluble in alcohol and insoluble in ether. Sodium bromate is produced by the introduction of bromide into a solution of sodium carbonate. It is used in conjunction with sodium bromide to extract gold form gold ores. Potassium bromate is produced by passing bromide into a solution of potassium hydroxide. However, for large scale production of potassium bromate an industrial electrolytic process is used. All over the world, where bakery products form a major part of household foodstuff, potassium bromate is used as a flour improver where it acts as a maturing agent. Potassium bromate is a popular food additive which has been used by the baking industry for almost a century. It has been used in baking since 1914 when patent was issued by United states patent office, (Gelroth) [3,4].

Potassium bromate acts principally in the last dough stage giving strength to the dough during late proofing and early baking process, (Mehmet). It has been reported to reduce the antioxidant power of red cells and also to induce oxidative stress and other related problem [5,6]. World Health Service (WHO) evaluate the bromate moiety under the WHO Guidelines for drinking water quality and again concluded that "The weight of evidence from rat bioas-

says clearly indicates that bromate has the potential to be a human carcinogen" [7]. The use of potassium bromate in flour milling and baking was banned in Nigeria by National Agency for food, drug and control (NAFDAC) in 2003 and its use infringes on the drug and related products registration decree 20 of 1990 and NAFDAC Decree 15 of 1993 [8].

There are several existing methods for the determination of bromate, all with their different complexities, colourimeteric method [9], spectrophometric method [10] capillary electrophoresis, [11] mass spectrometry flow injection analysis [12]. Whichever method that is used, the complexmatrixin samples of bakery products dictate that a degree of sample pre-treatment is required. Secondly no conventional spot method for spot checks at retail outlets has been developed. The aim of this study is to formulate rapid method for detection and estimation of bromate in serum and confectionaries consumed in Enugu, Nigeria.

Materials

The materials used for this study were; glass slides, Whatman no. 1 filter paper, hot air, oven, water bath, test tubes, stop watch, volumetric flasks, centrifuge, spectrophometer, new life electronic balance model NL2000 product of New life Medical instrument, England, measuring cylinder, pH meter, pipettes.

Reagents

The following reagents were used for the study; sulphuric acid and hydrochloric acid, both products of E. Merck Darstadt, Phenol, methyl-red, potassium iodide, product of British Drug House (BDH) chemical Ltd England. Promethazine hydrochloride (PTZ), product of British Drug house (BDH) Chemicals Ltd England was obtained from Enugu state, Nigeria, Central Medical store. Methyl blue powder (Technical dye) CAS 12063-86-2, product of Surechem product limited, England. Sodium tunstate, was procure from Bronila Laboratory, Enugu, Nigeria. Deionized water, potassium bromate, product of Guangdong Guangua Science Tech. co. Ltd, China. Bread procured from local street vendors in Enugu, Nigeria. All the reagents unless otherwise stated were procured from sigma Laboratory Zonal Representative in Jos, Nigeria. All the reagents are of analytical grade. Bread samples (four different bread products) were procured from a retail outlet in one of the markets in Enugu municipation.

Experimental animals; (forty) 40 healthy albino wistar rats of both sexes aged 2 - 3 months were obtained from the animal House the faculty of Veterinary Medicine, University of Nigeria, Nsukka (both LD_{50} and blood analysis). The rats were kept at the University of Nigeria, Enugu (old site) at a temperature of 28°C - 32°C at 13: 11, light: dark cycle. The weight range of the rats was 150 - 180g. They were kept in stainless steel wire mesh cages which were perforated beneath for the exit of the rats' wastes to prevent coprophagy. They were fed with standard commercially prepared pelleted feed of top-feed, Nsukka, Nigeria and the pyrogen free ad lithium.

Experiment conduct

Hazard Identification and Response Assessment: This is otherwise known as acute toxicity testing. The LD_{50} was calculated according to the method described by [13]. Twenty healthy albino rats of both sexes aged 2 - 3 months and weighing between 150 - 180g were divided into five groups (5) groups (labelled A-E). Each group contained four (4) wistar rats. The rats were fasted for 18h prior to dosing and each rat was given a single dose of potassium bromate ($KBrO_3$) orally using 22 - gauge oral feeding (Cannular). The volume of the dose depends on the size of the animals but did not exceed 1 mL/100 g body weight. Group A-D were administered with KBrO3 while group E served as control a, b, d received no $KBrO_3$, They, were administered pyrogen free water. All the rats had access to standard commercially prepared pelleted rat feed and pyrogen free water ad libitum.

