QUALITATIVE DETERMINATION OF POTASSIUM BROMATE IN SOME BREAD SAMPLES CONSUMED WITHIN ILORIN METROPOLIS

BY

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CERTIFICATION

This is to certify that, this project work was carried out and reported by BADMUS Faisat Oyindamola, ND/23/SLT/FT/0003, student of Department of Science Laboratory Technology, Institute of Applied Sciences (IAS) and has been read and approved as meeting the requirement for the award of National Diploma (ND)

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DEDICATION

This project work is dedicated to Almighty God for been there for me throughout the journey.

ACKNOWLEDGEMENT

My profound gratitude goes to Almighty God for the privilege given to me to complete this project work; He has been helping me from the beginning till the end of the program.

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My sincere appreciation goes to my beloved for being our back bone and supporter, without them I have no power to be here today. I sincerely thank them for been there for me in time of needs, encouragement, moral support, spiritually and financially. May God enrich their purse and grant them long life and prosperity to reap the fruits of their labour.

My profound gratitude also goes to my beloved family member, siblings, friends and course mates who have been supporting me in all ways to make this program successful. May the lord reward you all abundantly and meet you at the point of your needs (amen).

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ABSTRACT

In order to determine the compliance level of bakeries in Ilorin metropolis with national and international standards, bread samples from five (5) of these bakeries were analyzed for levels of potassium bromate, lead (Pb) and iron (Fe) in the samples using appropriate spectrophotometric techniques. From the results, the concentrations of potassium bromate ranged between 0.12 ±0.08 mg/kg and 7.28 ±2.14mg/kg, indicating that all the bread samples had bromate levels higher than the permissible limit (0.02mg/kg) set by FDA and (0.025mg/kg) set by NAFDAC. The concentration ranges of Pb and Fe in the samples were 0.23 ±0.10 mg/kg - 5.15 ±1.2mg/kg and 0.45 ±0.10 mg/kg - 6.40 ±2.20mg/kg respectively. 60% of the bakeries recorded higher Pb levels than the permissible limit while 50% of them recorded higher Fe levels than the permissible limit. It is thus deduced that some bakeries from the present study are operating at sub - standard levels. A significant difference was found in the potassium bromate levels of bread samples from different bakeries ($P<0.05$). It is therefore concluded that, exigent measure is required by the regulatory bodies to ensure that there is strict compliance to the ban of the use of potassium bromate as bread additive.

CHAPTER ONE

1.1 INTRODUCTION

Bread is a popular food consumed widely amongst all socioeconomic sets in the world (Alli *et al.,* 2013). Bread is a formulation of different ingredients such as wheat flour, sugar, salt, water, improvers and preservatives (Magomya *et al.,* 2013), via different processes comprising milling, mixing, fermenting, molding and baking (Emeje *et al.,* 2010). Bread is a good source of nutrients such as proteins, vitamins (Thiamin-B1, Niacin-B3, Folic acid-B9, Vitamin E and to some extent vitamin A, fibre and complex carbohydrate. It has a low level of fat and cholesterol (Alli *et al.,* 2013). Similarly, bread contains significant amounts of mineral elements such as calcium, magnesium, phosphorous and potassium but others like iodine, iron and sodium are present in minute amounts (Shanmugavel *et al.,* 2019).

Potassium bromate ($KBrO_3$) is a good and well-known bread improver that has been used for many years by different bakers around the world (Nakamura *et al.,* 2006). The use of this compound has been completely banned by different countries across the globe due to its deleterious health effects (Magomya *et al.,* 2013). In addition to bread consumers, bakery workers are also considered to be exposed to this compound via inhalation because of its hepatotoxic and nephrotoxic effects (Oloyede and Sunmonu, 2009). The joint committee of Food and Agricultural Organization (FAO) and the World Health Organization (WHO) has completely banned the use of potassium bromate in bread, due to its long-term toxicity and carcinogenicity (FAO/WHO, 1999). Potassium bromate is a potential class II carcinogen for humans (IARC, 1999) and has also proved to cause severe toxic effects to critical human organs such as kidney, liver, and brain (Ahmad *et al.,* 2015; Ben Saad *et al.,* 2016a&b).

