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Bromate Residual Contents In Some Brands of Bread And Bakery Products Collected From Benghazi`s Bakeries, Libya

Mariam Ambarak S. Busaadia[1], Nagwa H. S. Ahmida[1], Najma. H. Towier[2] and Randa. S. El-zwaey[1]

[1] Department of Environmental Health, Faculty of Public Health, University of Benghazi, Benghazi, Libya
[2] Chemistry Department, Art& Science Faculty-Tokra, University of Benghazi, Benghazi, Libya

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Corresponding author :
najmahtowier@gmail.com

ABSTRACT

Potassium bromate is an oxidizing agent that has been used as a food additive added to preserve flavour or improve the taste and appearance of food, mainly in the process of making bread and baked products. The continuous consumption of potassium bromate is hazardous to human health. The aim of this study is to assess the safety of some bread and baked products by determining the residual potassium bromate using a spectrophotometric method, in twenty nine bread and different bakery products samples included; croissant, toast and cake, collected from different bakeries in Benghazi, Libya. The obtained results indicated that, the level of residual potassium bromate in the analyzed bread and baked samples ranged from 0.6138µg/g to 1.558µg/g, with mean concentration equal to 0.9641± 0.4 µg/g. A significant differences were found in the potassium bromate levels of the different analysed samples (P<0.05). The results of this study showed that the contents of potassium bromate in all analysed baked products were higher than permissible limit sets by Food and Drug Administration (FDA) and by Libyan Standard Regulations. The excessive consumption of potassium bromate has a highly toxic effects to consumer`s health.

Introduction

Bread is a high nutritional value food, it is rich in proteins, vitamins and minerals. Bread is considered as one of the most popular foods consumed worldwide(Pagewiseet al., 2002). In Libya, bread is readily available at very low price. All people consume bread as an essential food item, irrespective to their age, living and socio economic status. It is extensively consumed in homes, schools, restaurant, and hotels during breakfast, evening snacks, and school tiffin(Ojeka et al., 2006).

The modern baking industry tends to offer the needs of consumers who demand of high quality, strengthening and stable products by using different ingredients and additives (Caruso et al., 2017). Therefore, the modern bakeries mainly sell bread, and other baked products, included; cookies, crackers, desserts, pizza, snack cakes and tortillas. Most of these products are consumed for breakfast or lunch (Grujic and Grujcic, 2016). In general, the making of bread and baked goods consists of mixing and kneading the major constituents of bread which included; flour, water, salt, yeast or another leavening agent and flour improvers. The bakery products usually contain other several ingredients include milk, egg, sugar, spice, fruit, vegetables, nuts and seeds (Abubakar el al., 2008; Airaodion et al., 2019).

There are several flour improvers that increase the volume and the texture of the bread (FAO/WHO 1992). An example of a flour improver, that has gained wide acceptance all over the world, is bromate salts, mainly potassium bromate. This substance is a white crystalline solid, has no taste and odor and is easily soluble in water(Gandikota et al., 2005). Bromate ion acts as a maturing agent and dough conditioner via oxidizing the thiol (sulfhydryl) functional groups of the flour`s gluten protein into disulphide bridges. This oxidation makes the dough viscoelastic as a result of retaining the carbon dioxide produced by the yeast, make bread rise in the oven, increase loaf volume and gain a good texture in the final product (Ugochukwu et al., 2019; Rosentrater et al., 2018; Starek and Starek-Swiechowicz, 2016; Sahi et al., 2014).

Several studies have shown the excessive consumption of potassium bromate has many dangerous effects. Acute effect of bromate toxicity includes nausea, vomiting, sore throat, abdominal pain, diarrhea. peripheral neuropathy, anemia, hypotension, renal insufficiency and central nervous system dysfunction including seizures. It can also cause Cancer (Elharti et al.,2011; Akunyili, 2005; Robert and William, 1996). The International Agency for Research on Cancer (IARC) has classified potassium bromate as a possible human carcinogen, and classified it as Class 2B (IARC, 1986; Rana et al., 2020). However, potassium bromate has also being found to affect the nutritional quality of bread via degradation of the essential vitamins and niacin which are the main vitamins available in bread (Paul, 1996).

