بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Instrumental Measurement of Potassium Bromate Residue in Bread and Flour Samples in Khartoum State

قياس متبقي برومات البوتاسيوم آلياً في عينات من الخبز والدقيق بولاية الخرطوم

A Thesis Submitted in the Partial Fulfilment for M.Sc. in Chemistry

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بسم الله الرحمن الرحيم

إستهلال

قال تعالي:

﴿وَلۡتَكُن مِّنكُمۡ أُمَّةٞ يَدۡعُونَ إِلَى ٱلۡخَيۡرِ وَيَأۡمُرُونَ بِٱلۡمَعۡرُوفِ وَيَنۡهَوۡنَ عَنِ ٱلۡمُنكَرِۚ وَأُوْلَٰٓئِكَ هُمُ ٱلۡمُفۡلِحُونَ﴾ (آل عمران - 104)

صدق الله العظيم

Dedication

To the soul of my father,

my mother,

and to my sisters

Acknowledgement

All praises and thanks to Almighty Allah, the Merciful, the only Creator of the Universe and source of all knowledge and wisdom, who blessed me with heath, strength and opportunity to complete this research.

My appreciation and thanks go to Dr. Omer Adam Mohammed Gibla for his guidance and encouragement during this work.

My thanks would extend to the staff of Public and Environmental Health college (University of Khartoum) laboratory and to all those who helped, encouraged or give me advice throughout the time of my work, especially my teachers and friends.

Abstract

The aim of this study was to investigate the presence of potassium bromate in bread and flour samples within Khartoum state. Twenty five bread samples and five flour samples were obtained from different outlets and bakeries in Khartoum state. The samples were analyzed using UV-Vis spectrophotometric and X-Ray Fluorescence techniques. The results of spectrophotometric measurements using oxidation of crystal violet method showed an average bromate content of 25.44 ppm for bread samples, and 6.4 ppm for flour samples. The results of X-Ray Fluorescence method showed an average bromate content of 26.14 ppm for bread samples, and 13.96 ppm for flour samples. The analysis showed that, all bread samples contain bromate residue with concentrations higher than the permissible guide line values.

المستخلص

الهدف من هذه الدراسة هوإستقصاء وجود متبقي برومات البوتاسيوم في عينات من الخبز والدقيق في ولاية الخرطوم.تم جمع خمسة وعشرين عينة من الخبز وخمس عينات من الدقيق من منافذ بيع ومخابز مختلفة في ولاية الخرطوم. وقد تم تحليل العينات باستخدام جهاز مطيافية الضوء المرئي وفوق البنفسجي وتقنية فلورة الأشعة السينية (XRF). وأظهرت نتائج المطيافية الضوئية باستخدام أكسدة صبغة الكريستال البنفسجي على نسبة متوسط برومات 25.44 جزء في المليون لعينات الخبز، و 6.4 جزء في المليون لعينات الدقيق. وأظهرت نتائج طريقة فلورة الأشعة السينية على نسبة متوسط برومات 26.14 جزء في المليون لعينات الخبز، و13.96 جزء في المليون لعينات الدقيق. وأظهر التحليل أن جميع عينات الخبز تحتوي على متبقي برومات بتراكيز أعلى من القيم المسموح بها.

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Chapter One

Introduction

1. Introduction

1.1Food additives

Food additives are substances that, become part of a food product when they are added during the processing or making of that food. Direct food additives are often added during processing to add nutrients, keep the product fresh (and/or) make the food more appealing. It may be man-made or natural. Natural food additives include herbs or spices to give flavor to foods, vinegar for pickling foods and salt to preserve meats. Indirect food additives are substances that may be found in food during or after it was processed. They are not added to the food on purpose. These additives are present in small amounts in the final product (Bush R.K., Taylor S.L. 2014).

Food additives have five main functions. They are used to give the food a smooth and consistent texture e.g.: emulsifiers prevent liquid products from separating, stabilizers and thickeners provide an even texture and anticaking agents allow substances to flow freely. They may be added to improve or preserve the nutrient value. Many foods and drinks are fortified and enriched to provide vitamins, minerals, and other nutrients. Examples of commonly fortified foods are flour, cereal, margarine, and milk. This helps to provide vitamins or minerals that may be low or lacking in the diet. All products that contain added nutrients must be labeled. Bacteria and other germs can cause food borne illnesses. Preservatives reduce the spoilage that these germs can cause. Certain additives help to keep the flavor in baked goods by preventing the fats and oils from going bad. Preservatives also keep fresh fruits from turning brown when they are exposed to air. Additives control the acid-base balance of foods and provide leavening. Some additives help to change the acid-base balance of foods to get a certain flavor or color. Leavening agents that release acids when they are heated react with baking soda to help biscuits, cakes, and other baked goods rise. Certain colors improve the appearance of foods. Many spices, as well as natural and man-made flavors, bring out the taste of food (Bush R.K., Taylor S.L. 2014).

1.1.1 Maximum level of usable dose

All food additives are restricted to contain a level, at which additives may be present in food. The maximum limits in the annexes are based on the food as sold unless otherwise specified. However, for dried and/or concentrated foods (including drinks), the maximum limits apply to the food as reconstituted following manufacturers' instructions, taking into account the minimum dilution factor. It is recognized that certain substances, for example phosphates and glutamates, are naturally present in certain foods. The quantitative limits apply to the amount of additive added. There is however, an exception in the case of sulphites, as the legislation requires that the specified quantitative limits include sulphites available from all sources and therefore take into account any natural occurrence of the substance. There are instances in the legislation where no numerical limit is set for additive use. This is because there is no need on safety grounds to set a maximum level. Rather a level of quantum satis (QS) is set. QS is defined in the legislation and means that additives shall be used in the food concerned in accordance with good manufacturing practice. This means that it must not be used at a level higher than is necessary to achieve the intended purpose and must not be used in a way that misleads the consumer (Food Standards Agency, 2015).

1.1.2 Side effects

Use of chemical food additives is a common practice in packaged and processed foods. Not all of them are safe. One such additive is potassium bromate (KBrO3) which, until over two decades ago, was routinely used in most parts of the world to treat flour for bread and bakery product (CSE Study 2016).

The U.S. Food and Drug Administration (FDA) have a list of food additives that are thought to be safe. Many of them have not been tested, but most scientists consider them to be safe. These substances are included in the "generally recognized as safe (GRAS)" list. This list contains about 700 items. Congress defines safe as "reasonable certainty that no harm will result from use" of an additive. Examples of items on this list are: guar gum, sugar, salt, and vinegar. The list is reviewed regularly. Some substances that are found to be harmful to people or animals may still be allowed, but only at the level of 1/100th of the amount that is considered harmful. For their own protection, people with any allergies or food intolerances should always check the ingredient list on the label. Sensitivity to any additive can be mild or severe (Bush R.K., Taylor S.L. 2014).

1.2 Potassium bromate

1.2.1 Physical properties

IUPAC Systematic Name: Potassium bromate.

Other name: Bromic acid, potassium salt.

Description: White crystals or granules.

Molecular formula**:** $KBrO_3$.

Relative molecular mass: 167.01g/mol.

Melting-point: Reaction at bout 350°C; decomposes at about 370°C with evolution of oxygen.

Density: 3.27 g/cm^3.

Solubility: Soluble in water; slightly soluble in acetone, dimethyl sulfoxide, ethanol, methanol and toluene (National Toxicology Program, 1991)

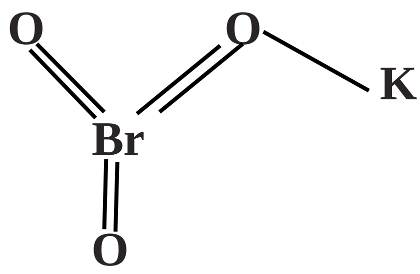

Fig (1-1) Structure of potassium bromate

1.2.2 Production

The available information indicated that potassium bromate was produced in Argentina, Brazil, China, Germany, India, Israel, Italy, Japan and Spain (Chemical Information Services, 1995).

