**AN INVESTIGATION INTO THE HEALTH DANGER OF POTASSIUM BROMATE CONTENT IN BREAD SAMPLES (A CASE STUDY OF ENUGU METROPOLIS)**

**ABSTRACT**

This work was carried out in the eastern part of Nigeria to investigate the presence of residual potassium bromate in bread and the health implications. 25 different brands of bread were sampled from Enugu metropolis. The samples were analyzed using a previously reported method (David 1976). A freshly prepared 1% potassium iodide in 0.1N hydrochloric acid was used to develop the colour. The result revealed that all 25 bread samples contained residual potassium bromate. Sample A, C, R, S, T, W and X did not show any visible colour change when treated with potassium iodide. Sample C and X; 26mg/kg has the lowest concentration of residual potassium bromate. Sample B; 146mg/kg has the highest quantity. Sample N and Y; 108mg/kg also recorded quantities above 100mg/kg. Potassium bromate usually used as a bread improver is a carcinogen. All bread samples are therefore considered unsafe for human consumption.

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**CHAPTER ONE**

1. **INTRODUCTION**

Bread is an important source of food prepared by cooking dough of flour and water, and possibly more ingredients. Dough is usually baked but in some cuisines, breads are steamed, fried or baked on an unoiled skillet. It may be leavened or unleavened salt, fat and leavening agents such as yeast and baking soda are common ingredients. Though bread usually contains several ingredients that would help improve the quality of the bread. Some of the basic identified ingredients apart from flour include: table salt, sugars, flavours and at least a floor improver such as potassium bromate (Vicki, 1997). Other ingredients may also include milk, egg, spice, fruit (such as raisins), vegetables (such as onions), nuts (such as walnuts) or seeds (such as poppy-seeds). The ever-growing popularity of bread may be connected with its convenience, high acceptability, high energy content and low level of blood cholesterol associated with its consumption (Gaman and Sherington, 1981). Water and flour are the major component in a bread recipe. They affect the bread texture and crumb properties.

 The major challenge in both flour milling industries and bakeries is the baking quality of flour, which is determined by the capacity of the dough prepared from it to retain gas. As a result of wide variation in the composition of flour, various treatments and supplements conditioning agents (flour/bread improver) are added for strength during mixing, extensibility for molding and also to increase loaf volume and texture.

Over the years, several improvers have been used but studies have shown some to be deleterious to health, thereby necessitating their ban. The use of potassium bromate has been a common choice among flour miller and bakers throughout the world because it is cheap and probably the most efficient oxidizing agent. It acts as a slow oxidizing agent throughout the fermentation proofing and baking process affecting the structure and the rheological properties of the dough. As a result, many bakeries use potassium bromate as an additive to assist in the raising process and to produce a texture in the finished product that is appealing to the public (Sherington, 1981).

**1.2 POTASSIUM BROMATE**

Potassium bromate is a flour improver that acts as a maturing agent. It acts principally in the late dough stage giving strength to the dough during the late proofing and early baking (Kurokawo *et al.,* 1990). Potassium bromate takes the form of white crystals or powder. Potassium bromate has been used as a dough conditional for the past 60 years. According to USDA, it improves dough processing properties, internal crumb quality and low volume in concentration from a few to 75ppm, the highest concentration permitted by law. The mechanism by which bromate acts in dough is complex and not well understood (Destefanis, 1992).

 In early 1990’s, the World Health Organization (WHO) discovered that potassium bromate if consumed has the capacity to cause such diseases as cancer, kidney failure and several other related diseases. The adverse effect of potassium bromate on health and its health effects are divided into two categories. The first category deals with effects related to non-cancer effect. This includes its effect on the nutritional quality of bread. It degrades vitamin A2, B1, B2 and niacin which are the main vitamins available in bread (IARC, 1999).

Studies (IARC, 1999) have shown significant difference in essential fatty acid content of flour treated with bromate or in bread made from flour containing bromate.

 In humans, potassium bromate can cause cough and sore throat when inhaled (Atkins, 1993). Abdominal pain, diarrhea, nausea, vomiting, kidney, are some of the other non-cancer health problems associated with ingestion of potassium bromate (Atkins, 1993). In the secondary category, numerous studies have revealed the potential of potassium bromate to cause cancer in experimental animals and in humans (CSPI, 1999); Watson, 2000). Using bromate as bread improver has been banned (Ekop *et al.,* 2008). Also, in Nigeria, the bromate use in bread making was banned in 1993 (Obot *et al.,* 2008). However, some bread makers have continued to include potassium bromate in their bread.

 Furthermore, a preliminary survey revealed that some other materials employed in bread making (e.g. water) and the environments where these bakeries are located are not free from contamination by heavy metals such as lead.

**1.3 JUSTIFICATION**

 Bread is one of the oldest, stable and most reliable source of food. Its consumption is very high in the eastern part of Nigeria where most of the bread makers has resorted to using potassium bromate as a maturing agent for flour and as a dough conditioner in order to improve the quality of the dough and allowing higher rising. Potassium bromate has been considered a category 2B (possibly carcinogenic to humans) carcinogenic by the International Agency for Research on cancer (IARC). In addition, it has negative effect on the nutritional quality of bread. It degrades vitamins A2, B1, B2, E and niacin in bread (IARC 1999).

 This study confirms the presence of residual amount of potassium bromate in different brands of bread samples in Enugu metropolis.