The rats were observed within 24 hours period for signs of cytotoxic effects such as dullness, depression, restlessness, diarrhea and death in extreme cases. After 24 hours the number of rats reacted, that is, showed signs of toxicity were counted into prohibit using Finney's table [14], table 1. The percentage deed for 0 and 100 were corrected before determining their prohibit using the formula below. The prohibit values, thus obtained were plotted against log- dose and then the dose corresponding to prohibit 5, that is 50%, was determined. The approximate standard error (standard deviation) at 95% confidence limit was calculated using the formula described by Finney (1971).

Approx.SE of $LD_{50} = (LogLD_{84}\text{-}LogLD_{16}) \div \sqrt{2N}$
Where SE = Standard error,
N = number of rats per group

Experiment B: To Determine Qualitative (spot test) For Presence of Bromate

Reagent: 0.05% potassium iodide in 2 MHCl. This was prepared by weighing 0.05 g of potassium iodide in 100 mL of 2 MHCl shaking gently to form a homogeneous solution. This was dispensed into brown containers. The reagent was used for testing bread samples directly and also the filtrate within 2 hours of preparation. The reagent was stored at room temperature and testing repeated every two weeks for 18 weeks. This was check for the effect of storage on the potency of the reagent. Principle: this is based on the reaction of bromate and iodide ions in an acid medium to produce triiodide.

Procedure

1. 0.10 mL of the 0.5% potassium iodide solution was dropped on the surface of the bread sample and examine for dark spot after 2 minutes.
2. For the filtrate. This was prepared as follows: 5g of bread sample was weighed and grinded into a powder in a mor tar. The powder was transferred into a 100 mL of distilled water and stirred thoroughly with magnetic stirrer for 5 minutes. It was then filtered through Whatman No. 1 filter paper.

2 mL of the filtrate was transferred into a clean, dry test tube and 0.10 mL of potassium iodide solution added. The Presence of colour change was noted after 2 minutes. Black spot indicates presence of bromate in the sample while absence implies presence of bromate or probably undetected level of bromate. The black spot was observed for 24 hours for possible fading or disappearance of the colour. The analytical performance of the spot postulate was determine by analyzing fifteen (15) samples, ten (10) positive and five (5) negative samples and using the 2x2 table techniques and calculating the sensitivity, specificity, efficiency and predictable values. Bread loaves from different bakeries purchased from the retail outlets within Enugu municipality and tested positive with the [15] technique.

Experiment C: Semi- Qualitative test for bromate

Principle

The test is based on the reaction between bromide and bromate ions in an acid medium of PH 3.5 to 4.5 to liberate bromide which bleaches the methyl red.

Protocol

Step 1: Two test tubes labelled A and B were set in a water bath at 65 ± 2oC, into tube A was added; 5 mL phenol solution, 2.5 mL potassium bromate solution, 2.5 Ml of bromated standard or sample and 0.10 mL of methyl red.

Step 2: Into tube B was added 2.5 mL of 0.3 M H_2SO_4, content of both tubes was mixed by transferring quickly the content of tube B into tube A and two times. The final mixture was placed inside the water bath and stop watch started immediately. The time taken for colour of methyl red to disappear was recorded. The time for the $KBrO_3$ standard solution with different concentrations were compared. These served as positive controls. A negative control test was set up using water as a sample. The time for the test samples were also compared with those of the $KBrO_3$ standard.

Interpretation: the time taken for the disappearance of methyl red colour is directly proportional to the concentration of bromate in the sample. That is, the lesser the concentration, the longer the time taken for disappearance.

Experiment D: Quantitative test for bromate

Twenty albino wistar rats weighing between 150 - 180g were divided into five (5) groups (1 to 5), four rats in each group. Group 1, 2, 3, 4 and 5, were given oral doses of 0,10,15,17, 20 mg/kg body weight of potassium bromate respectively. Group 1 is the control group. After 15 - 20 minutes of potassium bromate ingestion, blood samples were collected from each rat through the retro-bulbo plexus of the median contus into ethylenediamine tetracetic (EDTA) and a plain dry test tube. The samples in the plain dry test tubes were allowed to clot and the serum separated into very clean dry container. Two serum samples picked randomly from each group were analysed for bromate using both the promethazine hydrochloride and methylene blue methods. The EDTA samples (considered as post potassium bromate administration samples) were used for full blood count analysis.