The halogens are all soluble in water to some extent with tendency decreasing as the molecules become larger. Even iodine dissolves to a noticeable extent at room temperature as evidenced by the color it imparts to water.

Bromine is more soluble in water than iodine. A saturated aqueous solution of bromine at room temperature has a concentration of about 0.2 M Br_2. This accounts for the characteristic color of aqueous bromine solutions which ranges from pale yellow to dark reddish-orange. However, other colorless species also exist in the solution. Like chlorine and iodine, bromine reacts with water. The element disproportionate into two additional bromine-containing species:

$$Br_2\ (\ell) + 2\ H_2O\ (\ell) \leftrightarrow H_3O^+\ (aq) + Br^-\ (aq) + HOBr\ (aq)$$

Disproportionation is a redox process in which an ion is both oxidized and reduced. In hydrobromic acid (HBr) as strong acid, ionizes completely, the bromine is in oxidation state -1. The hypobromous acid (HOBr) is a weak acid which exists mainly in molecular form and in which the bromine is in oxidation state +1.

At room temperature this equilibrium lies far to the left. Molecular bromine is the predominant species in solution. Heating drives the reaction forward to some extent but making the solution basic causes a large shift in the equilibrium as hydronium ions and HOBr are neutralized:

$$H_3O^+\ (aq) + OH^-\ (aq) \rightarrow 2\ H_2O\ (\ell)$$

$$HOBr\ (aq) + OH^-\ (aq) \rightarrow H_2O\ (\ell) + BrO^-\ (aq)$$

Combining the three reactions shown so far, the overall reaction of bromine in basic aqueous solution is given as:

$$Br_2\ (\ell) + 2\ OH^-\ (aq) \rightarrow Br^-\ (aq) + BrO^-\ (aq) + H_2O\ (\ell)$$

The hypobromite ion, BrO^-, is not stable in basic aqueous solution at high temperatures. Heating causes it to disproportionate as well:

$$3\ BrO^-\ (aq) \rightarrow 2\ Br^-\ (aq) + BrO_3^-\ (aq)$$

The new species formed (BrO_3^-), has bromine in oxidation state +5. This reaction goes rapidly and essentially to completion above 50°C in alkaline solution. The reactions shown illustrate a synthetic pathway for obtaining potassium bromide and bromate from an aqueous solution of bromine. If the solution is made appropriately basic with potassium hydroxide and heated, the only species present in appreciable amounts should be potassium, bromide, and bromate ions. If the water is simply removed by evaporation, a mixture of the two solids will result. A method of separation is needed to recover each compound separately. Fortunately the two compounds differ in solubility at just about any reasonable temperature for an aqueous solution. When a solution containing potassium bromate and bromide is cooled to 0°C, nearly all bromate will precipitate, while all the bromide will stay in solution (Robert F. Bryan, Robert S. Boikess).

1.2.3 Uses in baking

Of all the baked goods bread is the most common product and involves four basic ingredients flour, yeast, salt and water. Bread has been a staple food in mans diet. Other than being a good source of starch that gives energy bread also contains dietary fiber and vitamins like thiamine, riboflavin, niacin, pyridoxine and folic acid. With respect to minerals, it contains large amounts of phosphorus, magnesium, calcium and potassium, and in lesser quantities of sodium, iron, or iodine. Due to these nutritional values nutritionists consider it to be essential part of diet.

In baking of bread all the ingredients are mixed properly to form the dough firstly then, it is allowed to rest where the yeast grows utilizing the carbohydrates breaking them down into simple sugars and produce alcohol and CO2 gas which is trapped in the dough forming pores in it resulting in rising of the dough. The risen dough is transferred to the oven where the heat penetrates the dough, as the gases expand the size of the dough increases giving its size and shape. Caramelization of the sugars takes place giving bread its taste and smell. To make good bread the dough must have elastic nature, ability to hold gases produced by yeast and the property to retain its shape. All this is done by the Gluten protein that is naturally found in the flour. Bonding between the gluten molecules is the result of oxidation, in olden days floor was aged by exposing it to the open air for oxidation for days to weeks and then added to dough, later chemical oxidizing agents have come into use that have cut down the ageing time. Potassium bromate is a potential oxidizing agent and also acts as a bleaching agent that imparts bond strength by improving the elasticity that results in soft, fluffy and White bread. (Preethi Grace G., 2016).

During the preparation of the dough, a network of protein molecules linked together by disulphide bonds is formed. The strength and elasticity of the network which gives the dough its characteristic properties is best when the network comprises of long chain proteins such as gluten. Short chain peptides such as glutathione which are react with gluten molecules breaking down the dough structure. This structural breakdown can be prevented by the addition of oxidizing agents such as potassium bromate (Cogswell, 1997). In the presence of any of this oxidizing agent, the glutathione is oxidized to glutathione disulphide and therefore cannot interfere with disulphide links of the gluten molecules (Kent, 1984)

Although banned for use in foods by many countries, in the USA Potassium bromate is typically used as a flour improver (E number E924). It acts to strengthen the dough and to allow higher rising. It is an oxidizing agent, and under the right conditions, will be completely used up in the baking bread. However, if too much is added or if the bread is not baked long enough or not at a high enough temperature, then residual amount will remain, which may be harmful if consumed (Kurokawa, et.al (1990).

Table (1-1) Bromate and replacer ingredients (Lallemand Baking Update, 1996)

COMPOUND	FUNCTION	CONSIDERATIONS
Potassium Bromate	Slow oxidizer	Used alone as the oxidative agent of choice
Ascorbic Acid	Intermediate oxidizer	Requires oxygen, so is less effective in continuous mix
Calcium Peroxide	Fast oxidizer	Usually used with ascorbic acid
ADA (Azodicarbonamide)	Fast oxidizer	Critical dosing with limited tolerance
Potassium and Calcium Iodate	Fast oxidizer	Critical dosing with limited tolerance
Oxidative Enzymes (lipoxygenase, glucose oxidase, sulfhydryl oxidase)	Form oxygen via hydrogen peroxide	Usually used with ascorbic acid
Carbohydrase Enzymes (fungal *alpha*- amylase, hemicellulase)	Act on starch and fiber during baking	Used with ascorbic acid to improve oven spring and freshness
Cysteine and Glutathione	Act on gluten during mixing to relax dough	Used with fast chemical oxidizers to shorten mix time and improve handling
Yeast Components	Natural source of glutathione and enzymes	Used with fast chemical oxidizers and with enzymes for an all-natural system

1.2.4 Exposure to potassium bromate

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 27 000 workers in the United States were potentially exposed to potassium bromate.

Occupational exposure to potassium bromate may occur during its production and during its use as a dough conditioner and food additive. In a survey of retail bread samples in the United Kingdom in 1989, potassium bromate was found in all six unwrapped breads analyzed, with a median concentration of 35 μg/kg (range, 17–317 μg/kg), and in seven of 22 wrapped breads, with a median concentration of < 12 μg/kg (range, < 12–238 μg/kg). In a second survey of the same brands in 1992, all samples contained less than the detection limit of 12 μg/kg flour (Dennis et al., 1994).

Ozonization of surface waters containing bromide ion can result in the formation of bromate (Glaze, 1986).

No international guidelines for potassium bromate in drinking-water have been established (WHO, 1993).

1.2.5 Effects on nutritional value

Treatment of flour with potassium bromate at a concentration of 45 mg/kg did not cause any decrease in its content of thiamine, riboflavin or nicotinic acid (Ford et al., 1959).