In Nigeria, despite the ban on the use of the compound and the high burden of fatalities from chronic diseases such as chronic kidney disease (CKD), chronic liver disease (CLD), cardiovascular disease (CVD), and other cancer-related death, it was discovered that 92% of bread samples in the country contained potassium bromate (Emeje *et al.,* 2010). Seven (7) different population-based cross-sectional studies alone reported the incidence of CKD in Nigeria to be within 2.5% to 24.3% (Chukwuonye *et al.,* 2018). Both CKD and CLD have enormous financial burdens on the families of subjects (Ulasi and Ijoma, 2010; Chukwuonye and Oviasu, 2012). Therefore, it is believed that a good knowledge of the health consequences associated with the use of potassium bromate in food products, particularly bread and other confectionaries, will drastically reduce the incidence of these chronic diseases. It is considered that maintaining good health as well as prevention of diseases is an essential tool for consumers of food, particularly breads and other flour-based products, thus the need for healthier bread products free from potassium bromate or any other banned substances.

Numerous studies conducted after the ban reported high levels of potassium bromate in bread products produced and consumed in Nigeria (Alli *et al.,* 2013; Emeje *et al.,* 2015; Dada *et al.,* 2017; Nosa *et al.,* 2018; Uduak, 2019; Uwah and Ikwebe, 2020; Abduljalil *et al.,* 2021; Dagari *et al.,* 2022; Lateefat *et al.,* 2022). Consequently, this review evaluates different studies conducted in the country to ascertain the prevalent of use of Potassium bromate and therefore safety of the bread products and to also determine the bakers' compliance with the specified regulations. Furthermore, this paper assesses the possible reasons for the use of potassium bromate by the Nigerian bread bakers (intentional or unintentional?) and also addresses what regulatory bodies need to do to improve the bakers' compliance with a view to considerably decreasing or abstaining from the use of KBrO3 as bread improver.

1.2 Statement of Problem

Potassium bromate, ($KBrO_3$) is an oxidizing agent commonly used by bakers to enhance bread quality. It causes flour maturation and strengthens the gluten network thereby improving gas retention and product volume. $KBrO_3$ acts as a maturing agent and dough conditioner by oxidizing the sulfhydryl groups of the gluten protein in flour into disulphide bridges making it less extensible and more elastic; this makes the dough viscoelastic such that it can retain the carbon dioxide gas produced by the yeast. The overall effect of potassium bromate is improvement in bread texture and increase in loaf volume. In 1986, the international agency for research on cancer classified potassium bromate $KBrO_3$ as a category 2B carcinogen (possibly carcinogenic to humans) based on sufficient evidence that $KBrO_3$ induces cancer in experimental animals. This finding led to the ban of bromate in flour and bakery products by many countries of the world. The countries that banned the use of potassium bromate include; Argentina, Brazil, Canada, South Korea, UK, Australia, Peru and Uganda. The substance was also banned in Sri Lanka in 2001, Nigeria in 2004, China in 2005 and India in 2016. Countries like the U.S.A did not place a total ban on the use of $KBrO_3$ but rather set maximum limits for its use as food additive. The maximum concentration of potassium bromates allowed in bread by the US Food and Drug Agency (FDA) is 0.02 μg/g (0.02 mg/kg).

Heavy metals are potential environmental contaminants with the capability of finding their way into the food we eat and causing human health problems. They are given special attention throughout the world due to their ubiquitous nature and toxic effects even at very low concentrations. Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported. The major route for humans' exposure to heavy metals is through the food pathway. Contamination of bread by heavy metals could arise from flour which may have been produced from contaminated raw materials. Water used for bread making could also be a source of heavy metal contamination. Studies revealed that the kind of baking fuel used for bread production could also be responsible for heavy metal contamination.

1.3 Justification

Due to the possible adverse effects of potassium bromate and heavy metals in humans, it is important to identify major sources of contamination so that the necessary steps could be taken to eliminate or minimize them. Since bread is consumed by the majority of Nigerians, it is absolutely necessary to assess whether the way bread is processed and sold on the open markets could lead contamination by heavy metals. Results from this study will hopefully provide the requisite information on food safety and how their contamination with toxic substances may be eliminated.

1.4 Aim and Objectives

The aim of this project is to analyse the quantitative and qualitative evaluation of potassium bromate in selected bread samples produced within Ilorin metropolis.

The specific objectives are:

• To determine the qualitative and quantitative determination of potassium bromide using spectrophotometry analysis.

To determine the concentration of heavy metals such as Cd, Pb, Fe, and Zn in selected bread sample sold at area in the Ilorin Metropolis.

• To compare the result of the analysis to NAFDAC

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Chemistry of Potassium Bromate ($KBrO_3$)

Potassium bromate is an IUPAC systematic name given to a chemical compound with the formula $KBrO_3$, a relative molecular mass of 167.01 and a density of 3.27g/cm^3 (Finar, 1973). $KBrO_3$ has a considerably high melting point of about 350ºC; and decomposes at about 370oC with emission of oxygen gas and toxic fume (Kurokawa *et al.,* 1990). Potassium bromate is a colourless and odourless crystal which is highly soluble in water; and slightly soluble in acetone, diethyl sulfoxide, ethanol, methanol and toluene (IARC, 1986).