Reagents

0.02 M of promethazine hydrochloride (PTZ) was prepared by weighing and dissolving the appropriate amount in deionized water. Stock potassium bromate solution was also prepared in deionized water. 0.02g/dL methylene blue was used for the study. HCl (36%) was used without any further treatment. The protein precipitant working solution was obtained by mixing the sodium tungstate solution and 0.11 M HCl in a ratio of 1:9. This was always prepared before use and discarded after four hours.

Procedures

Stage 1: Sample preparation

(a) **Serum**

1. 0.5 mL of test sample was added into a clean test tube, then 9.5 mL of protein precipitant working solution was added, the mixture was allowed to stand for 10 minutes, shaking intermittently, It was centrifuged at 2800g revolution for 10 minutes then filter with Whatman No.1 filter paper and the supernatant collected.

(b) **Bread**

2. Bread sample was placed in hot air oven at 50°C for 6 hours and then grinded into fine powder in a mortar, 5g of the powder was transferred into 100 mL of distilled water and stirred thoroughly with magnetic stirrer for 5 minutes. It was then filtered with Whatman No 1 filter paper.

Preparation of standard calibration curve

The calibration curves for methylene blue and promethylene blue and promethazine hydrochloride were obtained by analyzing different $KBrO_3$ concentrations ranging from 0.0 to 5.0 µg/L, obtain by diluting the stock solution appropriately), using the procedures described in this study for methylene blue and promethazine hydrochloride. The absorbance was plotted against the concentration values. The curve obtained was used to derive the bromate levels in the bread and serum samples. For both the calibration curve and tests triplicate readings were taken and an average calculated and recorded.

Stage 2: Sample analysis

Methodology

1. Promethazine hydrochloride method

Principle

The method is based on the reaction of bromate and promethazine in acid medium to produce a red pink compound which absorbance is measured spectrophometrically at wavelength 515 wavelength.

Procedure

4.4 mL of the filtrate was added into 10 mL clean test tubes, then 1 mL of 0.02M promethazine hydrochloride was added, 2.0 mL of 12 M HCl was added to the mixture, the content of the test tube was mixed for 1 minute and colour developed measured spectrophometrically at 5.5 snm. The concentration were derived from the calibration curve already done.

2. Methylene Blue Method

Principle

This is based on the bromate oxidation of methylene blue dye in acid medium which changes the colour of the solution to faint golden yellow which absorbance is measured at 745 wavelengths.

Procedure

4.4 mL of the filtrate was added into a 10ml clean test tubes, then 0.25 mL of methylene blue solution was added, 4.3 mL of 0.2 M HCl was added to the mixture. The content of the test tube was allowed to stand for 5minute, the colour developed measured spectrophotometrically at 745 nm. The concentrations were derived from KBrO3 calibration curve already done. In the study methylene blue using spectrophotometry was developed to measure bromate in bread and serum samples. The method was compared with promethazine hydrochloride method as described by [15], measuring same test samples by both methods. Calibration curve for both methods were first prepared from which the concentrations of the test samples were determined.

Experiment E: Estimation of haematological parameters in the experimental animals

The EDTA samples collected during experiment D were analyzed for heamoglobin concentration, haematocrit (HCT), mean cell haemoglobin concentration, white cell count (total and differential), platelets count. Pre-administration EDTA samples were collected and analysed accordingly three days before administration of potassium bromate. These samples were analysed using QBC auto read plus haematology analyser product of druckers diagnostics, USA, manual method described by [16].

Data generated from haematogical parameters were presented as mean with standard deviation (SD). Statistical analysis were done using statistical package for social sciences (SPSS), MODEL VERSION 16.0. Probability values < 0.05 were considered statistically significant.

Results

Qualitative test (spot test)

All bread samples tested positive by the quantitative method, showing the presence of black spots. The black spot did not fade even after 24 hours. The filtrate of the same bread samples when applied on Whatman No. 1 filter paper before dropping the potassium iodide reagent gave no black spot. Similarly, when the reagent dropped directly negative results were obtained. The same samples were also tested using flooding method, described in the promethazine hydrochloride method and similar results were obtained. However, the colour for promethazine hydrochloride method was pink. The potassium iodide reagent gave consistently positive results throughout its storage period of three months when applied directly on the loaf of bread. The methylene blue reagent developed for quantitative test was also tested using the quantitative procedure. The result showed the disappearance of the blue colour to the background colour almost similar to that of the bread sample. The analytical performance was measured by determining eight out of ten of the positive sample were reported positive while four out of five negative samples were reported negative. This implies a false negative of two and false positive of one.