During baking process(at ~400^{O}C), the toxic bromate ion is degraded completely via reduction to harmless bromide ion (Mahmud et al., 2021). Abu-Obaid et al show that bromate ions decompose during baking at 150-200°C, due to the presence of metals, such as Fe, Mg, Zn, Mn, Cu, Al, in flour that act as activators (Abu-Obaid et al., 2016). However, when an excessive quantities of bromate salts or the bread are not cooked enough, or the baking process is not carried out at a sufficiently high temperature, then a residual quantities of toxic bromate ion will remain. In 1993, World Health Organization (WHO) declared that large amounts of bromate were detected in 75% of the loaves tested in the United Kingdom (UK), this was followed by its prohibition in the UK (Mahmud et al., 2021; WHO, 1995). Now day, potassium bromate has been rejected from the list of acceptable additives for flour treatment in several countries including; the European Union, Canada, Brazil, China, Australia, New Zealand and Nigeria (Rana et al., 2020; El Ati-Hellal et al., 2017). On the other hand, potassium bromate is still used in the other countries in the world with strictly limitations regulated by different global regulations. In Japan, the highest acceptable level of potassium bromated sets at 10 mg/kg of flour(Abu-Obaid et al., 2016). While in United States, the Food and Drug Administration (US-FDA) sets the maximum allowable limit at 50 mg/kg of flour (US-FDA, 2018). Even more, WHO and US-FDA have stipulated the amount of bromate ion, that be used as bread improver, should not excess than 0.02 mg/kg of bread produced (US-FDA, 2018; Shanmugavel el al., 2020; Irogbeyi et al., 2019; Johnson et al., 2013; Okafor et al., 2011).

As a result of the health related toxic effects of bromate ion, it is important to carefully monitor the levels of this ion in baked products (Abdulla et al., 2009), in order to recognize whether the concentrations are within the safe value. Thus several studies concerned with analysis of bromate ion in various baked products were carried out in different countries around the world, included; Bangladesh (Mahmud et al., 2021), India (Rana et al., 2020), Nigeria (Ugochukwu et al., 2019; Airaodion et al., 2019; Aletan, 2019; Naze et al., 2018; Alli et al., 2013), Tunisia (El Ati-Hellal et al., 2017), Palestine (Abu-Obaid et al., 2015), Sudan (Nehal et al., 2015), Saudi Arabia (Mehder, 2015), Ethiopia (Ergetie and Hymete, 2012) and Morocco (El harti et al., 2011). These studies indicated the continued using of potassium bromate in the baked product-making processes. In these Published works, the authors have been used different analysis methods, included; X-ray fluorescence (Nehal et al., 2015), redox titration method and mostly spectrophotometric techniques, to determine bromate ion in different backed product samples.

In Libya, although, Libyan National Center For Standardization and Metrology (LNCSM) has banned the importation and using of potassium bromate as a bread improver and food additive (LNCSM, 2015), Alhanash et al. have detected this substance in different brands of bread collected from different bakeries in Tajoura region-Tripoli (Alhanash et al., 2020). The levels of potassium bromate in the collected samples were 300 to 1333 times more than the maximum allowed level set by USA-FAD (Shanmugavel el al., 2020; Okafor et al., 2011).

This study was conducted to determine the bromate contents in some selected brands of bread and baked products that collected from different bakeries in Benghazi, Libya, using spectrophotometric method. The obtained results will used to assess the safety of baked products for human consumption by checking the level of compliance with National Libyan Standard Regulations by city bakers regarding the ban on potassium bromate.

Experimental

Materials and Method:

Sampling:

A total of twenty nine (29) of widely consumed baked products were collected randomly from 18 different bakeries located in Benghazi city during August 2021. These baked products included four different types (white bread, brown bread, croissant, toast and cake). These types were the mostly purchased by the consumers. Each brand was randomly selected three times. The central portion of each sample was taken and dried in an oven for an hour at temperature of 70ºC. The dried crusts of the same baked product samples was powdered and well mixed to get the homogenous representative sample of each selected product. The samples were given different code numbers such as Bw1 to Bw10 for ten samples of white bread, Bb1 to Bb4 for four samples of black bread, C1 to C5 for five samples of croissant, T1 to T5 for five samples of toast and K1 to K4 for four samples of cake. These samples were stored in the well closed plastic packaging till further analysis.