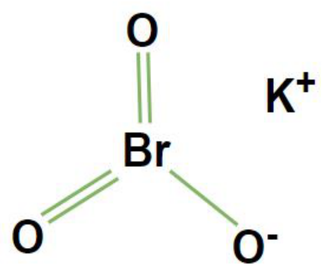

Fig 1: Chemical Structure of Potassium Bromate

Producing countries and Uses of Potassium Bromate ($KBrO_3$): In the last 27 years ago, countries such as Argentina, Brazil, China, Germany, India, Israel, Italy, Japan and Spain were the main producers and marketers of potassium bromate (Chemical Information Services, 1995). Potassium bromate ($KBrO_3$) is commonly used for the following purposes:

i. **As flour enhancing agent**: $KBrO_3$ is considered to be a strong oxidizing agent and one of the best and cheapest dough improvers, hence it is commonly used as a flour-enhancing agent in many countries across the globe for the past 90 years (Oloyede and Sunmonu, 2009; Emeje *et al.,* 2010; Shanmugavel *et al.,* 2019). The production and marketing of potassium bromate are geared towards its primary use as a maturing agent for flour and as a dough conditioner despite its detrimental health effects. Potassium bromate has a strong influence on the structure and rheological characteristics of dough because it causes flour maturation and strengthens the gluten network and also helps in the retention of gas and product volume (Ojeka *et al.,* 2006). During bread baking process, potassium bromate is almost completely converted to potassium bromide. Potassium bromate acts mainly in the late dough stage, thereby giving strength and elasticity to the dough during the baking process thus stimulating the rise of bread. These properties make potassium bromate widely used as a flour-enhancing agent in most of the baking industries despite its ban (Ojeka *et al.,* 2006).

ii. **As food additive**: $KBrO_3$ is also used as an additive in beer malting, cheese making, and as an ingredient in fish paste products (Ahmad and Mahmood, 2014).

iii. **Production of pharmaceuticals and cosmetics**: $KBrO_3$ is used in the production of pharmaceuticals and cosmetic products (skin bleaching agents) and also as a component of cold wave hair solutions (Gandikota and MacRitchie, 2005; Alberto and Charles, 2007; Oloyede and Sunmonu, 2009).

iv. **Laboratory reagent**: Besides its use during the fermentation and baking process, KBrO3 is also used as a table reagent and oxidizing agent in the laboratories (Gandikota and MacRitchie, 2005).

Likewise, $KBrO_3$ is a byproduct of water disinfection produced when waters containing bromide are ozonized (treated with ozone), thus frequently detected in tap and bottled water (Dongmei *et al.,* 2015; Altoom *et al.,* 2018). Liu and Mou (2004) stated that “bromate is one of the most prevalent disinfection by-products of surface water”. Ozonation is an effective and efficient means of disinfection due to its ability to clear microbes leading to significant decrease in the level of carcinogenic trihalomethanes (THMs). The Ozonation process introduces ozone in the water which can leads to hydrobromic acid formation with increased content of bromide which subsequently forms brominated organic by-products and bromate (Fiessinger *et al.,* 1985).

2.2 Detrimental health effects of potassium bromate

The undesirable and detrimental health effects of potassium bromate were observed and reported since the mid-1980s. Kurokawa *et al.,* (1990) reported that $KBrO_3$ poisoning cases in humans are common due to its widespread use and consumption in homes, though the occurrence of such poisoning varied with geographical trends. Most of the $KBrO_3$ poisoning cases in Western countries occurred by accidental ingestion, mostly among children, while in West African countries is more frequently ingested through different foods containing $KBrO_3$ (Kurokawa *et al.,* 1990). Due to this, precautionary measures were adopted by several countries in the world amongst them is its complete prohibition as a dough improver (Omotoso, 2021). Potassium bromate was reported to cause the following deleterious health effects:

i. Acute and sub-acute toxicity: Although, the toxic or lethal dose of potassium bromate in humans is not precisely known (Kurokawa *et al.,* 1990). The human lethal dose has been estimated to be 5 to 500mg/kg of body weight (Gradus *et al.,* 1984; Kuwahara *et al.,* 1984) while Watanabe *et al.,* (2001) reported human lethal oral doses of 154 to 385mg/kg of body weight. Also, Watanabe *et al.,* (2001) reported a serious poisoning at doses of 46 to 92mg/kg body weight. This lethal dose has caused serious symptoms for 15 months old child (Quick *et al.,* 1975). Data from two (2) potassium bromate poisoning cases which occurred in Japan, showed that 9 of the 24 adults died within 3-5 days after ingestion of 5-500mg/kg b.w of $KBrO_3$ (Kuwahara *et al.,* 1984). Also, a severe irreversible sensorineural hearing loss has been reported within 4-16 hours after ingestion of same dose in nearly all of the adults in the 2 cases. Studies conducted for decades by Gosselin *et al.,* (1976) revealed that ingestion of 57-133g of 2% solution of potassium bromate caused nausea and vomiting, usually with epigastric and/or abdominal pain; diarrhoea and haematemesis in children of 1.5-3 years of age. Therefore, in the acute stage of poisoning, the main symptoms include vomiting, diarrhoea and abdominal pain. Consequently, manifestations such as oliguria, anuria, deafness, thrombocytopenia, hypotension, vertigo, and depression of the central nervous system followed (Gosselin *et* *al.,* 1976). Additionally, in both children and adults there have been reported cases of acute renal failure from mild to severe anuric forms (Starek and Starek-Swiechowicz, 2016). Also, Quick *et al.,* (1975) and Warshaw *et al.,* (1985) reported a strong irritating action of $KBrO_3$ on gastric mucosa.

ii. Hepatotoxic, neurotoxic, nephrotoxic and mutagenic effects: Numerous studies shows that longterm exposure to small and high dosages of $KBrO_3$ caused severe damage to the liver tissue architecture through alteration of liver tissues, congestion of the central vein and sinusoidal dilatation as well as liver cells necrosis (Abuelgasim *et al.,* 2008; Akanji *et al.,* 2008; Dimkpa *et al.,* 2013; Olutoyin *et al.,* 2013; Oyewo *et al.,* 2013; Bayomy *et al.,* 2016; Akinola *et al.,* 2020; Hassan *et al.,* 2020). Similarly, $KBrO_3$ can cause vacuolation and sinusoidal dilatation of liver cells with concomitant reduction of antioxidant enzymes and enhancement of xanthine oxidase and lipid peroxidase activities (Khan *et al.,* 2003; Omer *et al.,* 2008). Toxicological studies conducted by Young *et al.,* (2001); Ahmad and Mahmood (2012); Saad *et al.,* (2017) revealed that $KBrO_3$ affect the neurobehavioral status of different animal models (rats, mice and guinea pigs). A similar study by Chuu *et al.,* (2000) showed that KBrO3 prompt injurious effect on auditory brainstem response while it caused oto-neurotoxicity mostly through the peripheral auditory nerve relatively than via the central brainstem intoxication. The nephrotoxic effect caused by $KBrO_3$ is considered to be due to its ability to initiate the production of reactive oxygen species (ROS), lipid peroxidation and 8-hydroxyguanosine modification in renal DNA (Sai *et al.,* 1992; Chipman *et al.,* 1998; Spassova *et al.,* 2015). Two (2) separate studies conducted in Saudi Arabia and Sultanate of Oman by Ajarem *et al.,* (2016) and Ali *et al.,* (2018) respectively revealed that the amount of ROS induced by $KBrO_3$ significantly surpasses the cellular antioxidant defense capacity, thus leading to manifest nephrotoxicity in humans and animals. The kidney is the main target organs of $KBrO_3$, therefore its toxic effect in humans ascend from acute toxic to renal failure, though the exact mechanism(s) by which $KBrO_3$ induce acute renal toxicity and means to overwhelm the antioxidant defense system its toxic effect using natural products are still unknown (Ben Saad *et al.,* 2016a; Abdel-Latif *et al.,* 2021). Furthermore, it has been proposed by Ballmaier and Epe (1995) that its carcinogenic effect is due to the formation of ROS induced by the compound. Also, Kraus *et al.,* (1995) hypothesized that $KBrO_3$ induces tumor formation through DNA strand breaks whereas Kaya and Topaktaş (2007) confirmed the hypothesis by their finding that the compound commonly triggers chromatid and chromosome breaks (chromosomal aberrations). Consequently, Yamaguchi *et al.,* (2008) concluded that due to positive results in the Ames test, and chromosome aberration and micronucleus test conducted, $KBrO_3$ has been classified as a genotoxic carcinogen. Several previous studies have proven that potassium bromate is a mutagenic and carcinogenic in different life forms (Abdelraheem and Kambal, 2007; Akintonwa *et al.,* 2010; Elhaddad *et al.,* 2020). The mutagenic effect of $KBrO_3$ is an indication that it has the ability to induce tumor formation through a genotoxic mode of action (Brock *et al.,* 1999). Consequently, the International Agency for Research on Cancer (IARC) categorized potassium bromate as a potential class II carcinogen for humans (IARC, 1999). Several studies have provided evidence that $KBrO_3$ in many consumer products poses mild to severe toxicity to critical organs in humans which include kidneys, liver and brain (Ahmad and Mahmood, 2012; Ahmad *et al.,* 2013; Ahmad *et al.,* 2015; Ben Saad *et al.,* 2016a&b). iii. Growth and Reproductive effects: Although there are scarce studies on the reproductive effect of $KBrO_3$, however, Elsheikh *et al.,* (2016) concluded that pre-pubertal exposure of male rats to $KBrO_3$ hinders growth, lowers testicular weight, alters testicular histology as well as impairs spermatogenesis, which predicts infertility or even sterility in the future.