In cell' A' are the true positive (TP)
In cell 'B' are the false positive (FP)
In cell s'C' are false negative (FN)
In cell 'D' are the true negative (TN)

Calculations

The various parameters were calculated using the appropriate formulae as shown in table 2 below.

i. Sensitive (%) = TP/TP + FN x 100. Substituting; Sensitive (%) = 10/10 + 0 x 100%

ii. Specificity (%) = TN/TN + FP x 100. Substituting; Specificity (%) = 4/4 + 1 x 100 = 80%

iii. Efficiency (%) =TP + TN/TP + TN + FP + FN x 100. Substituting; Efficiency (%) = 10 + 4/10 + 4 + 0 + 1 x 100 = 93.3%

iv. Predictive values.

Positive Predictive Value (%) = TP/TP + FPx100. Substituting; Positive Predictive Value = 10/10 + 1 x 100 = 90.9%

Negative Predictive Value (%) = TN/TN + FN x 100. Substituting; Negative Predictive Value = 4/4 + 0 x 100 = 100.

Sensitive (%) is the percentage of all bromate samples that had positive result while specificity (%) is the percentage of all the non-bromated samples that had negative result. The predictive value is a measure (%) of the times that the value (positive or negative) is the true value and the efficiency represent the percentage of the times that the spot postulate gave the correct answer compared to the total number of tests done.

Semi-Quantitative test

The time taken for methyl red colour to disappear for different concentrations of KBrO3 STANDARDS decreased with increase in the concentration of the standards. This implies that the less the concentration, the longer the time taken for disappearance. The result is also presented in Table 3. Table 3 shows the different time taken for different concentrations of potassium bromate to cause disappearance of the methyl red colour.

Table 4 shows the difference time taken for different concentration of potassium bromate to cause disappearance of the methyl re colour. The time (minutes) were 20, 15, 12, 8 and 5 for potassium bromate (mg/dL) concentrations of 50, 100, 200, 400 and 500 respectively.

Comparison of the concentration of bromate (µg/L) in samples using promethazine and methylene methods is shown in table 5 below.

The statistical values from table 6, shows no significant difference between the pre and post-administration of pyrogen-free water $P > 0.05$.

Haemoglobin, absolute eosinophil and lymphocyte counts for post administration of potassium bromate samples from table 7, were significantly decreased $P < 0.05$.

Table 8 shows haemoglobin and absolute lymphocyte counts for post administration of potassium bromate samples were significantly decreased ($p < 0.05$), while absolute eosinophil counts is significantly increased ($p < 0.05$). other parameters did not vary significantly.

Table 9 below, shows total white blood cell count, absolute neutrophil and lymphocyte counts for the post administration of potassium bromate samples were significantly decreased $P < 0.05$, while other parameters did vary significantly ($p < 0.05$).

Significant differences were observed in MCHC (increased) and total white cell count (decreased) ($p < 0.05$) from table 10, below.

The concentration of samples A, B, C and D are 3.64, 2.93, 2.93, 3.64 respectively as given by the promethazine method. Their concentrations as given by the regression equation of methylene blue method are 1.98, 0.94, 1.46 and 1.46 respectively.

Cage	Number of rats	Dose of $KBrO_3$ mg/kg body weight	Number of dose
A	4	10.00	1.0
B	4	20.00	1.0
C	4	30.00	1.0
D	4	40.00	1.0
E	4	0.00	1.0

Table 1: Total Dose of potassium bromate given orally to rats.

Test results	Bromate bread	Non-bromated bread	Total
Positive	A.10	B.1	9
Negative	B.0	D.4	6
Total	10	5	15

Table 2: Showing results 15 bread tested by spot postulate.

$KBrO_3$ Standard (mg/dL)	50.0	100.0	300.0	400.0	500.0
Timing started (minutes)	0.0	0.05	0.10	0.15	0.20
Timing (minutes)	0.20	0.20	0.22	0.23	0.25
Time taken (minutes)	20.0	15.0	12.0	8.0	5.0

Table 3: Clearance Time for Different Concentration of Bromate.

Sample	Sample 1	Sample 2	Sample 3	Sample 4	Control
Timing started (minutes	0.00	0.005	0.10	0.15	0.25
Timing stopped (minutes)	0.35	0.40	0.45	0.50	0.60
Time taken (minutes	> 35.0	> 35.0	> 35.0	> 35.0	> 35.0

Table 4: Shows the difference time taken for different concentration of potassium bromate to cause disappearance of the methyl re colour. The time (minutes) were 20, 15, 12, 8 and 5 for potassium bromate (mg/dL) concentrations of 50, 100, 200, 400 and 500 respectively.