Chemicals and Reagents:

All chemicals and reagents used in this study were of analytical grade and were purchased from qualified companies. These chemicals included; Hydrochloric acid and Potassium iodide (Merck-Darmstadt, Germany) and potassium bromate (Scharlau, 99.8%). De-ionized water was used for all solution preparations and dilutions. All glassware containers were cleaned by prior overnight soaking in diluted HNO_3 (10%) before a final rinse with distilled water.

Potassium bromate (100 µg/ml stock standard solution), was prepared by dissolving accurately 10 mg of the substance in 100 ml of distilled water.

Potassium iodide solution (0.5%), was prepared by dissolving 0.5g of iodate-free potassium iodide solid in 100mL 0.1N hydrochloric acid solution.

Apparatus

All the absorbance measurements were carried out using a single beam Spectrophotometer (*UV-VISSpectrophotometer*, *SPECORD* 40, *Analytik Jena*, *Germany*) with two 1.0 cm matched cells.

The analysis of bread and baked products samples were carried out in Chemical Analysis Laboratory of Environmental Health Department, Faculty of Public Health, University of Benghazi.

Procedure

Preparation of Potassium bromate Calibration Curve

Accurately measured volumes from stock standard solution of pure potassium bromate, which equivalent to 0.5-30 µg/ml were transferred to a series of 25 ml volumetric flasks. Then the volume was made up with the extract of bread-free of potassium bromate. Accurate, 1mL of each serial standard was transferred to clean test tube, and mixed with 1mL of potassium iodide solution. The absorbance of each standard solution was measured at 540 nm using UV-VIS spectrophotometer, against blank solution of diluted bread extract mixed with potassium iodide solution. A calibration curve was drawn by plotting the absorbance against e potassium bromate concentrations, and the regression equation was generated (Ojo et al., 2013).

Procedure for Analysis of potassium bromate in Baked Products

One gram of each representative powdered sample was weighed and transferred into a test tube, 10 ml of distilled water was added and shaken vigorously using vortex. The test tube was allowed to stand for 20 min at a room temperature (28 ± 5 ºC), then the solution was filtered using a Whatman no 1 filter paper. One milliliter of the clear aliquot of each sample were transferred into another separate test tubes, then 1 ml of 0.5% freshly prepared potassium iodide solution in 0.1N HCl was added to the solution and mixed well using vortex. The absorbance of the solution was measured at 540 nm in 1cm cell against the corresponding baked product extract diluted with 0.1 N HCl, in blank cell (Emeje et al., 2010; Ojo et al., 2013). The Analysis was done on three replicates for each of the representative samples. The amount of potassium bromate (µg/mL) in each sample is calculated using thecorresponding regression equation of the calibration curve prepared from the standard solutions of potassium bromate. Then, the concentration of potassium bromate (µg/g) in each sample is calculated, using equation {1}.

$$KBrO_3\text{Concentration}\left(\frac{\mu g}{g}\right) = \frac{KBrO_3\text{Concentration }(\mu g/mL)}{\text{sample weigh (g)}} \times \text{dilution factor} \qquad \text{Equation } \{1\}$$

Statistical analysis

All the experimental analysis were done in triplicate determinations. The results were presented as mean (µg/g) (ppm) ±standard deviation (mean ±sd) and range (min-max). The comparisons between the different baked products were performed by the One-Dimensional Variance Analysis (One-way ANOVA) test, followed by Least Significant Difference (LSD) test. All the statistical analysis were carried out using Statistical Package For Social Analysis (SPSS) (SPSS version 19.0; IBM, Chicago, III., USA) Program, adopting the significance level of 5% ($P < 0.05$).

Results and discussion

In this study, the concentration of potassium bromate in the collected baked samples was determined by spectrophotometic method. This method based on the reaction of bromate ions with iodide ions in acid medium to produced iodine, according to equation {2}. The produced Iodine formed blue color complex in presence of starch from baked products (Abu-Obaid et al., 2016).

$$BrO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + 3H_2O + Br^- \qquad \text{Equation } \{2\}$$

As shown in Figure 1, the absorbance at 540 nm increased linearly with the bromate ion concentration in the range from 0.25 to 15.0 µg/ml, with a correlation coefficient of 0.994. The corresponding regression equation was A=0.061C+0.009, where A and C were the absorbance and potassium bromate concentration, respectively.