The compound binds to the iodine receptors, thus reducing iodine uptake by the thyroid gland and consequently causing iodine deficiency which is the leading factor for growth retardation (Fisher and Bull, 2006). The iodine deficiency induced by $KBrO_3$ affects the biosynthesis of the thyroid hormones and; hence negatively affects the development and function of the gonads (Crockford, 2009). Likewise, Peltola *et al.,* (1992); Chlubek (2003) and Sahoo *et al.,* (2008) reported that the testis contains sufficient polyunsaturated fatty acids, but insufficient antioxidant defense molecules, thus the iodine deficiency caused by $KBrO_3$ can vulnerably expose the testis to oxidative stress. Additionally, $KBrO_3$ decreases the quality of oocyte due to the OS caused by it (Yamada *et al.,* 2018), and also resulted in the inhibition of mitochondrial spreading throughout the cytoplasm and abnormal mitochondrial aggregation (Nagai *et al.,* 2006; Udagawa *et al.,* 2014). The excess free radicals due to $KBrO_3$ can lead to alterations in the spermatogenic cycle, hypogonadism, reduction of sperm production, alterations of reproductive hormones and degeneration in seminiferous tubules (Khan and Ahmed, 2009). Large amounts of $KBrO_3$ decrease the number of oocytes at the stage of metaphase II and increase abnormal mitochondrial distribution (Yamada *et al.,* 2018).

2.3 Effect of Potassium Bromate on Humans (Children and Adults)

Consumption of potassium bromate has serious health complications on humans as children and adults. A report of children of 17 months to 6 years who presented with sedation, lethargy and depression of the central nervous system following an inadvertent exposure to about 20mg to 1g/kg b.w of $KBrO_3$ (Shanmugavel *et al.,* 2019). Thereafter, 18 out of the 31 subjects presented with irreversible deafness which occurred within 4 to 16 hours whereas kidney toxicity also occurred, a condition that subsequently led to 26 of them developing with kidney failure. A similar case was also reported in Belgium by De Vriese *et al.,* (1997) where a 17-year-old exhibited vomiting, severe abdominal pain and diarrhoea within 30 minutes after ingestion of cold wave neutralizer (containing 10% $KBrO_3$) in her attempt to commit suicide. A few hours after, the victim displayed further complications namely tinnitus and dizziness, with complete loss of hearing. Other complications such as intravascular haemolysis and increased oxidative stress in the renal tubules, leading to kidney failure were confirmed after laboratory examinations.

The consumption of potassium bromate affects not only children but also adults. The first human $KBrO_3$ poisoning case was reported in 1971 by Ohashi *et al.,* (1971). In another development, 13 individuals working with a cold wave neutralizer were poisoned with $KBrO_3$. Ohashi *et al.,* (1971) reported that the 13 victims manifested with acute renal failure and extended oliguria and subsequently 7 individuals amongst them died due to renal failure, thus resulting in a 54% mortality rate. Furthermore, Kurakowa *et al.,* (1990) reported a case of women (professional hairdressers) who consumed potassium bromate to commit suicide and all of them showed vomiting and diarrhea with abdominal pain, a few hours later oliguria, anuria, deafness, vertigo, hypotension, depression of central nervous system and thrombocytopenia followed. Similarly, Kumar and Pankaj (2012) reported that 9 bakery workers in India presented with abdominal pain, vomiting and diarrhea following potassium bromate poisoning. All the 9 workers were observed with severe gastritis, leading to hematemesis, and subsequently 24-48h after the intake, acute renal failure ensured. Severe vomiting, abdominal pain and diarrhea also developed in a 44-year-old man who ingested potassium bromate in an attempt to commit suicide and several hours after, the subject had auditory disturbance and renal failure (Hamada *et al.,* 1990). Human exposure to $KBrO_3$ is associated with ototoxicity, sore throat, nausea, abdominal pain, cough and nephrotoxicity (Uduak, 2019; Magomya *et al.,* 2020). Summarily, nausea, vomiting, abdominal pain and diarrhea are the characteristic acute symptoms that are displayed shortly after ingestion of $KBrO_3$ whereas acute renal failure changing from mild to severe anuric forms have been reported in both children and adults (IARC, 1999). Oliguria and death from renal failure whereas partial hearing loss and total deafness have also been observed in both children and adults (Quick *et al.,* 1975; Gradus *et al.,* 1984).