**1.4 AIMS AND OBJECTIVES**

To determine the presence of potassium bromate in different brands of bread sampled from Enugu metropolis.

To determine the health implications of potassium bromate in different brands of bread sampled from Enugu metropolis.

**CHAPTER TWO**

1. **LITERATURE REVIEW**

In the mid-1970’s, the close correlation between mutagenicity and carcinogenicity of many chemicals became striking to those engaged in studies on carcinogenesis.

 Accordingly, a cooperative program was designed to evaluate the predictability of mutagenicity tests for carcinogenicity. This program commenced in 1974 under the auspices of the Ministry of Health and Welfare of Japan. To date, 85 bioassays using rats and or mice have been conducted on 51 chemicals, including medical drugs, pesticides, and food additives. Potassium bromate was selected as one of the chemicals for carcinogenicity testing because of its positive mutagenicity and widespread use as a food additive. It is used mainly in the maturation of flour because of its oxidizing properties. This present evaluation however, is to determine the presence of residual potassium bromated in finished product, specifically, bread.

* 1. **CHEMISTRY AND PRODUCTION OF POTASSIUM BROMATE**

 O

 Br

O O K+

Potassium bromate (KBrO3, CAS No. 7758-01-02, molecular weight 167.00g/mol; density, 3.27g/cm3) exists as white crystals, crystalline powder, or granules. It is highly soluble in ethanol, and almost insoluble in acetone; it is very stable when dissolved in water at room temperature. KBrO3 decomposes at temperature above 370oC (melting point: 350oC), with the evolution of oxygen and toxic fumes.

Potassium bromate (KBrO3) is produced by passing bromine into a solution of potassium hydroxide. An industrial electrolytic process is used for large scale production. Alternatively it can be created as a by – product of potassium bromide production by absorption of bromine from ocean water into potassium carbonate.

**2.2 USES OF POTASSIUM BROMATE IN BREAD**

 Potassium bromate (KBrO3) is typically used as a flour improver (E-number E924), strengthening the dough and allowing for higher rising. It is an oxidizing agent and under the right condition will be completely used up in the baking process. However, if too much is added or if the bread is not baked long enough or not at a high enough temperature then a residual amount will remain, which may be harmful if consumed. Bromate is considered a category 2B (possibly carcinogenic to humans) carcinogen by the International Agency for Research on Cancer (IARC).

**2.3 TOXICITY AND CARCINOGENICITY OF POTASSIUM BROMATE (KBrO3)**

 Potassium bromate (KBrO3) is an oxidizing agency that has been used as a food additive, mainly in the bread making process. Although adverse effects are not evidence in animals fed bread – based diets made from flour treated with KBrO3, the agent is carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally. It has been demonstrated that KBrO3 reduces renal cell tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid. In addition, experiments aimed at dedicating the mode of carcinogenic action have revealed that KBrO3 is a complete carcinogen, possessing both initiating and promoting activities for rat renal tumorigenesis. However, the potential seems to be weak in mice and hamsters. In contrast to its weak mutagenic activity in microbial assays, KBrO3 showed relatively strong potential inducing chromosome aberrations both in vitro and in vivo.

 Glutathione and cysteine degrade KBrO3 in vitro; in turn the KBrO3 has inhibitory effects on reducing lipid peroxidation in the rat kidney. Active oxygen radicals generated from KBrO3 were implicated in its toxic and carcinogenic effects, especially because KBrO3 produced 8 – hydroxydeoxy-guanosine in the rat kidney (Y. kurokawa, 1982).

 According to a research work by O. B. Oloyede and T. O. Sunmonu; Food and chemical toxicity, 2009. Consumption of bread samples containing KBrO3 portend serious damaging effects on the liver and kidney cells as evidenced by reduced activities of AST, ALT and ALP in the affected tissues. Consumption of such bread samples may result in liver and kidney damage and as such should be avoided.

 This was a conclusion deduced after an experiment with albino rats sourced from the Animal Holding Unit of the Department of Biochemistry University of Ilorin, Ilorin Nigeria. There was a pronounced reduction in the ALP activities of the kidney relative to the liver enzyme activity of the infected rats, and this may be attributed to the fact that the liver has a major role of detoxification and hence capable of handling toxic compounds such as potassium bromate.

The transaminases (AST and ALT) are well – known enzymes used as biomarkers to predict possible toxicity (Rahman et al, 2001). Generally, damage to liver cells will result in elevation of both these transaminases in the serum (Wolf et al, 1972). In the present study, the observed decrease in the activities of liver AST and ALT followed by the concomitant increase in serum AST and ALT activities suggest that there may be a leakage of these enzymes from the liver to the serum (Hanley *et al.,* 1986), Abdel Tawwab *et al.,* (2001) and Mousa and Khattab (2003) in separate studies ascribed the reduction in transaminases activity to liver necrosis caused by the toxicants and a possible damage to the hepatocytes. The reduction in the activities of the ALT and AST in the liver may be due to the interference with protein metabolism in the cells or inhibition of the enzyme (Karmer et al, 1995). There may also be leakage of the enzymes from the liver into the serum accounting in the serum.

All these are evidence of possible damage to the liver of rat caused by the potassium bromate in bread samples.