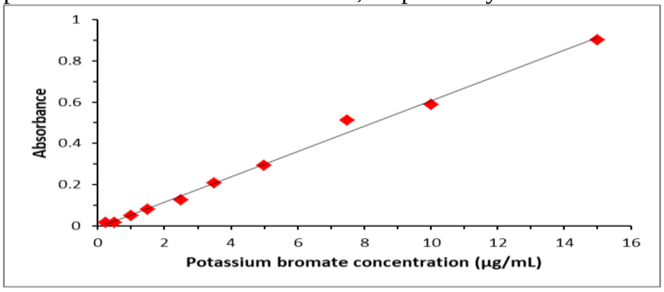

Fig (1): The standard curve of potassium bromate (0.25-15 µg/ml) using potassium iodide solution. The absorbance is measured at 520 nm. The linear regression equation is $A = 0.061\,c + 0.009$, ($R^2 = 0.994$).

The results of potassium bromate in bread and baked products samples (included; croissant, toast and cake) collected from different bakeries located in Benghazi city were demonstrated in Table 1.

Table 1: Potassium bromate concentrations in some baked products samples collected from different bakeries in Benghazi city (mean ±sd)

Baked Product	Sample Code	Potassium bromate Content* (µg/g)
White Bread	Bw1	0.3223 ±0.02
	Bw2	1.842 ±0.1
	Bw3	0.6830 ±0.04
	Bw4	0.9945 ±0.06
	Bw5	0.8306 ±0.06
	Bw6	0.1899 ±0.02
	Bw7	0.5519 ±0.07
	Bw8	0.9751 ±0.05
	Bw9	0.6557 ±0.2
	Bw10	0.3645 ±0.04
Brown Bread	Bb1	1.276 ±0.04
	Bb2	0.2076 ±0.05
	Bb3	0.3716 ±0.04
	Bb4	0.2213 ±0.03
	Mean ±SD	**0.6779 ±0.47**
	(Range) Min-Max	**(1.652) 0.1899-1.842**
Croissant	C1	0.3606 ±0.07
	C2	0.4317 ±0.1
	C3	0.5792 ±0.1
	C4	1.180 ±0.4
	C5	0.6885 ±0.07
	C6	0.4426 ±0.02
	Mean ±SD	**0.6135 ±0.3**
	(Range) Min-Max	**(0.8195) 0.3606-1.1801**
Toast	T1	2.0397 ±0.2
	T2	0.7323 ±0.1
	T3	0.6721 ±0.4
	T4	1.0817 ±0.04
	T5	0.5079 ±0.02
	Mean ±SD	**1.0067 ±0.6**
	(Range) Min-Max	**(1.538) 0.5079-2.0397**
Cake	K1	2.0989 ±0.2
	K2	1.426 ±0.2
	K3	1.549 ±0.01
	K4	1.158 ±0.05
	Mean ±SD	**1.558 ±0.4**
	(Range) Min-Max	**(0.9409) 1.1582 -2.0989**

*each value is the mean of triplicate determinations

The obtained Results in Table (1), showed that all the baked samples contained potassium bromated ranged from 0.6138 to 1.558 µg/g, with a mean concentration equal to 0.9641 ±0.4µg/g. The highest potassium bromate mean concentration was detected in cake samples. While the lowest mean concentration of this substance was in bread samples. A significant difference of potassium bromate contents was seen between all baked samples (*P*=0.00).