2.4 Effect of Potassium Bromate on the Environment

Presently no data is available on the bioaccumulation factor of $KBrO_3$ either in plants or animals. But Flury and Papritz (1993) have extensively discussed the bromate ecotoxicity where they reported that aquatic animals such as juveniles of various fish species, crustaceans and flatworms can significantly be affected with a $KBrO_3$ lethal concentration of 31 mg/l - 2258 mg/l. Therefore, from the result of this study scholars such as Hutchinson *et al.,* (1997) and Markert (1994) concluded that $KBrO_3$ has low to moderate potential for aquatic toxicity although it also affects sensitive organisms.

Furthermore, some industries release $KBrO_3$ into freshwater which is believed to substantially affect sensitive organisms such as fish, freshwater algae and invertebrates and estuarine and marine crustaceans (Shanmugavel *et al.,* 2019). Butler *et al.,* (2005); Versteegh *et al.,* (1993) also reported that $KBrO_3$ producing industries or industries that use the compound as a raw material contaminate the groundwater that is near them, thereby polluting the environment which will affect many animals including humans.

2.5 Effect of Potassium Bromate on the Nutritional Composition of the Bread

Several studies show that potassium bromate affect the nutritional composition of bread via its strong capacity to destroy vital available vitamins in the bread namely vitamins A1, B1, B2, E and niacin (Magomya *et al.,* 2020). The use of $KBrO_3$ as a bread improver in bread formulation has significant effects on the nutritional value of bread because it degrades essential vitamins (A2, thiamine, riboflavin and niacin) and decreases essential fatty acids (FAO/WHO, 1999; Airaodion *et al.,* 2019; Magomya *et al.,* 2020). Tanaka *et al.,* (1980) revealed that potassium bromate directly affects the starch content of the bread via improving the extent of gelatinization, viscosity and swelling of starch. It is widely reported that $KBrO_3$ remove sulfhydryl groups of gluten proteins in dough by oxidizing them into disulfide bonds (Bloksma and Bushuk, 1988). Additionally, Shanmugavel *et al.,* (2019) reported that maturating agents such as bromate modify the sulfhydryl groups of protein molecules present in the flour to disulfides in order to enhance the effects of the dough and bread. The presence of potassium bromate in the dough makes the protein less reactive, leading to greater residual protein extractability during the course of bread baking (Kurokawa *et al.,* 1990; Kawasaki *et al.,* 2002). Similarly, Panozzo *et al.,* (1994) discovered that addition of $KBrO_3$ affects the extractability of lipids, though not on the general composition of lipids. Potassium bromate does not modify the lipids content of the flour; thus, the compound has less effect on the lipids than proteins, but assists in the formation of a protein-lipid complex to enhance bread quality (Panozzo *et al.,* 1994).

2.6 Effects of Baking Condition on Potassium Bromate Content in Bread

Studies show that baking temperature has a significant effect on the quantity of potassium bromate in the bread. An experimental study by Abu-Obaid *et al.,* (2016) showed that potassium bromate in bread decomposes at a temperature range of 150ºC to 250ºC, unlike in distilled water where the compound decomposes at 350ºC to 400ºC, suggesting that the decomposition of $KBrO_3$ in bread at a low temperature may be due to the metal ions (Fe, Mg, Zn, Mn, Cu and Al) presence in the flour which serve as catalysts.

Another study by Nakamura *et al.,* (2006) which measured the effect of processing conditions on the $KBrO_3$ residue in the bread concluded that baking the bread at a temperature greater than 120oC for more than 30 minutes will not totally degrade the compound, but can moderately degrade it, thereby resulting in lower residual $KBrO_3$. Likewise, Congswell (1997) and Emeje *et al.,* (2010) believed that the baking process reduces the toxic $KBrO_3$ to potassium bromide (KBr) which is harmless. But in the event that an extreme quantity of the compound is used during bread formulation or if the bread is not properly baked (baked for a shorter period of time or not at higher temperature), then $KBrO_3$ can be detected in the bread which is toxic (Bushuk and Hlynka, 1960).