**2.4 BRIEF HISTORY OF BREAD**

 Bread is one of the oldest prepared foods. Evidence from 30,000 years ago in Europe revealed starch residue on rocks used for pounding plants (Diakonov, I. M. 1999). It is possible that during this time, starch extract from the roots of plants, such as cattails and ferns, was spread on a flat rock placed over a fire and cooked into a primitive form of flatbread. Around 10,000BC, with the dawn of the Neolithic age and the spread of agriculture, grains became the mainstay of making bread. Yeast spores are ubiquitous, including the surface of cereal grains, so any dough left to rest will become naturally leavened (McGee, Harold, 2004).

 There were multiple sources of leavening available for early bread. Airborne yeasts could be harnessed by leaving uncooked dough exposed to air for some time before cooking. Pliny the Elder reported that the Gauls and Iberians used for foam skimmed from bear to produce “a lighter kind of bread than other people”.

 In 1961, the Chorleywood bread process was developed which used the intense mechanical working of dough to dramatically reduce the fermentation period and the time taken to produce a loaf. The process, whose high-energy mixing process allows for the use of lower protein grains, is now widely used around the world in large factories. As a result, bread can be produced very quickly and at low cost to the manufacturer and consumer (Chorleywood, 1961).

 However, there has been some criticism of the effect on nutritional value (Chorleywood, 1961). Doughs are usually baked, but in some cuisnes breads are streamed (e.g. Mantou), fried (e.g. puri), or baked on an unoiled frying pan (e.g. tortillas). It may be leavened or unleavened (e.g. Matzo). Salt, fat and leavening agents such as milk, egg, sugar, spice, fruit (such as raisins), vegetables (such as poppy).

 The soft inner part of bread is known to bakers and other culinary professionals as the crumb, which is not to be confused with small bits of bread that often fall off, called crumbs. The outer portion of the bread is called the crust. The crumbs texture is greatly determined by the quality of the pores in the bread.

**2.5 FUNCTIONAL INGREDIENTS IN BREAD PRODUCTION**

 Quality control of your bread production should start with an understanding of what the ingredient do. Using good technical skills, the baker can manipulate the inconsistencies of ingredients to produce a uniform, consistent product. When discussing other ingredients, usage levels are based in comparison to flour. This percentage is called the “Banker’s percent”. When using Baker’s percent, the total flour will always add up to 100%.

**2.5.1 FLOUR**

 Flour is a product made from grain that has been ground to powdery consistency. Flour provided the primary structure to the final baked bread. While wheat flour is most commonly used for breads, flours made from rye, barley, maize, and other grains are also commonly available. Each of these grains provides the starch and protein needed to form bread.

 The protein content of the flour is the best indicator of the quality of the bread dough and the finished bread. While bread can be made from all purpose wheat flour, especially bread flour containing more protein (12 – 14%), is recommended for high quality bread. If one uses flour with a lower protein content (9 – 11%) to produce bread, a shorter mixing time will be required to produce or develop gluten strength properly. An extended mixing time leads to produce or develop gluten strength properly. An extended mixing time leads to oxidation of the dough, which gives the finished product a whiter crumb, instead of the cream colour preferred by most artisan bakers (Jeffrey Hamelmon 2004). High gluten white flour will require more mix time than a white flour with a lower gluten content.

**2.5.2 LIQUIDS**

 Water or some other liquid, is used to form the flour into a paste or dough. The weight of liquid required varied between recipes, but a ratio of 3 parts liquid to 5 – parts flour is common for just breads (Laurie Ashton, 2009). Recipes that use steam as the primary leavening method may have a liquid content in excess of 1 part liquid to 1 part flour. Instead of water, other types of liquid, such as clarify products, fruit juices, or beer may be used; they contribute additional sweeteners, fats or leavening components as well as water.

**2.5.3 YEAST**

 Much bread is leavened by yeast. The yeast most commonly used for leavening bread is *saccharomyes* *cerevisiae*, the same species used for brewing alcoholic beverages. This yeasts ferments some of the carbohydrates in the flour, including any sugar, producing carbon dioxide. Commercially produced baker’s yeast is the most preferred for the leavening of dough. Baker’s yeast has the advantage of producing uniform, quick, and reliable results, because it is obtained from a pure culture. Many artisan bakers produce their own yeast by preparing a growth culture that they then use in the making of bread. When the culture is kept in the right conditions, it will continue to grow and provide leavening for many years.

 Both the baker’s yeast and the sourdough methods of baking bread follow the same pattern.

Sourdough: This is a type of bread produced by a long fermentation of dough using naturally occurring and lactobacilli. In comparison with breads made with cultivated yeast, it usually has a mildly sour taste because of the lactic acid produced by the lactobacilli.

**2.5.4 FATS OR SHORTENINGS**

 Fats, such as butter, vegetable oils, lard or that contained in eggs, affect the development of gluten in breads by coating and lubricating the individual strands of protein. They also help to hold the structure together. If too much fat is included in bread dough, the lubrication effect will cause the protein structures to divide. A fat content of approximately 3% by weight is the concentration that will produce the greatest leavening action (Young, Linda, 2007). In addition to their effects on leavening, fats also serve to tenderize breads and preserve freshness.

**2.5.5 BREAD IMPROVERS**

 Bread improvers and dough conditioners are often used in producing commercial breads to reduce to time needed for rising and to improve texture and volume. Chemical substances commonly used as bread improvers include ascorbic acid, hydrochloride, sodium metabisulfate, ammonium chloride, various phosphates, amylase and protease.