Wheat flours treated with potassium bromate at a concentration of 25 mg/kg and stored for 12 months did not show any greater decrease in tocopherol content than flour, either untreated or treated with ascorbic acid and stored under the same conditions, not more than 35-50% (Menger, 1957).

At high levels of use, about 200 mg/kg, bromate has no significant effect on the thiamine, riboflavin or nicotinic acid content of flour or of bread made from it. No statistically significant differences have been found in essential fatty acid content in flour treated with 200 mg/kg potassium bromate or in bread made from such flour (Ministry of Agriculture, Fisheries and Food, UK, 1974).

Potassium bromate completely destroys folic acid in solution in 10 days (British Food Manufacturing Industries Research Association, 1980).

1.2.6 Banning of potassium bromate

In 2007, Chinese authorities pulled a batch of imported snack chips from store shelves because they believed the chips contained potassium bromate, a food additive banned in China. Potassium bromate is also illegal in the European Union, Canada, Brazil and elsewhere because it causes cancer in rats and mice (Luke Yoquinto, 2012).

In the United States, however, it has remained legal since it was first patented for use in baking bread, in 1914.In 1982; researchers in Japan published the first series of studies showing that potassium bromate causes cancer in the thyroids, kidneys and other body parts of rats and mice. As a result of these findings, countries around the world banned the additive, but the U.S. Food and Drug Administration held back, in part because the amount of potassium bromate that remains in bread after baking should be negligible: less than 20 parts per billion. According to information published by baking industry trade groups, it is "well within the normal production control measures in any modern bakery to ensure that bromate residues are well below 20 ppb."

However, whenever bromated flour isn't baked for long enough or at a high enough temperature, or if too much potassium bromate is added in the first place, this harmful additive can potentially be found in the final product in far greater quantities. Today, many small and commercial bakeries voluntarily avoid using bromated flour. However, it's still found in many fast food buns and some flours, among other products (Luke Yoquinto, 2012).

1.2.7 Absorption, distribution, metabolism and excretion

After some mice received diets containing 79% bread crumbs made from flour treated with 50 or 75 mg/kg (potassium bromate, concentrations of 1 and 2 mg/kg bromine, respectively, were detected in adipose tissue (Ginocchio et al., 1979).

Bromine did not accumulate in the adipose tissue of Wistar-derived Porton rats fed for 104 weeks on diets composed of 79% bread crumbs made from flour treated with 75 mg/kg potassium bromide (Fisher et al., 1979). In a further study, male Wistar rats, six to eight weeks of age, were given 100 mg/kg bw potassium bromate by gavage in an aqueous solution as bromate. Animals were killed at various times after treatment, and bromate was assayed in the stomach, small intestinal contents, plasma, and bladder urine. Bromate was found to be rapidly absorbed and eliminated (or degraded): 2 hour after administration, bromate was no longer detected in plasma, and 4 h after treatment, bromate was no longer detected in bladder urine or small intestine. Twenty-four hours after administration of potassium bromate at a dose ≤ 2.5 mg/kg bw, bromate was not detected in urine. At doses of 5–100 mg/kg bw, the concentrations of bromate in urine after 24 h increased proportionally with dose (Fujii et al., 1984).

Absorption and distribution studies of bromate in experimental animals have been carried out at very high doses (100 mg/kg). There is a real need to obtain experimental data and develop a pharmacokinetic model for distribution of bromate in the body at doses between those obtained from drinking water at the MCL (about 0.0003 mg/kg) and those that induce cancer in the rat (about 3 mg/kg), a margin of exposure of about 10,000. There is preliminary data indicating that a potentially important portion of ingested bromate at low intakes can be chemically reduced prior to leaving the GI tract, and perhaps in the bloodstream (Awwa Research Foundation, 2006).

1.2.8 Effect of temperature

The effect of temperature on bromate ions was studied in the range from 50˚C to 400˚C in which all potassium bromate ions decomposed. The effect of temperature was studied at 50 ppm of bromate solution for each sample. The results are shown in Figure (1-2) (the absorbance measured at pH 1 and iodide concentration 0.1 M). As Figure (1-2) shows, the absorbance of iodine (I2) decreased upon increasing the temperature during the first 3 min after mixing the bromate ions. The absorbance was in the range from 350˚C - 400˚C ≈ zero, this meaning that the bromate ions decomposes and converts to KBr and O2 from heating as in equation:

$$2KBrO_3 \rightarrow 2KBr + 3O_2$$

Bromate decomposes in distilled water at (350˚C - 400˚C) but in bread decomposes within the range of 150˚C - 250˚C. The decomposition of bromate ions in bread at low temperatures may be due to the presence of metal ions in flour, which acts as a catalyze (**Ahmad** Abu-Obaidt, et al., 2016).

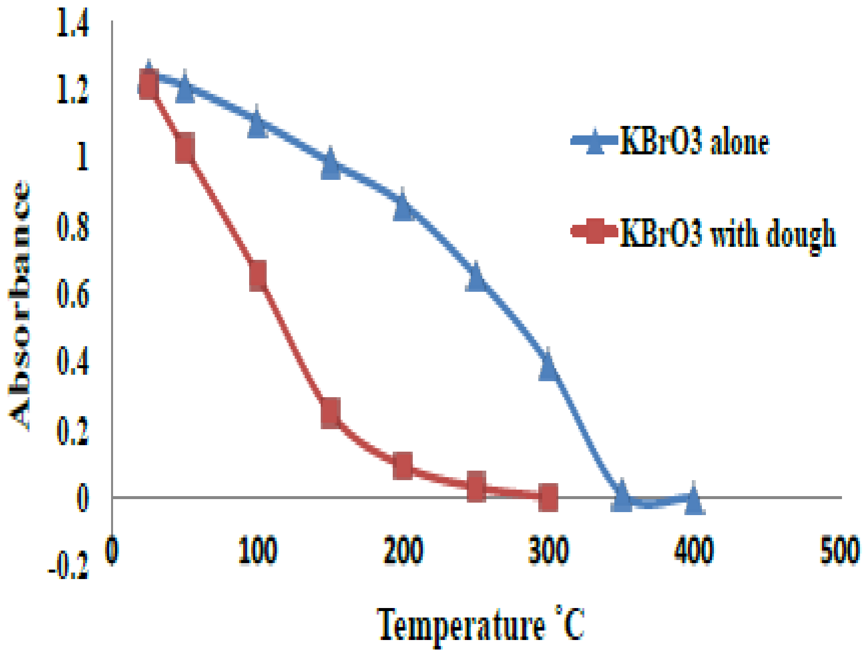

Fig (1-2) Effect of temperature on Potassium Bromate

1.2.9 Cancer in humans and experimental animals

No data were available for carcinogenicity of potassium bromate in human, but Potassium bromate was tested for carcinogenicity in one experiment in rats by oral administration. It produced a high incidence of renal tubular tumours (adenomas and/or carcinomas) in animals of each sex, an increased incidence of mesotheliomas of the peritoneum of males and tumours of the thyroid in female rats. Experiments in mice and rats fed diets containing bread baked from flour containing potassium bromate were inadequate for evaluation. In two experiments with rats, potassium bromate exerted an enhancing effect on the induction by N-nitroso ethyl hydroxyl ethylamine of kidney tumours and dysplastic foci (IARC, 1986).

1.2.10Toxic effects on human and experimental system

Potassium bromate dust is irritating to the mucous membranes of the upper respiratory tract, but there was no evidence of any cumulative effect on the lung of workers after 18 years of exposure to levels as high as 15 mg/cu m (Bingham et al., 2001).

In children 1.5–3 years of age, ingestion of 2–4 oz (57–133 g) of a 2% solution of potassium bromate caused nausea and vomiting, usually with epigastric and/or abdominal pain; diarrhea and haematemesis occurred in some cases (IARC, 1999).