Sample	Promethazine method (concentration (µg/L))	Methylene blue method (concentration (µg/L))	Difference between the values obtained
A	3.64	1.98	1.66
B	2.93	0.94	1.99
C	2.93	1.46	1.47
C	3.64	1.46	2.18

Table 5: Comparison of the concentration of bromate (µg/L) in samples using promethazine and methylene methods.

Effect of potassium bromate on some haematological parameters.

The results are presented as Mean ± Standard Deviation (SD) for different pre and post administation groups and then compared using student t-test.

Parameters	Pre-Administration	Post-Administration	P-Value	Remark
Haematocrit (%)	43.0(1.2)	43.5 (0.6)	0.47	NSD
Haemoglobin ((g/dL)	14.3 (0.1)	14.4 (0.2)	0.65	NSD
MCHC (g/dL)	33.3 (0.6)	33.5 (0.6)	0.58	NSD
TWBC (x 10^9/L)	7.38 (0.14)	7.28 (0.20)	0.45	NSD
Neutrophil (x 10^9/L)	1.88 (0.02)	1.88 (0.14)	0.95	NSD
EosinophiL (x 10^9/L)	0.338 (0.003)	0.344 (0.019)	0.57	NSD
Monocyte (x 10^9/L)	0.354 (0.007)	0.336 (0.024)	0.20	NSD
Basophil (x 10^9/L)	0.000 (0.000)	0.000 (0.000)	-	NSD
Lymphocyte (x 10^9/L)	7.33 (0.09)	7.23 (0.14)	0.10	NSD
Platelet (x 10^9/L)	2.863 (12.12) E2	3.002 (4.50) E2	-13.9	NSD

Table 6: Mean (SD) Of Haematological Parameters Of Group 1 Pre And Post Administration Of Pyogen Free Water.

KEY

MCHC = Mean Cell Haemoglobin

NSD =No Significant difference

SD =Significant difference

Statistical analysis shows no significant difference between the pre and post administration of pyrogen-free water P>0.05.

Parameters	Pre-Administration	Post-Administration	P-Value	Remark
Haematocrit (%)	43.0(1.2)	40.5 (01.7)	0.06	NSD
Haemoglobin ((g/dL)	14.7 (0.2)	13.9 (0.4)	0.01	SD
MCHC (g/dL)	34.2 (0.4)	34.2 (0.5)	1.00	NSD
TWBC (x 10^9/L)	7.36 (0.12)	7.00 (0.12)	0.005	SD
Neutrophil (x 10^9/L)	2.03 (0.02)	1.96 (0.05)	0.56	NSD
Eosinophil (x 10^9/L)	0.378 (0.003)	0.351 (0.006))	0.0005	SD
Monocyte (x 10^9/L)	0.375 (0.006)	0.377 (0.005)	0.65	NSD
Basophil (x 10^9/L)	0.000 (0.000)	0.005 (0.006)	0.16	NSD
Lymphocyte (x 10^9/L)	7.43 (0.20)	6.98 (0.04)	0.01	SD
Platelet (x 10^9/L)	3.040 (7.66)	3.010 (1.62)	0.49	NSD

Table 7: Mean (SD) of Aematological Parameters of Group 2 Pre And Post Administration of 10 mg/kg of Potassium Bromate.

Parameters	Pre-Administration	Post-Administration	P-Value	Remark
Haematocrit (%)	42.5(1.7)	40.0 (1.7)	0.06	NSD
Haemoglobin ((g/dL)	14.7 (0.1)	14.3 (0.1)	0.003	SD
MCHC (g/dL)	34.6 (1.1)	35.8 (0.8)	0.15	NSD
TWBC (x 10^9/L)	7.10 (0.23)	7.08 (0.09)	0.85	NSD
Neutrophil (x 10^9/L)	1.80 (0.01)	1.83 (0.08)	0.50	NSD
Eosinophil (x 10^9/L)	0.378 (0.01)	0.337 (0.01)	0.004	SD
Monocyte (x 10^9/L)	0.339 (0.01)	0.337(0.04)	0.85	NSD
Basophil (x 10^9/L)	0.005 (0.006)	0.005 (0.006)	1.00	NSD
Lymphocyte (x 10^9/L)	7.15 (0.17)	7.31 (0.12)	0.20	NSD
Platelet (x 10^9/L)	2.7900E2 (12.82)	2.7830E2 (9.35)	0.93	NSD

Table 8: Mean (SD) of Aematological Parameters of Group 3 Pre and Post Administration of 15 mg/kg of Potassium Bromate.