In bread samples,the mean concentration of potassium bromate was 0.6779 ±0.5 µg/g, with a range between 0.1899-1.8415 µg/g. The highest bromate level was detected in sample **Bw2** (1.842 ±0.1µg/g), whereas the lowest level was detected in sample **Bw6** (0.1899 ±0.02µg/g). Our results revealed that the white bread samples contained the highest amounts of bromate, in comparing to brown bread samples. However, brown bread samples contained low content of bromate expect sample Bb1 (1.276 ±0.04 µg/g). The highest bromate level in the croissant samples was detected in sample **C4** (1.180 ±0.4µg/g), whereas the lowest level was detected in sample **C1** (0.3606 ±0.07µg/g). In Toast samples, the highest bromate level was shown in sample **T1** **(**2.0397 ±0.2 µg/g) , whereas the lowest level was detected in sample **T5** (0.5079 ±0.02 µg/g). The mean concentration of potassium bromate in cake samples is 1.558 ±0.4 µg/g, and ranged between 1.1582-2.0989 µg/g. The highest bromate concentration was detected in sample **K1**, whereas the lowest concentration was detected in sample **K4**. These quantities of potassium bromate in bread and baked products samples collected from bakeries in Benghazi may be due low baking temperature, baking time, and large quantity of potassium bromate used in flour. Therefore, consumers and bakers of these products are at risk of exposure to potassium bromate, due to bioaccumulation over a period of time (Mahmud et al., 2020; Abu-Obaid et al., 2019).

The results of our study, concerning the contents of potassium bromate in some baked products, were similar to other related studies reported in international literature from other countries of the world. However, the potassium bromate concentration range of the collected baked products from different bakers in Benghazi, were much lower than the concentration range of potassium bromate detected in baked samples collected from one region in **Tripoli-Libya,** named **Tajora**(6.0-26.67 µg/g) (Alhanash et al., 2020), India (10.72-39.73 µg/g) (Rana et al., 2020), Tunisia (5.95-49.31 µg/g) (El Ati-Hella et al.,2017), Nigeria (3.6-9.2 µg/g) (Alli et al., 2013), Ethiopia (6.61-9.97 µg/g) (Ergetie and Hymete, 2012), Morocco (did not exceed 5 µg/g) (El Harti et al. 2011) and Iraq (11.09-67.45 µg/g) (Abdulla et al.,2009). While, the concentration range of potassium bromate in our baked products was higher than potassium bromate concentration range of different baked products collected from Saudi Arabia markets (0.15-0.46µg/g) (Mehder et al., 2015).

Figure 2, show that the mean concentrations of potassium bromate in all baked samples were extremely high than the permissible safe level of potassium bromate recommended by US-FDA and WHO, in bread and baked products (Shanmugavel el al., 2020; Irogbeyi et al., 2019; Johnson et al., 2013; Okafor et al., 2011).

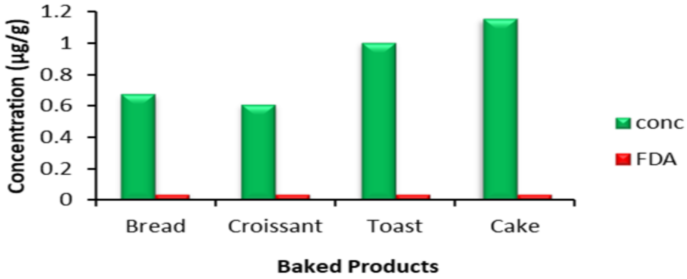

Fig. 2: Comparative the mean concentrations(µg/g) of potassium bromate in some baked products samples with the maximum allowed limit recommended by FDA.

However, In food industry processes, some food and food products (such as flour) can formulated with potassium bromate residues below the level of 0.02 mg/kg in the finished products. Therefore, American Bakeries Association and American Institute of Baking International (ABA/AIBI) recommended the warning label is necessary when bromated flour used (American Bakeries Association and American Institute of Baking International.ABA/AIBI, 2008). On the other hand, Libyan National Center for Standardization and Metrology has banned the importation of bromate salts and using them as flour treatment agents. However, all samples under investigation contained varying detectable quantities of potassium bromate (LNCSM, 2015). The high contents of potassium bromate in the analysed baked samples may be due to using of imported bromated flour.

Conclusions

This study reveals that potassium bromate found in all bread and different baked products samples, which collected from different bakeries of Benghazi City. This substance has been found at a concentration much above the allowable permissible level of potassium bromate value set by US-FDA and Libyan Standard National Regulation in bread. Therefore bread consumers and bakers are at risk of exposure to potassium bromate. In Libya, the authorities need to monitoring the bakeries and producers by continuous surveillance to ascertain compliance to standard and safety of humans, and enforcement of the ban on use of potassium bromate in baking industry. Even more, Libyan authorities should prevent the importation of bromated flour.

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