**2.5.6 SALT**

 Salt is one of the most common additives used in bread production. In addition to enhancing flavour and restricting yeast activity, salt affects the crumb and the overall texture by stabilizing and strengthening the gluten (Silverton Mancy 1996). Some artisan bakers are foregoing early addition of salt of the dough, and are waiting until after a 20-minute “rest”. This is known as an autolyse (Reinhart, Peter (2001) and is done with refined and whole – grain flours.

LEAVENNG: This is the process of adding gas to dough before or during baking to produce lighter, more easily chewed bread.

CHEMICAL LEAVENING: A simple technique for leavening bread is the use of gas-producing chemicals. There are two common methods. The first is to use baking powder or a self-rising flour that include baking powder. The second is to include an acidic ingredient such as buttermilk and add baking soda, the reaction of the acid with the soda produces gas.

Chemically, leavened bread is called quick breads and soda breads. This method is commonly used to make muffins, pancakes, Americans style biscuit and quick breads such as banana bread.

**2.6 PREVIOUSLY REPORTED WORK ON TOXICITY AND CARCINOGENICITY OF POTASSIUM BROMATE**

An evaluation conducted by International Agency for Research on Cancer (IARC), 1986. 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 esotheliomas 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*-nitrosoethylhydroxyethylamine of kidney tumours and dysplastic foci (IARC, 1986).

Kurokawa in 1986 studied the causes of cancer in experimental animals and concluded that potassium bromate is a carcinogen capable of causing renal tubular tumours in rats and therefore toxic to humans. Groups of 20 or 24 male Fischer 344 rats, seven weeks of age, were given potassium bromate (food-additive grade) in their drinking-water at concentrations of 0, 15, 30, 60, 125, 250 or 500 mg/L (ppm) for 104 weeks. When water intake and body weight were accounted for, the intakes represented doses of 0, 0.9, 1.7, 3.3, 7.3, 16 and 43 mg/kg per day. Because the group receiving 500 mg/L had lower body weights and higher water consumption than other groups, their total intake of potassium bromate was approximately three times greater than that of the group given 250 ppm. The survival of rats receiving 500 ppm was significantly shorter (*p* < 0.01) at 83 {12 weeks than that of controls at 100} 3.3 weeks, but the lengths of survival of controls and other treated groups were comparable. The incidences of renal tubular tumours (adenomas and denocarcinomas) were significantly increased at doses of 125, 250 and 500 ppm, and the incidences of tubules with a typical hyperplasia were significantly increased at 30 ppm and higher. The incidences of thyroid follicular tumours and mesotheliomas of the peritoneum were also increased at the high dose of 500 ppm (Kurokawa *et al*., 1986).

In a research work by DeAngelo 1998, KBrO3 was found to be mutagenic in the Ames test and to cause chromosomal aberrations in Chinese hamster fibroblasts. KBrO3 had no initiating activity in the rat kidney when given as a single dose followed by a promoter, neither initiated nor promoted skin tumors in a skin paint study, and did not induce renal tumor development after 1 or 4 weekly subcutaneous injections in neonatal rats and mice. Bromate is absorbed rapidly from the digestive track and excreted in the urine as bromate (30-35%) and bromide (-40%) within 2 hours after administration.

Bromate is thought to produce its toxic response through oxidative damage resulting from increased levels of lipid peroxide. Bromate forms oxygen radicals, which are known to damage DNA, as evidenced by increased 8-hydroxy deoxyguanine levels in response to oxidative damage. Bromate must undergo cellular metabolism to cause DNA damage because DNA damage did not occur directly in an *iii vitro* mixture of DNA and bromated. The mechanism of action for bromate-induced carcinogenesis may include lipid peroxidation, which generates oxygen radicals that induce DNA damage. The apparent species differences in the induction of renal cell tumors are correlated with the different levels of lipid peroxidation. The results of the present study agree with those of previous reports that KBrO3, is carcinogenic in the rat kidney, thyroid, and mesothelium; the present study also showed that KBrO3, is a renal carcinogen in the male mouse. KBrO3, was carcinogenic in rodents at concentrations in water as low as 0.02 g/L (20 ppm; 1.5 mgkg/day). The development of tumors from chronic exposure to KBrO3, is thought to be due to bromate metabolism with subsequent lipid peroxidation and generation of oxygen radicals that causes DNA damage.

In Nigeria, on March 4, 2003, bakers were ordered to end the use of potassium bromate in bread making banned since 1993 (Akunyili, 2003). The chemical is an additive that bakers add to bread dough, during the mixing process, to make it stronger and moldable. In trying to defend the use of the additive in making bread, Theresa Cogswell of the American Society of Bakery Engineers said it is used to help bread rise in the oven and to create a good texture in the finished product. The catch though, is that even with the benefit bakers seem to derive from the use of potassium bromate, there are concerns that it has deleterious effects on humans when consumed in certain quantities. Of course bread is the staple breakfast for many; in fact, the average person eats bread about five times a week. This means that the average person may just be consuming unsafe levels of potassium bromate. In light of this and cognizant of the fact that the additive "is termed a cancer threat"[CSPI Newsroom, July 19, 1999], concern about its use is right. The Director-General of NAFDAC, Dr. Dora Akunyili stated that toxicology studies show that potassium bromate degrades the essential vitamins in bread. She added that research shows that bread treated with bromate proved carcinogenic in oral administration in rats but that in humans, it reportedly brought about cough and sore throat on inhalation, abdominal pain, diarrhea, nausea, vomiting, kidney failure, hearing loss and redness in the eyes on ingestion [Daily Independent Online, March 17, 2003] Researchers became concerned about the additive when in a 1982 study; they discovered that Potassium Bromate brought about the development of various types of tumors on the lab animals like tumors of the thyroid and kidneys. This development caught the attention of the American Food and Drug Administration; as a result, in 1992 and again in 1998, the agency conducted rounds of tests on baked goods. The tests revealed that many baked goods, in the country, contained potassium bromate at levels considered unsafe for humans. In 1990, the United Kingdom banned the use of the additive in baking because of health concerns; four years later, in 1994, Canada followed suit. Surprisingly, it turned out that Nigeria was also following the findings that point to the health risks inherent in the consumption of goods baked with the additive because in 1994, NAFDAC banned its use for making bread in the country.