Parameters	Pre-Administration	Post-Administration	P-Value	Remark
Haematocrit (%)	42.0 (0.0)	42.0 (1.2)	1.00	NSD
Haemoglobin (g/dL)	14.5 (0.1)	14.7 (0.2)	0.09	SD
MCHC (g/dL)	34.4 (0.1)	34.9 (0.6)	0.14	NSD
TWBC (x 10^9/l)	7.08 (0.09)	6.91 (0.05)	0.02	SD
Neutrophil (x 10^9/L)	1.96 (0.05)	1.84 (0.01)	0.01	SD
Eosinophil (x 10^9/L)	0.360 (0.006)	0.366 (0.01)	0.45	NSD
Monocyte (x 10^9/L)	0.353 (0.066)	0.348 (0.044)	0.90	NSD
Basophil (x 10^9/L)	0.000	0.0000	-	-
Lymphocyte (x 10^9/L)	7.05 (0.20)	6.78 (0.09)	0.01	SD
Platelet (x 10^9/L)	2.895E2 (13.914)	2.897E2 (23.556)	0.97	NSD

Table 9: Mean (SD) of Aematological Parameters of Group4 Pre and Post Administration of 17 mg/kg of Potassium Bromate.

Parameters	Pre-Administration	Post-Administration	P-Value	Remark
Haematocrit (%)	44.0 (1.2)	43.5 (0.6)	0.47	NSD
Haemoglobin (g/dL)	14.8 (0.5)	14.8 (0.1)	1.00	NSD
MCHC (g/dL)	33.7 (0.2)	34.1 (0.2)	0.02	SD
TWBC (x 10^9/L)	6.98 (0.03)	6.88 (0.02)	0.002	SD
Neutrophil (x 10^9/L)	1.82 (0.04)	1.78 (0.02)	0.43	NSD
Eosinophil (x 10^9/L)	0.330 (0.012)	0.319 (0.024)	0.49	NSD
Monocyte (x 10^9/L)	0.307(0.012)	0.315 (0.019)	0.52	NSD
Basophil (x 10^9/L)	0.005(0.006)	0.005(0.006)	1.00	NSD
Lymphocyte (x 10^9/L)	7.13 (0.09))	7.09 (0.10)	0.56	NSD
Platelet (x 10^9/l)	2.989E2 (13.22)	2.8990E2 (10.74))	0.33	NS

Table 10: Mean (SD) of Aematological Parameters of Group 5 Pre and Post Administration of 20 mg/kg of Potassium Bromate.

Discussion

Potassium bromate has been established to cause cancer and other related health problems [17,18]. Subsequently it has been ban in many countries including Nigeria as food additive in bakery products, [8]. However, some bakers in Nigeria and elsewhere still resort to its use illegally, [19]. In the study with methylene blue method using spectrophotometry, the reaction readings were taken after 5 minutes. The first stage of the reaction was rapid disappearance of the blue colour, followed by gradual development of light yellow colour. The differences between the values obtained from methylene blue and promethazine hydrochloride methods show a significant difference in magnitude. This wide margin in magnitude may be associated with interfering substances with the methylene blue not considered within the scope of this study. The effect of bromate on blood cell counts as reported by some researchers, [5,20] were observed not to be immediate for monocyte that showed consistent decrease with increasing concentration of bromate. The manifestation of the effect of bromate on blood cell counts as shown by this study requires prolong exposure or is an indirect effect resulting from the impact on the haemopoeitic organs. The later may be more likely when the report of [21] is considered.

Blood film for all the groups showed normochromic red cells, slight spherocytosis and poikilocytosis with few schistocytes. Most platelets were seen in small clumps and this may be attributed to probably slight delay in anticoagulating the blood.

Conclusion

Apart from the interference of extraneous factors, whose presence did not interfere with the positivity and sensitivity of the spot and semi-quantitative, the quantitative procedure may be applied in bromate estimation in bread with caution. Changes in blood parameter was not immediate, it requires prolong exposure to bromate. Blood film for all the groups showed normochromic red cells, slight spherocytosis and poikilocytosis with few schistocytes

Recommendation

The simplicity and low cost of the spot method makes it a very desirable method for detecting bromate in bakery products at retail especially in the developing countries like Nigeria. The semi-quantitative method if properly harnessed and adopted is less demanding, as much skill, reagents and electricity are not needed and therefore recommended for field work.

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