Several authors report the effects of acute oral exposure in children to potassium bromate following accidental ingestion of home permanent hair wave neutralizing solution. The age of the children ranged from 17 months to 6 years. When estimated, doses ranged from 20 mg BrO_3^-/kg to 1,000 mg BrO_3^-/kg. In all cases, the initial symptoms appeared to include abdominal pain, vomiting, or other gastrointestinal effects. CNS effects such as sedation, lethargy, and CNS depression appeared to be early symptoms of bromate poisoning after doses of about 70 mg/kg or higher. Irreversible deafness is also an effect of bromate exposure. One review of bromate auto toxicity found that deafness occurred in 18 of 31 cases, usually within 4-16 hours of exposure (USEPA, 2001).

In both children and adults, oliguria and death from renal failure have been observed (Dunsky, 1947; Ohashi et al., 1971; Gradus et al., 1984). Partial hearing loss and complete deafness have also been reported (Matsumoto, 1973; Quick et al., 1975; Gradus et al., 1984). The toxic or lethal dose of potassium bromate in humans has not been accurately established (Kurokawa et al., 1990), but a dose of 500 mg caused serious symptoms in a 15-month-old child (Quick et al., 1975).

De Vriese et al. (1997) described the clinical symptoms of a patient who ingested 300 mL of a cold-wave neutralizer consisting of 10%

potassium bromate. They also reviewed 49 other cases of human exposure published since 1947. The characteristic symptoms after ingestion of various solutions containing bromate include nausea, vomiting, abdominal pain and diarrhea shortly after ingestion. Acute renal failures varying from mild to severe forms have been reported in both children and adults. Nine cases of adult poisoning (33%) resulted in death. Severe irreversible sensorineural hearing loss within 4–16 h of ingestion was recorded in almost all of the adults but in only a few children.

Toxicological studies on bromate-treated flour and bread are done in experimental system into short-term studies and long-term studies.

1.2.10.1 Short-term studies

Bread made from flour treated with 14 mg/kg and with 100 mg/kg of potassium bromate was fed to two groups of six male and 20 female rats each of these diets continued over three generations, the entire experiment lasting 10 months. The health, behaviour, weight gain and reproductive performance remained normal throughout. Histological study of the tissues showed no abnormalities and analyses of brain and liver showed no accumulation of bromine. Eighteen rats were fed a diet containing 84% of flour treated with potassium bromate at a level of about 75 mg/kg for a period of four weeks. Growth and reproductive performance were normal. Bread made from flour treated with 200 mg/kg of potassium bromate was fed to 12 rats for 16 days and the flour itself to 16 rats for 10weeks without adverse effects (Ford et al., 1959).

Three dogs were fed for 12 weeks a diet containing 84% of bread made from flour treated with 75 mg/kg potassium bromate. No ill-effects were observed. Five dogs were fed for six to 14 weeks flour treated at a level of 75 mg/kg potassium bromate. Two dogs were fed for 16 days with bread made from flour treated at a level of 200 mg/kg with potassium bromate. No ill-effects were observed (Ford et al., 1959).

Three dogs fed for six weeks on diets containing flour treated with 70 mg/kg potassium bromate showed no ill-effects or "running fits". Four dogs fed for 17 months on bread made from flour containing 200 mg/kg potassium bromate showed no adverse effects attributable to the diet (Impey et al., 1961). Three monkeys fed for eight weeks on a diet containing 84% of bread made from flour treated with 75 mg/kg potassium bromate showed no adverse effects (Ford et al., 1959).

1.2.10.2 Long-term studies

Groups of mice fed flour treated with 15 mg/kg potassium bromate showed no ill-effects over eight generations (Ford et al., 1959).Groups of 60 male and 60 female mice were fed for 80 weeks on five diets containing 79% breadcrumbs; the bread used was prepared from untreated flour (control), from flour treated with 50 mg/kg or 75 mg/kg potassium bromate, or from flour treated with 50 mg/kg bromate plus one of two mixtures of other commonly used flour additives (ascorbic acid, benzoyl peroxide and chlorine dioxide).

Appearance, behavior, health and survival were similar in test and control groups and there was no evidence that any of the treatments affected the incidence of neoplasm, the incidence of malignant tumours being similar in control and test groups. Anemia was observed in males of all groups (including controls) and in females at 18months. No dose-related differences in blood biochemistry were found in male mice; in the females, dose-related increases in blood glucose levels were observed at one and 12 months but not at 18 months.

Renal concentration and dilution tests and urinalysis were indicative of normal renal function. Some dose-related differences in the weights of heart, pituitary and uterus were found but, when expressed relative to body weight, the values for heart and uterus were not dose related. The relative weights of pituitary, brain, kidneys and thyroid showed dose-related changes in males only, with relative weights of heart and pituitary being lowered and kidneys and thyroid elevated. These changes were not associated with any histopathological abnormalities. No significant dose-related accumulation of covalently-bound bromine was observed in adipose tissue (Ginocchio et al., 1979).

Twenty rats were fed for two years with flour treated at a level of 627 mg/kg potassium bromate. Weight gain, general health and survival rate were not significantly different from those of controls. Five generations of rats were fed bread made from flour treated with potassium bromate at a level of 15 mg/kg. No effects on weight gain, reproductive performance or survival were observed (Ford et al., 1959).

Five groups of 60 male and 60 female rats were fed for 104 weeks on five diets containing 79% breadcrumbs; the bread was prepared from untreated flour (control), from flour treated with 50 mg/kg or 75 mg/kg potassium bromate, or from flour treated with 50 mg/kg bromate plus one of two mixtures of other flour additives. Appearance, behavior and health were similar in test and control groups. The death rate was lower in the test groups than in controls in the females and the males of the high-dose group had fewer deaths than the other groups taken together. No evidence of carcinogenicity or of chronic toxicity was attributable to the compounds under test at the dose levels used. There was no evidence of accumulation of covalently-bound bromine in the adipose tissue (Fisher et al., 1979).

1.2.11 Environmental fate

Potassium bromate can be assumed to have a negligible vapor pressure, and it is therefore not expected to partition to air. However, bromate may be associated with aerosols. Similar to many inorganic salts, potassium bromate is highly soluble in water and dissociates rapidly (primarily ionic bonds) to release the bromate ion, which is the moiety of interest in the ecological component of this assessment. As typified by many inorganic ions found primarily in anionic form in water, the bromate oxyanion is expected to have a high geochemical mobility in oxic waters (i.e., pH between 5 and 9; redox potential [Eh] between 0.5 and 1 V). As a possible consequence of this expected behavior, there is a lack of impetus for researchers to study the speciation and bioavailability of bromate in solution.

No studies have been found on interactions between bromate and colloidal organic matter, for example. However, available thermodynamic stability constants for bromate–inorganic ligand complexes suggest that this anion would be weakly complexes in natural waters. The inorganic complexation of this ion was found to be negligible (<<1%). Seawater and more mineralized waters are expected to also weakly complex bromate because of the tendency of chemical stability constants to decrease with increasing ionic strength.

Considering its mobility in water, relatively little bromate is expected to partition to sediments and soils. Bromate ions found in sediments and soils are expected to be mobile in these compartments. For example, Butler et al. (2005) reported a case in the United Kingdom of groundwater contamination by bromate in a chalk aquifer following an industrial spillage, indicating that bromate can, under some circumstances, pass through soil into groundwater.

Natural bromate reduction may occur in waters with low oxygen concentrations, according to the following reaction:

6 CH2O + 4 BrO3− → 6 H2O + 6 CO2 + 4 Br− [6]

Butler et al. (2005a) indicated that the rate of reduction may be slow, according to studies on these processes performed in laboratories.

There are indications from high dose acute exposures that bromate can under some conditions cause hearing loss (Environment Canada, September 2010).