A research work by M.G. Abubakar on the analysis of bromate in bread to assess the response of bakers on the bromate ban proved that most bakers still use potassium bromate as bread improver. He concluded that all bread samples analyzed contained potassium bromate and are therefore unsafe for consumption because of its toxicological effects on human (WHO 1989). Fifteen different bread samples were randomly collected from various local bakeries located in Sokoto metropolis. Presence of potassium bromate was determined using Redox-titrimetric method (Vogel, 1961). This involved the titration of the bread sample solutions (digested and undigested) in mixture with potassium iodide (3g), 4N Hydrochloric acid (5mls) and iodine solution (2mls) with 0.1N Sodium thio-sulphate to a colourless end point. The result obtained from the bread analysis showed that a great number of bread makers in Sokoto metropolis still use potassium bromate as a bread improver. All the fifteen samples tested indicated the presence of high concentration of bromate. The results implied that consumers of marketed bread in Sokoto metropolis are still being exposed to this toxic substance not withstanding several existing legislations outlawing its use. ). In Nigeria, use of potassium bromate in flour milling and baking has been banned by NAFDAC since 1993 (Akunyili, 2004).

**Observations in Man:**

 A number of case studies of acute human intoxication with potassium bromate have been reported following accidental ingestion or attempted suicide. In autopsy cases, degeneration of kidney tubules and liver parenchymal cells, and acute myocarditis were the principal pathological changes observed (Paul, 1966; Stewart et al., 1969; Niwa et al., 1979; Norris, 1965; Quick et al., 1975).

In humans, potassium bromate causes cough and sore throat on

inhalation, abdominal pain, diarrhoea, nausea, vomiting, kidney failure, hearing loss as well as redness and pain in both eye and skin (Akunyili, 2005)

**CHAPTER THREE**

1. **MATERIALS AND METHODS**
	1. **MATERIALS**

**3.1.1 Equipments**

25 breads samples

Test tubes

Beakers

Spatula

Colorimeter

Pipette

Conical flask

Measuring cylinder

**3.1.2 REAGENTS**

Potassium bromate

Distilled water

Potassium iodide

Hydrochloric acid.

* 1. **METHODS**
		1. **SAMPLE COLLECTION AND PREPARATION**

Bread samples were purchased from open markets, bus stops and from bread vendors in Enugu Metropolis, Nigeria. A total of 25 different brands of breads were used in this study.

* + 1. **REAGENT PREPARATION**

0.1M potassium bromate (KBrO3) 1g of potassium crystal was weighed and dissolved in 1000ml of distilled water.

0.1N of hydrochloric acid

This was prepared by using the formular:

 Molarity X Molar Mass x 100

Specific gravity x percentage purity.

Where;

Mole = 0.1M

Molar mass = 36.465g/mol

Specific gravity = 118

% purity = 36%

**Standard Curve Preparation**

 Required concentration of pure potassium bromate for blanking were prepared in the range 0,50,100,150,200,500,600, 700, 800 and 1000. From the above value, six replicate determinations were used to calibrate the colorimetry curve. The original values were calculated by using the formular:

C0V0 = CRVR, V0 = CRVR  C0

Where C0 = original concentration (1000ml)

 V0 = original volume

 CR = Required Concentration (0-1000ml)

 VR = Required volume (50ml)

**TABLE 1: Bread Samples and Location**

|  |  |
| --- | --- |
| **Sample** | **Manufacture Location** |
| A | Trans-Ekulu G.R.A |
| B | Amechi Awkunanu |
| C | Asata |
| D | Presidential Road |
| E | Maryland Ugwuaji |
| F | Egbene Abakpa Nike |
| G | Amechi Akwunanu |
| H | Emene |
| I | Akwunanu |
| J | Emene |
| K | Ogui |
| L | Mayor Amechi |
| M | Coal Camp |
| N | Thinkers Corner |
| O | Rangers Avenue |
| P | Rangers Avenue |
| Q | New Heaven |
| R | New Heaven |
| S | New Layout Road |
| T | Trans-Ekulu |
| U | 9 mile Corner |
| V | Nike |
| W | New Layout Road |
| X | Ekulu Avenue |
| Y | Emene |

**3.3.1 QUANTITATIVE ANALYSIS OF POTASSIUM BROMATE IN BREAD**

 A 1.0g quantity was weighed out from the crumb part of each bread sample using an electronic weighing balance. This was transferred into different test tubes. 10ml of distilled water was added; the mixture was shaken and allowed to stand for 20 minutes at 28±100C. a 5.0ml volume was decanted from the test tube 5.0ml quantity of freshly prepared 1% potassium iodide solution in 0.1N hydrochloric acid was added. Colour change was observed from milky to light purple with variations in the degree of colour change. The absorbance of the sample was taken at 620nm in a colorimeter and converted to concentration using a calibration curve constructed for potassium bromate using the pure sample (David 1976).