Nakamura *et al.,* (2006) also reported that a baking lid has a significant effect in reducing the $KBrO_3$ content in the bread than the baking temperature or time, or even changing bread formulation. This is so because the findings of their experiment revealed that no residue of potassium bromate was detected in the bread baked with a baking lid (Pullman-type bread), but it was detected in the open top-type bread (bread baked without a lid) (Nakamura *et al.,* 2006). Shanmugavel *et al.,* (2019) explained that their finding may be explained by the proposition that $KBrO_3$ in the upper section of the crust that rises out of the baking pan is related to a decline in the reactive efficiency due to drying of the crust or rapid thermal denaturation of the protein.

Moreover, Himata *et al.,* (1997) reported that the baking procedure and flour sample have a significant effect on the quantity of $KBrO_3$ in the bread. However, if a large amount of $KBrO_3$ is added and is not baked at high temperatures or for a longer time, then a residual amount of the compound will remain in the bread which can be harmful if ingested (Kurokawa *et al.,* 1990). This assertion has been corroborated by a study conducted more than 6 decades ago by Bushuk and Hlynkal (1960) who found 31ppm of $KBrO_3$ in the dough (after about 4 hours of fermentation) out of an initial 40ppm of the compound added. They further reported that the residual bromate content reduced to 21ppm after 5 minutes of baking which also disappeared after additional 10 minutes of baking. Additionally, Ronald (2006) stated that during the baking process, potassium bromate is being converted to bromide which is less harmful. In support of this assertion, Lee and Tkachuk (1960) reported that potassium bromate of 30mg/kg was converted to bromide in bread prepared from the flour after a bulk fermentation process. Several studies concluded that the baking process changes the chemical composition of $KBrO_3$ and makes it a harmless compound (bromide), but if a large amount of it is added and the bread is not baked at a longer time or at high temperature, then a residual amount of $KBrO_3$ will remain in the bread.

2.7 Studies conducted on the use of potassium bromate in bread products in Nigeria despite its ban

Nigeria is the most populous country in Africa and 6th in the world with an estimated population of 210,431,790 (Statista, 2022b). The country is divided into six (6) geopolitical zones with an estimated 400 ethnic groups and 450 languages (Okorie *et al.,* 2013). The 6 geopolitical zones are North Central, North East, North West, South East, South-South and South West.

Bread is one of the basic foods consumed widely among all ethnic and socioeconomic groups in Nigeria due to its affordability (Alli *et al.,* 2013); therefore, bakeries are sited all over the country to meet the demands of the populace. No exact or documented data is available either from government or nongovernmental organizations on the actual number of bakeries in the country. Available statistics show that the value of the Nigerian baked goods market amounted to almost N203 million as of 2021 (Statista, 2022b). In 2003, Nigeria's apex drug and food regulatory agency; the National Agency for Food and Drug Administration and Control (NAFDAC) banned the use of KBrO3 in bread due to its deleterious health effects (NAFDAC, 2003). Since then, several studies had been conducted across the 6 geopolitical zones including FCT to ascertain the safety of the products and to also determine the bakers' compliance with the specified Agency's regulations (particularly the ban). Despite the ban on the use of $KBrO_3$, many studies conducted across the nation detected a high level of the compound in the bread products produced and consumed in the country (Emeje *et al.,* 2015; Dada *et al.,* 2016; Uduak, 2019; Uwah and Ikwebe, 2020). These studies are summarized and presented in Table 1 based on the six (6) geopolitical zones of the country and Federal Capital Territory (FCT). Our findings showed that numerous studies on potassium bromate content in bread were conducted in twenty-five (25) states and FCT using different methods. These methods included spectrophotometry, Congo red oxidation, Redox titration, Mohr's methods, HPLC, AAS, crystal violet method, AOAC's method and the Iodometric titration method. The 25 states represent 69.44% of the total number of states in the country where studies are being carried out. A total of Forty-seven (47) different studies (majority quantitative) were reported across the country which were compiled and presented in Table 1. All the analysed studies were conducted within year 2006 (3 years after the ban) to 2022, which conceivably showed that Nigerian bread bakers are still using the compound despite the ban. In Rivers, four (4) independent studies were reported while in other 7 states it was reported by at least 3 different researchers in diverse locations of the states. In all the studies conducted only 2 studies (4.25%) have not reported the presence of potassium bromate in their samples. Similarly, forty-five (45) studies (95.74% of the total studies) conducted reported the presence of potassium bromate in the bread samples analysed. A unique study by Oyefuga *et al.,* (2012) showed the presence of potassium bromate in the entire 1,512 bread samples analysed. This is a unique among all the studies conducted due to the large sample size and long period of sampling and analysis. In the study, six (6) geographically distributed bakeries were selected in a particular local government area while 6 bread samples were randomly selected per bakery on daily basis for six weeks (42 days).