1.2.12 Methods of detecting residual bromate levels

There are several existing methods for the determination of bromate, all with their advantages and disadvantages:

• Flow through fluorescence methods, based on derivation reactions, has limits of detection of around 1 ppb but are not are simple. These methods also involve the use of quite extensive sample pre-treatments.

• IC/Mass spectrometry provides detection limits of around 5 parts per trillion (ppt) so is easily sensitive enough, but the instrumentation required is relatively complex and expensive.

• Ion Chromatography (IC), involving the use of suppressed conductivity detection is useful, as a relatively small amount of sample pre-treatment is required. However suitably low limits of detection are not always possible and the matrix effect of chloride is pronounced.

• A spectrophotometric method, based on the reaction of bromate with crystal violet dye in a hydrochloric acid medium in which bromate is a strong oxidant.

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

With irreversible oxidation indicators, the quantity of bromate solution consumed by the dyestuff indicator is exceedingly small and the indicator is bleached in the presence of 2M HCl used for this

$$10Cl^- + 2BrO3^- + 12H^+ \rightarrow 5Cl_2 + Br_2 + 6H_2O$$

Crystal violet is a triarylmethane dye. It is a leuco compound (colorless) converted to tertiary alcohol (color base) on oxidation. In the presence of acid the colorless benzenoid form changed to the quinonoid dye due to salt formation. The salt is easily reconverted into the leuco – base.

It is purple in weakly acid solution, green in strong acid solution and finally yellow. The color changes may be adduced in the light of proton addition. The molar absorptivity of crystal violet dye is 9.70 x10^5 dm^3/ mol.cm at λ_{max}, 485 nm. (Ade Kujore and Josep-Miquel Serret, 2010).

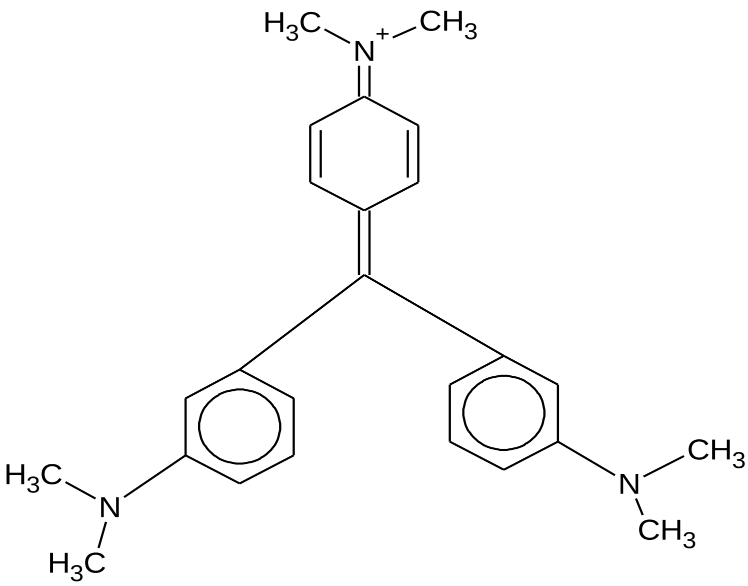

Figure (1-3) Structure of Crystal Violet

1.3 Previous studies

Twenty three samples of bread from eastern part of Nigeria was qualitatively and quantitatively analyzed by (M.O. Emeje.et.al, 2010) using spectrophotometric method and they have found that the least quantity of potassium bromate detected was 1.16μg/ml and the maximum quantity was 10.44 μg/ml. The maximum amount of potassium bromate allowed in bread by The Food and Drug Administration (FDA) is 0.02μg/ml. The amount of potassium bromate found in all bread samples was more than (0.02 μg/ml); this implied that, none of the bread samples marketed in eastern part of Nigeria is safe for human consumption.

Centre for Science and Environment (CSE study, May 2016) decided to check the levels of potassium bromate in breads and bakery products produced in India Tests conducted by CSE’s Pollution Monitoring Laboratory (PML). The PML collected a total of 38 bread and bakery samples from retail shops, bakeries and fast food outlets in Delhi in May-June 2015. The samples included popular varieties of white bread, whole wheat/atta bread, brown bread, multigrain bread, sandwich bread, pav, bun, ready-to-eat burger bread and ready-to-eat pizza bread. The tests were conducted on UV-visible spectrophotometer using a published method which can detect the presence of both potassium bromate and potassium iodate, as both of them oxidize the dye producing the same color. They found that Over 84 per cent (32/38) samples tested were found having residues of potassium bromate/iodate in the range of 1.15–22.54 ppm .All tested product categories were found with residues of potassium bromate/iodate. All samples of white bread, pav, bun and ready-to-eat pizza bread were found to contain potassium bromate/iodate. Over 79 per cent (19/24) samples of bread and about 75 per cent samples of ready-to-eat burger breads were also positive. The highest level of potassium bromate/iodate was present in sandwich bread. This was followed by pav, bun and white bread. Even the average levels were high in these products. Products of Perfect Bread, Harvest Gold and Britannia were found with high average levels of potassium bromate/iodate. Harvest Gold sandwich bread had highest concentration. Products of all seven popular fast food outlets selling pizza and burger were found positive with potassium bromate/iodate – but at levels lower than those found in bread, pav and bun.

(Ojo Rotimi Johnson et.al,2013) made A survey of available loaves of bread and flour samples in karu, Nasarawa state in Northern part of Nigeria, were carried out in bakeries, fast foods outlets, open markets, bus stops and flour distributors' shops in Karu local government. Fourteen different types of bread samples freshly baked and well packaged by their bakers were selected based on their availability and popularity among the majority of the consumers; also four most popular wheat flour brands among the bakers were selected. Potassium bromate contents of the bread and wheat flour samples were analyzed by spectrophotometric method and they found that the least concentration of potassium bromate in bread samples is (0.5µg/g) while the highest concentration was found to be (8.4µg/g). These levels are higher than 0.02µg/g, permitted by (FDA), but lower than the levels permitted by China (50µg/g) and Japan (10µg/g).

Application of UV- Vis spectroscopy for determining bromate in bread was used to analyze fifteen samples of bread, made from flour treated with potassium bromate, were collected from different regions in Hawler, Iraq. The residual bromate level in the analyzed bread samples were in the range from 6.66mg/L to 67.45mg/L (Narmeen S.Abdulla and Media A. Hassan , 2009).

Wavelength-dispersive x-ray fluorescence (WDXRF) was applied to determine bromide (Br^-) as an indication of pre-baking bromate addition in bread. The proposed methodology needed a minimum sample preparation procedure because it was carried out directly on solid samples. The calibration of Br in bread obtained showed low detection limit and high sensitivity to distinguish precisely (Br^-) concentrations greater than natural (Br^-). The excellent performance of the present methodology would be useful to identify pre-baking bromation in bread, which can be used to help set up a program to control bromation in bread. Application of this methodology to bakery control caused an important reduction of bromate use in province of Córdoba, Argentina (Roberto Daniel Perez & Alberto Edel Leon, Jun 2012)

(A.M Magomya et.al, 2013) analyzed fifteen different bread brands produced in Zaria metropolis of Northern Nigeria to determine potassium bromate content using the iodometric titration method. The study revealed the presence of potassium bromate in all the samples analyzed, the concentrations ranged from 2.46 to 13.60mg/kg, all the values were above the allowed limit by FDA.

A rapid and reliable spectrophotometric method was validated to determine the level of bromate in bread(J. El harti,et al.,2011) this method is based on the red-ox reaction between bromate and promethazin in acidic medium. This produced a red-pink product with maximum absorption at 515nm. The calibration curve was linear (r = 0.9989) over the range 0.5 μg/ml – 4.5 μg/ml of bromate, the proposed method has been successfully applied to determination of bromate in commercial bread.