 The pure sample for the calibration curve was prepared by weighing out 1.0g of potassium bromate using a weighing balance, and dissolved in 1000ml of distilled water. Different concentrations were made by solving for original volume (V0) using the formular V0 = CRVR with Required volume (VR). And original concentration (C0) constant, different values were obtained for the original volume (2.5, 5, 7.5, 10, 12.5, 25, 30, 35 40 and 50ml) with each corresponding to the varying required concentration (CR). They were all made up to 50ml with 2.5ml and 50ml having the lowest and highest concentration respectively.

 A 50ml quantity of freshly prepared 1g of potassium iodide solution in 0.1N HCl was added to each pure sample. The absorbance of the sample was taken at 620nm in a colorimeter. The result was used to plot a graph of absorbance against concentration. Hence, a calibration curve for potassium bromate was constructed using the concentration; 50, 150, 500, 600, 70 and 800.

**3.3.2 QUALITATIVE ANALYSIS OF POTASSIUM BROMATE IN BREAD**

 Small sample from each bread brand was measured out into different test tubes. Water was added to wet the samples. 0.5ml of 1% potassium iodide solution in 2M HCl was added. The test tubes were labeled, covered with foils and allowed to stand for a day. The appearances of black spots on the samples indicate the presence of potassium bromate in the bread samples.

**CHAPTER FOUR**

**4.0 Results and Discussion**

Potassium bromate complexed with potassium iodide gave up a purple colouration. The colour change ranged from very light purple to purple with increase in concentration. The degree of colour change correlates with the level of potassium bromate present (HHDEIFT, 1998). A result showing the colour identification of potassium bromate in the 25 bread samples is represented in the table 4.1. Seven of the bread samples (A, C, R, S, T, W, and X) did not show any visible colour change when treated with potassium iodide. It is they are free of potassium bromate or that potassium bromate was present in the samples in residual amount that could not be detected by the reagent. All the other samples indicated positive result for the presence of potassium bromate (David 1976).

Table 4.1 also shows the presence of black spot on the samples when treated with 0.5N potassium iodide solution in 2M of hydrochloric acid. Black spot was observed on the samples indicating the presence of potassium bromate. From the results represented in table 4.1 below, six of the bread samples (A, C, H, R, T, and X) did not show any visible black spot. This also implies that the above may be free of potassium bromate or that potassium bromate was present in the samples in residual amount that could not be detected by the reagent. All the other bread samples had black spot which indicates positive result for the presence of potassium bromate in varying degrees.

The quantitative amount of potassium bromate found in each bread sample was also represented in table 4.1. The absorbance of the sample was taken at 620nm in a colorimeter.

A pure potassium bromate sample was used to generate a calibration curve. The absorbance of the samples were then converted to concentration (ppm) using the calibration curve. The least quantity of potassium bromate detected was 0.007ug/g (26ppm) and the maximum quantity was 0.039ug/g (146ppm). This implies that none of the 25 bread brands marketed in Enugu Metropolis is free of potassium bromate and therefore not safe for human consumption (Alkins, 1993). There are basically two ways by which humans may get poisoned with potassium bromate, by ingestion when it is present in food such as bread and by inhalation. It is therefore, not safe for the bread consumer and the factory worker who works in a bakery where the substance is used as a bread improver.

Potassium bromate is a flour improver that acts as a maturing agent. According to USDA, potassium bromate improves dough processing properties and interval crumb quality. During the preparation of the dough, a network of protein molecules linked together by disulphide bond 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 is present as well, 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)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Samples**  | **Bread sample Colour reaction with KI** | **Black spot** | **Quantity of KBrO3 found** | **Quantity of KBrO3 (mg/kg)** |
| A | No visible colour |  | 0.009 | 34 |
| B | Dark purple | + | 0.039 | 146 |
| C | No visible colour |  | 0.007 | 26 |
| D | Light purple | + | 0.012 | 46 |
| E | Dark purple | + | 0.022 | 84 |
| F | Purple | + | 0.018 | 68 |
| G | Purple | + | 0.013 | 50 |
| H | Purple |  | 0.013 | 50 |
| I | Purple | + | 0.020 | 76 |
| J | Light purple | + | 0.012 | 46 |
| K | Light purple | + | 0.011 | 42 |
| L | Purple | + | 0.02 | 76 |
| M | Purple | + | 0.014 | 53 |
| N | Dark purple | + | 0.028 | 106 |
| O | Purple | + | 0.025 | 94 |
| P | Purple | + | 0.016 | 60 |
| Q | Light purple | + | 0.012 | 46 |
| R | No visible colour |  | 0.008 | 31 |
| S | No visible colour | + | 0.01 | 38 |
| T | No visible colour |  | 0.01 | 38 |
| U | Purple | + | 0.016 | 60 |
| V | Light purple | + | 0.013 | 50 |
| W | No visible colour | + | 0.010 | 38 |
| X | No visible colour |  | 0.007 | 26 |
| Y | Dark purple | + | 0.028 | 106 |

**TABLE 4.1: Quantitative and Qualitative Analysis of Potassium Bromate in Bread.**

**TABLE 4.2 Colometric Reading for Standard Curve**

The figures obtained below is use to plot a standard curve of absorbance against concentration of potassium bromate.

|  |  |
| --- | --- |
| **CONCENTRATION** | **ABSORBANCE** |
| Blank (0) | 0 |
| 50 | 0.01 |
| 150 | 0.04 |
| 500 | 0.14 |
| 600 | 0.16 |
| 700 | 0.18 |
| 800 | 0.21 |

**FIGURE 4.2: Graph of Absorbance against Concentration**

**Health risk assessment of potassium bromate**

Human dietary exposure or estimated daily intake (EDI) was used to assess risk levels of potassium bromate via dietary intake of bread in Enugu. The EDI values were used to determine the chronic hazard quotient (HQ) values. The hazard ratio (HR) values were used to predict carcinogenic risk.