Objectives of the study

The aim of this study is:

- To investigate the presence of potassium bromate in bread and flour samples within Khartoum state.
- To quantify the amount of bromate residue in the samples

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Collection of samples

Twenty five bread samples and five flour samples were collected from different outlets and bakeries in Khartoum state. Representative samples were obtained from Omdurman; Khartoum and Khartoum North cities. Four of the flour samples were obtained from products that are widely consumed in Khartoum state and the fifth sample was obtained from raw wheat. Duplicate samples from each location were analyzed for residual bromate level.

2.2 Chemicals

- **Potassium bromate**

Obtained from National Public Health Laboratory (Sudan), made in England with 99.9%minimum assay

- **Hydrochloric Acid**

Assay: 35-38%. Mumbai – India. Product No.38507

- **Crystal Violet Dye**

Dye content: Min88%. Techno pharmchem

Bahadurgarh, India.

2.3 Instruments

- U.V microprocessor spectrophotometer

Microprocessor Spectrophotometer Model – 2306, Electronics India, Parwanoo (HP) India, Detector: Silicon Photodiode Wave length range: 320 – 1100 nm, Light source: Tungsten Halogen Lamp.

- X-Ray Fluorescence

Model CANBERRA series 35 plus, with Cd^{109} source and Silicon detector, designed and manufactured in USA.

- Centrifuge

Hettich (ZENTRIFUGEN) model: EBA – 21.manufactored in Germany. Maximum capacity 6×50 ml and speed (RPM) 18000.

2.4 Methods of Analysis

2.4.1 Samples treatment

Bread samples were dried at room temperature for 7 days and grounded to fine powder with mortar and pestle.

2.4.2 Determination of bromate residue by UV-visible spectrophotometer

5g of each powdered sample was weighed into a clean 250 ml beaker and 50 ml of distilled water was added. The mixture was centrifuged at 4000 rpm for 10minutes and the liquid fraction was filtered and diluted to 100 ml in a volumetric flask. 4 ml of aliquot of each sample was transferred to 25 ml volumetric flasks. 5 ml of $5x10^{-4}$ mol/dm^3 solution of solution of crystal violet dye was added, followed by 10 ml of

2M HCl solution. The volume was then completed to the mark with distilled water; and shaken gently prior to colorimetric analysis. A portion of sample solution was transferred into 1cm quartz cell to measure the absorbance at 485 nm.

The working standard solutions were prepared in the range, 12, 24, 36, 48, 60 and 72 ppm respectively. The BrO_3^- residue was then calculated (Ojeka E. O.et al, 2006).

2.4.3 Determination of bromate residue using X-Ray Fluorescence

Five composite samples from the twenty five bread sample were made by mixing 1g from every five samples to give a total of 5g.

Each sample was pressed into a pellets form using 11 ton pressing machine. The diameter of each pellet was about 4.9 cm and the mass about 1g, the pellets were presented to XRF spectrophotometer system, each of them was measured at 600 v^- for 2000sec.

Chapter Three

Results and Discussion

3. Results and discussion

3.1Results

Table (3-1) Absorbance of the standard solutions

Concentration(ppm)	Absorbance
12	0.021
24	0.039
36	0.056
48	0.073
60	0.087
72	0.099

Y=0.0015X

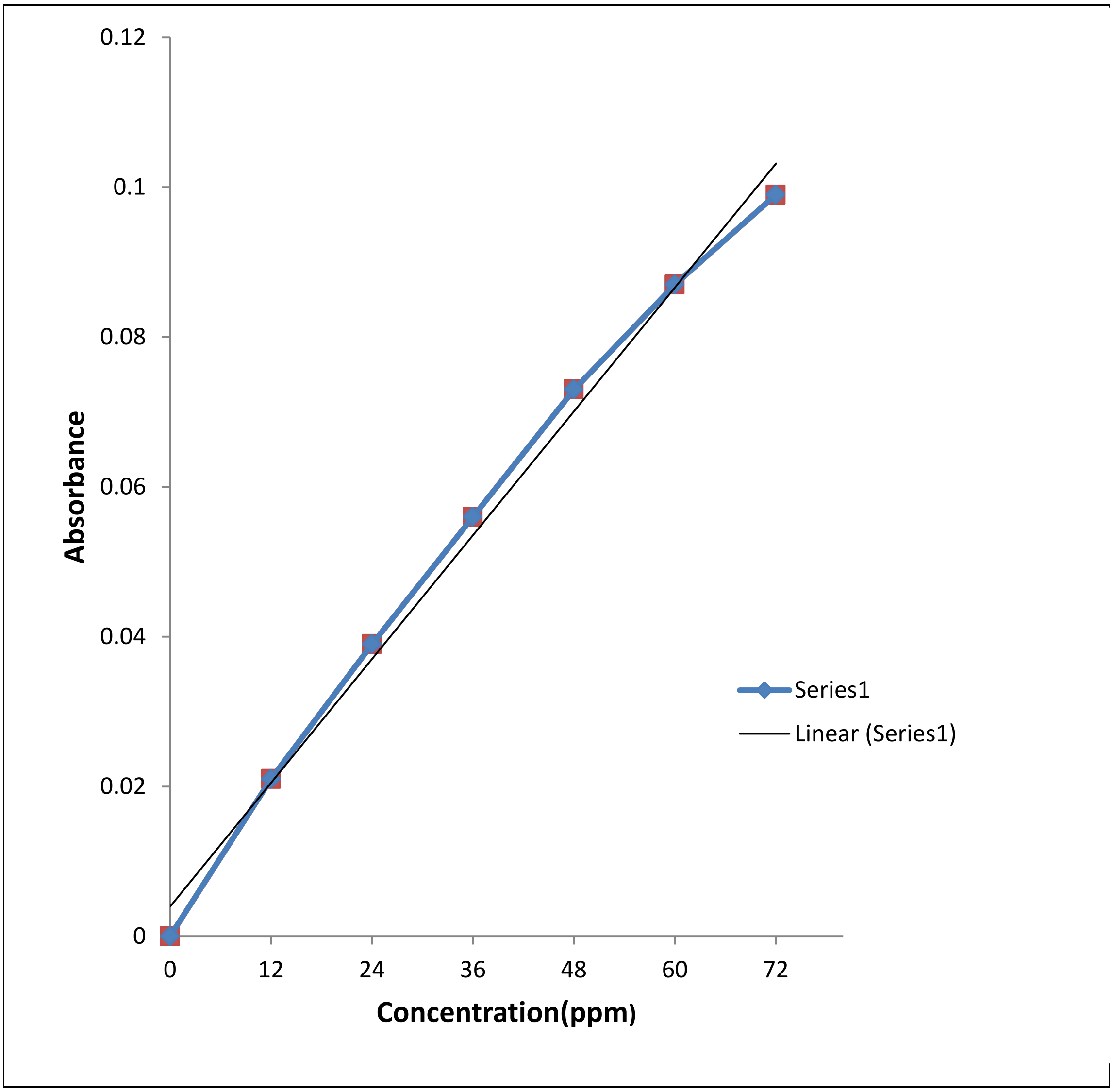

Figure (3-1) Standard curve for potassium bromate solutions

Table (3-2) UV-Vis spectrophotometer results of bread samples

Sample No.	Absorbance	Concentration(ppm)
1B	0.01	6.67
2B	0.012	8.00
3B	0.008	5.33
4B	0.030	20.00
5B	0.004	2.67
6B	0.034	22.67
7B	0.063	42.00
8B	0.055	36.67
9B	0.071	47.33
10B	0.061	40.67
11B	0.057	38.00
12B	0.033	22.00
13B	0.048	32.00
14B	0.070	46.67
15B	0.025	16.67
16B	0.057	38.00
17B	0.056	37.33
18B	0.043	28.67
19B	0.041	27.33
20B	0.062	41.33
21B	0.025	16.67
22B	0.009	6.00
23B	0.027	18.00
24B	0.029	19.33
25B	0.024	16.00
Mean		25.44

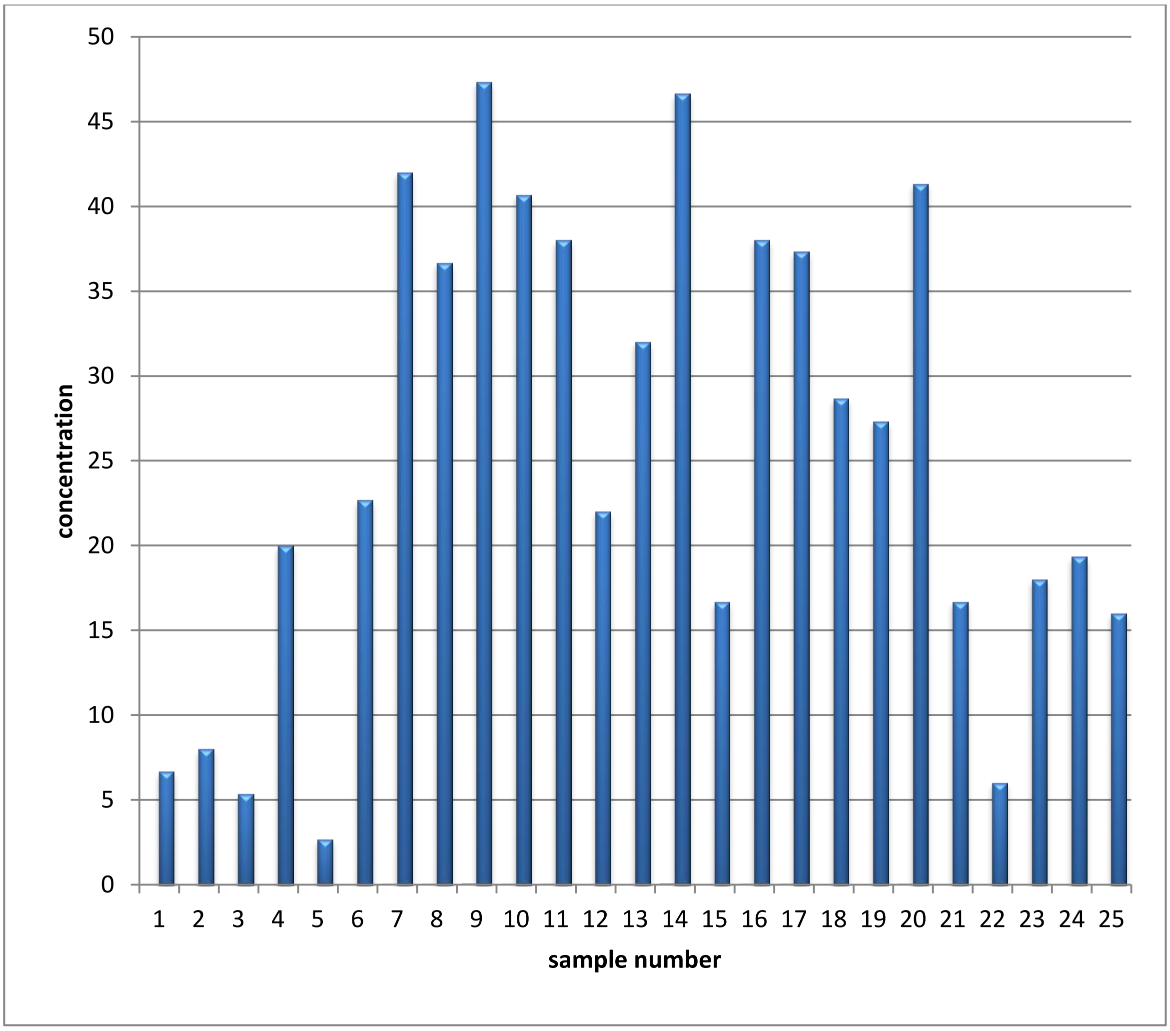

Figure (3-2) UV-Vis spectrophotometer results of bread samples

Table (3-3) UV-Vis spectrophotometer results of flour samples

Sample No.	Absorbance	Concentration(ppm)
1F	0.008	5.33
2F	0.013	8.67
3F	0.006	4.00
4F	0.019	12.67
5F	0.002	1.33
Mean		6.4

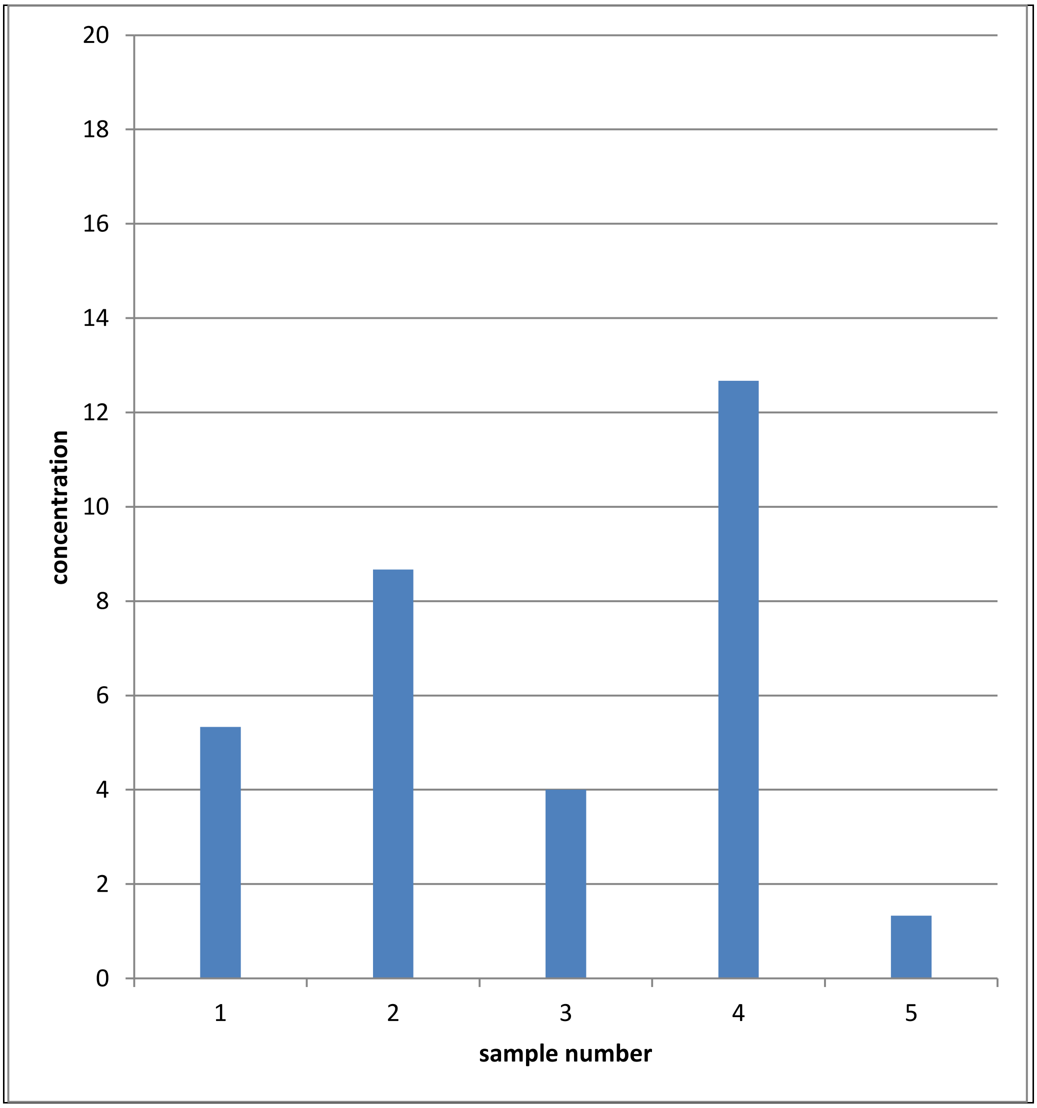

Figure (3-3) UV-Vis spectrophotometer results of flour sample

Table (3-4) X-Ray fluorescence results of bread samples

Sample No	**Br%**	**Br(ppm)**
B11*	0.333	33.30
B22	0.290	29.00
B33	0.280	28.00
B44	0.200	20.00
B55	0.204	20.40
Mean		26.14