**Estimated daily intake of potassium bromate**

The daily intake of potassium bromate depends on the potassium bromate concentration in bread, the daily bread consumption, and the average body weight of adults, which influences the tolerance of contaminants. Based on these assumptions, EDI was calculated as follows:

EDIingestion = C × D/BW

Where; EDI ingestion– estimated daily intake via oral route (mg/kg bw per day), C – average potassium bromate concentration in bread samples (mg), D – daily intake of bread (kg), and BW – average body weight per person (kg).

From the survey, bread is a traditional breakfast food, frequently eaten as snacks. The average bread consumption during each meal was 0.6 kg. The average body weight of adults was 70 kg, which is the default human body weight. The average concentration of potassium bromate (in mg) in all bread samples was used in computing the estimated daily intake (EDI) over a long-time consumption. The short-term (acute) exposure to potassium bromate was assessed since bread consumers may likely experienced symptoms of acute toxicity via oral exposure. The estimated short-term intake (ESTI in mg/kg BW per day) was calculated as follows;

ESTI Chighest × D/Bw

Where ESTI – estimated short-term intake (ESTI in mg/kg BW per day), Chighest– highest Potassium bromate concentration in bread samples (mg). D – Daily intake of bread (kg), and BW – average body weight per person (kg). The average of the highest concentrations of potassium bromate in the different bread samples was used to calculate the estimated short-term (acute) intake. During baking, some of the potassium bromate escape from the bread in the form of vapor. The estimated daily intake via inhalation was not calculated because the concentration of potassium bromate in the air during baking was not measured. Also, the reference concen- tration value (RFC) of potassium bromate via inhalation is unknown.

**Determination of non-carcinogenic dietary health risk**

The non-carcinogenic risk resulting from potassium bromate consumption of each bread type was determined using the non-hazard quotient (HQ) value. The values of the chronic hazard quotient (cHQ) were determined using the following formula;

cHQ= EDIingestion/ADI

Where cHQ – chronic hazard quotient through ingestion (no unit), EDI ingestion is the estimated daily intake through oral route (mg/ kg-day), and ADI – acceptable daily intake (in mg/kg per day). ADI is the estimated maximum daily intake of a toxicant over a lifetime that will cause no adverse effects at any stage in human life span. An acceptable daily intake (ADI) of 0.02 μg/g (0.02 mg/kg) po- tassium bromate in bead, recommended by the US Food and Drug Agency (FDA) was used to estimate the chronic risk of po- tassium bromate in various bread types. The short-term (acute) health risk (acute hazard quotient, aHQ) was computed as follows;

aHQ = ESTI/RfD

Where aHQ is the acute hazard quotient, ESTI is the estimated short-term intake (ESTI in mg/kg BW per day), and RfD is the reference dose of potassium bromate (mg/kg-day). RfD is an estimate of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The reference dose (RfD) of bromate considered to be without deleterious non-cancer effect is 0.004 mg/kg/day. Hazard quotient (HQ) < 1 indicates that the risk is acceptable, and HQ > 1 shows that the risk is unacceptable (has the potential to harm the human body). The hazard index (HI) is the sum of more than one hazard quotient for multiple chemical substances or multiple exposure pathways. Only one chemical substance (potassium bromate) and one exposure pathway (oral pathway) were considered, so the hazard index was not computed.

**Carcinogenic dietary risk**

Carcinogenic risks estimate the probability that an individual will contract cancer over a lifetime of exposure to potassium bromate in bread. The hazard ratio (HR) for carcinogenic risk was determined as follows;

HR = EDIingestion/CBC

Where EDI ingestion is the estimated daily intake via ingestion and CBC is the cancer benchmark concentration. The CBC for the carcinogenic effect of potassium bromate was determined from the risk of 1 in 1,000,000 resulting from a lifetime exposure. This value was determined as follow;

CBC=RL × BW/CR × OSF

Where RL is the maximum acceptable risk level (1 10—6), which is the probability of developing cancer over the lifetime due to potassium bromate exposure, BW is the average body weight (default bodyweight, 70 kg), CR is the food consumption rate (g/day) and OSF is the oral slope factor (mg/kg/d). This OSF value was obtained from the integrated risk information system (IRIS). The oral slope factor for potassium bromate is 7x10—1 per mg/kg-day. This value is the cancer potency estimate for kidney renal tubule tumors, mesotheliomas, and thyroid follicular cell tumors. Hazard ratio (HR) greater than one is indicative of potential risk to human health.