B*=bread

Table (3-5) X-Ray fluorescence results of flour samples

Sample No	Br%	Br(ppm)
F11*	0.184	18.40
F22	0.138	13.80
F33	0.104	10.40
F44	0.170	17.00
F55	0.102	10.20
Mean		13.96

F*=flour

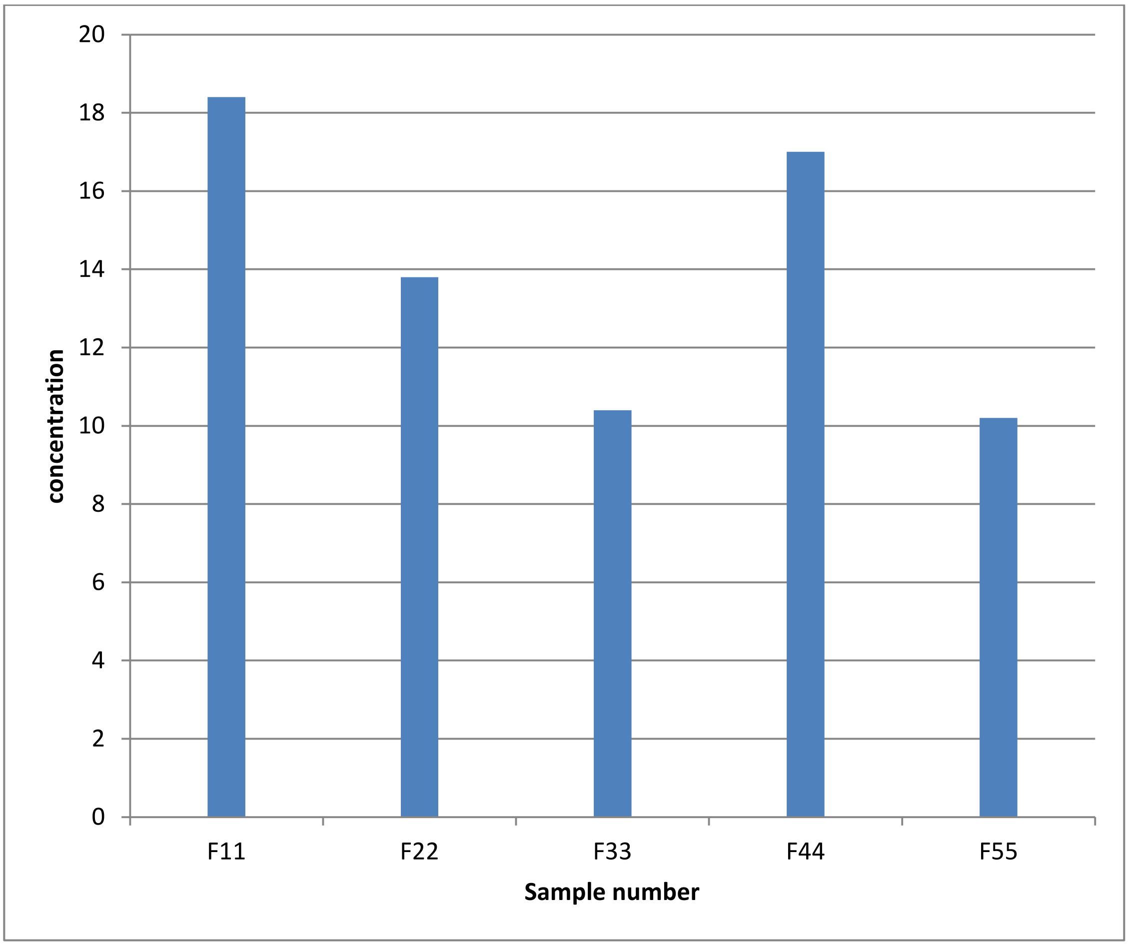

Figure (3-4) X-Ray fluorescence results of flour samples

3.2 Discussion

Table (3-2) shows the amount of potassium bromate residue found in bread samples using UV-Vis spectrophotometer, the least quantity of potassium bromate residue was found to be 2.67 mg/kg in sample (No.5B) and the maximum content was 47.33 mg/kg in sample (No.9B), with mean value of 25.44 mg/kg.

These results are similar to those reported by (Abdulla. N. S. et al, 2009) in which they analyzed 15 bread samples from Hawler city of Iraq by spectrophotometric method and found potassium bromate in all the bread samples in the range of 11.09 – 67.45 ppm.

Table (3-3) shows the amount of potassium bromate residue found in flour samples using UV-Vis spectrophotometer, the least quantity of potassium bromate residue was found to be 1.33 mg/kg in sample (No.5F) and the maximum quantity was 12.67 mg/kg in sample (No.4F), with a mean value of 6.4 mg/kg.

The maximum amount of potassium bromate allowed in bread by the FDA is (0.02 ppm), (10ppm) by Japan and (50ppm) by China (Ekop et al., 2008). All bread samples were higher than the levels permitted by FDA, but five samples were lower than the levels permitted by Japan. All samples were lower than the level permitted by China.

Table (3-4) shows the amount of potassium bromate residue found in bread samples using X-Ray fluorescence, the least quantity of potassium bromate residue was found to be 20 mg/kg in sample (No.B44) and the maximum quantity was 33.3 mg/kg in sample (No.B11), with a mean value of 26.14 mg/kg.

These results are similar to those reported by (Nehal, F. A. Mohammed, et al, 2015) in which they analyzed 9 bread samples from

(Khartoum –Sudan) using x-ray fluorescence spectrophotometer and found potassium bromate in all the bread samples in the range of 4.15 – 90.98 ppm. The Mean level of bromide value was found to be 15.6444 ppm in dry bread samples.

Table (3-5) shows the amount of potassium bromate residue found in flour samples using X-Ray fluorescence, the least quantity of potassium bromate residue was found to be 10.2 mg/kg in sample (No.F55) and the maximum quantity was 18.4 mg/kg in sample (No.F11), with a mean value of 13.96 mg/kg.

X-ray fluorescence analysis showed bromide content in the raw flour sample. This may need more investigations to determine if grains already contain bromide ions as a natural material or not. This may also be a result of instrumental error, in such case the bromate content would be less than the recorded values

Conclusion

The results of this study show that, all the analyzed bread samples contain potassium bromate above the accepted limit. It was found that (UV-Vis) spectrophotometer and XRF methods were very effective for the determination of bromate in food.

By the use of XRF, bromate ion can be determined directly without using complicated procedures, and still the sensitivity of determination is higher than that of conventional methods. This study also shows the simplicity of spectrophotometer and it's importance in routine checks by the regulatory authorities.

The mean concentration values of bromate residue determined using UV-Vis spectrophotometer and XRF methods were almost nearly the same (25.44 &26.14mg/kg) respectively.

Recommendations

Millers and bakers should be encouraged to comply with set standards of Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) guidelines acceptable worldwide, therefore, there is a need for continuous surveillance and enforcement of the ban on use of potassium bromate in baking industry in this study area by the governmental authorities. Therefore it may be suggested that alternative bread improvers such as ascorbic acid should replace potassium bromate which is currently being used by some bakeries (Ayo et al., 2002).

More research on potassium bromate in bread and flour should be performed.

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