**Analysis**

In the current study, the acute and chronic hazard quotient (HQ) values of the various bread types were very high, indicating a possible health risk to bread consumers in Enugu. These high HQ values may be due to the high concentrations of potassium bromate in analyzed bread samples. For instance, simple bread, milk bread, and French bread with the highest concentrations of potassium bromate had the highest HQ values. The high HQ values of the various bread types in the current study may be the reason for the expression of symptoms such as diarrhea and abdominal pain experienced by some bakers after consuming bread. Other symptoms like painful eyes, cough, sore throat, nausea, and dizziness were probably due to exposure to potassium bromate in the vapor during baking.

**Estimated daily intake, estimated acute intake, acute and chronic hazard quotient of potassium bromate concentration in different bread types.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bread Type | Highest KBrO3Concentration (mg/kg) | Average KBrO3concentration (mg/kg) | Average daily bread consumption (kg/ day) | Body weight (BW, kg) | Estimated Daily intake of KBrO3 (mg/kg/day) | Estimated short- term intake (mg/ kg/day) | Acceptable daily dose (mg/kg/day) | Reference dose (mg/kg/day) | Chronic hazard | Acute hazard quotient (aHQ) |
| French | 9548.5 | 3524.62 | 0.6 | 70 | 31.83 | 81.87 | 0.02 | 0.004 | 1591.50 | 2,387,125 |
| breadMilk bread | 10148.5 | 3914 | 0.6 | 70 | 36.89 | 87.01 | 0.02 | 0.004 | 1844.89 | 2,537,125 |
| Simple | 9148.5 | 4889 | 0.6 | 70 | 49.19 | 63.01 | 0.02 | 0.004 | 2459.36 | 2,287,125 |
| bread |  |  |  |  |  |  |  |  |  |  |
| Wheat | 3098.5 | 3301.5 | 0.6 | 70 | 11.56 | 78.44 | 0.02 | 0.004 | 577.93 | 774,625 |
| breed |  |  |  |  |  |  |  |  |  |  |
| Local | 698.5 | 639 | 0.6 | 70 | 5.56 | 6.01 | 0.02 | 0.004 | 277.93 | 174,625 |
| bread |  |  |  |  |  |  |  |  |  |  |

**Estimated cancer hazard ratio (HR).**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Bread type** | **Maximum acceptable** | **Food consumption rate (CR, g/day)** | **Body weight (BW, kg)** | **Oral slope factor (mg/kg/d)** | **Cancer benchmark concentration (CBC)** | **Estimated Daily intake of KBrO3(mg/kg/day)** | **Hazard Ratio (HR)** |
| French bread | 0.000001 | 0.6 | 70 | 0.7 | 0.00017 | 31.83 | 190980.00 |
| Milk bread | 0.000001 | 0.6 | 70 | 0.7 | 0.00017 | 36.89 | 221387.10 |
| Simple bread | 0.000001 | 0.6 | 70 | 0.7 | 0.00017 | 49.19 | 295122.90 |
| Wheat breed | 0.000001 | 0.6 | 70 | 0.7 | 0.00017 | 11.56 | 69351.43 |
| Local bread | 0.000001 | 0.6 | 70 | 0.7 | 0.00017 | 5.56 | 33351.43 |

In the present study, the Hazard ratio (HR) of all the different bread types showed a possible carcinogenic risk to consumers. For instance, the hazard ratios for simple, milk, and French bread suggest that the chances of having cancer from an average daily con- sumption of either simple bread or milk bread, or French bread are approximately 290,000 in 1,000,000 or 220,000 in 1,000,000 or 190,000 in 1,000,000. In the bloodstream, potassium bromate occurs as highly reactive oxides and free radicals that may affect DNA and play a role in carcinogenesis. For instance, potassium bromate induces and promotes the development of renal cell tumors in rats . In Cameroon for example, kidney cancer is the third urogenital cancer, common among women, and renal cell carcinoma is the predominant histological type and Although these authors linked the incidence of kidney cancer to clinical factors like smoking, hypertension, and diabetes, such studies should also consider the toxicological effects of chemical contaminants on humans.

The current study was limited only to the health risks of potassium bromate via the dietary intake of bread because the researchers never had the resources and equipment to measure the concentrations of potassium in vapor during baking. Also, the reference concentration value used for computing the estimated daily intake of potassium bromate via inhalation is unknown.

**CHAPTER FIVE**

**Conclusion and Recommendation**

**Conclusion**

The result of this study shows that all the 25 bread samples analyzed contained potassium bromate which is harmful to human if consumed. Bakers are therefore strongly advised against the use of potassium bromate as bread improver for the safety of the consumers and the factory workers. This study also underscores the importance of routine checks by the regulatory authorities in order to ensure that bakers always comply with rules and regulations and thereby safe guard the life of unsuspecting Nigerians.

**Recommendation**

 Potassium bromate (KBrO3) is an oxidizing agent that has been used as a food additive, mainly in the bread-making process. Potassium bromate if consumed has the capacity to cause such diseases as cancer, kidney failure and several other related diseases. It also have negative effect on the nutritional quality of bread; it degrades vitamins A2, B1, B2, E and niacin, which are the main vitamins available in bread (IARC, 1999).

 Bread makers and Bakers are therefore strongly advised against the use of potassium bromate as bread improver. They should source alternative bread improvers such as yeast, potassium iodate (KIO3), Ascorbic acid etc..

In a study by Ayo *et al.,* (2002), Ascorbic acid compared favourably with potassium bromate in improving the loaf volume of bread. On an equivalent cost basis, ascorbic acid can be considered a more effective improver even though bromate can be considered a higher loaf volume on equivalent weight basis.

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