2.8 Studies on the potassium bromate content in bread ingredients

Several studies were conducted to evaluate the presence of potassium bromate in bread ingredients particularly wheat flour, yeast and brands of bread improvers. Our findings show that few studies were conducted on potassium bromate contents in the bread ingredients, despite the high level of $KBrO_3$ in bread products reported in many studies.

states	Number of samples analyzed	Conc. of KBrO3 in Flour Samples	Conc. of KBrO3 in bread Samples	Number of samples tested positive	authors
Akwa Ibom	4	0.11 - 0.27 mg/100g	0.11 - 0.38 mg/100g	All	Aletan and Okon (2018)
Anambra	12	0.17 - 0.87 mg/kg	0.27 – 3.78 mg/kg	All	Irogbeyi *et al.,* (2019)
Nasarawa	4	0.83 - 1.42 µg/g	0.50 - 8.40 µg/g	All	Johnson *et al.,* (2013)
Ogun	12	3.15 - 14.59 µg/g	8.67 - 58.28 µg/g	All	Abiodun *et al.,* (2015)
Sokoto	5	0.36 - 0.533 mg/kg	0.03 - 0.67mg/kg	All	Abduljalil *et al.,* (2021)
Taraba	4	2.94 - 6.86 µg/g	2.51 - 11.52µg/g	All	Magomya *et al.,* (2020)

The majority of the reported studies analyzed bread products only; however, few of them analyzed other raw materials. Studies by Ekop *et al.,* (2008); Abiodun *et al.,* (2015); Aletan and Okon (2018); Irogbeyi *et al.,* (2019); Magomya *et al.,* (2020) and Abduljalil *et al.,* (2021) are among the few that analyzed the level of potassium bromate in wheat flour and bread improvers. Their findings indicate the presence of potassium bromate in all wheat flour and bread improvers analyzed. These studies were conducted in five (5) states of the country and are presented in Table 2. The studies analyzed the content of potassium bromate in wheat flour in addition to the bread products and the results showed that the concentration of potassium bromate in wheat flour is lower than in bread products, despite the effect of temperature on the compound as reported by Abu-Obaid *et al.,* (2016). This may signify the possible addition of potassium bromate during the formulation which may increase the content of the compound in the finished products. A more recent study carried out by Abduljalil *et al.,* (2021) discovered a high concentration of $KBrO_3$ in five (5) different bread improvers used by bread bakers in Sokoto State. This study is unique because as it is the only available study that analyzed other brands of bread improvers beside potassium bromate itself. Being potassium bromate as the best and cheapest flour-enhancing agent (dough improver) amongst others, it may be possible that the manufacturers of such improvers may use the compound in disguise for bakers' satisfaction, and to garner high patronage of their products. It may also be of economic benefits to them, since the compound ($KBrO_3$) is cheaper.

2.9 The use of potassium bromates by Nigerian bread bakers: intentional or unintentional?

The high potassium bromate content in bread products reported by numerous studies conducted across the federation may be due to the following factors if not intentionally added by the Nigerian bakers:

i. Naturally Occurring bromine in flour: Many foods including wheat flour have a naturally occurring concentration of bromine in a range between 1-10mg/kg which in some foods may substantially be more. The naturally occurring bromine (Br -) in wheat flour was estimated to be within 2.4 to 7.7mg/kg (Bushway *et al.,* 1998). Bromine is dissolved in water and converted to bromate (Atkins, 1993). This may be the reason why the joint FAO/WHO Committee on food additives temporarily accepted the level of KBrO3 in flour to be within 0 to 60mg/kg with a view that bread products prepared from processed flour may contain minute residue of potassium bromate (FAO/WHO, 1999). Later the joint committee of FAO/WHO stated that the acceptable limit of $KBrO_3$ in flour was withdrawn due to the long-term toxicity and carcinogenicity of the compound. Therefore, many studies recommended that the concentration of $KBrO_3$ obtained in wheat flour may not be necessarily indicated that the flour was intentionally brominated by the producers. Furthermore, it has been reported that gaseous germicides are usually used for wheat cultivation in order to increase the tenability of the wheat. Thus, such a germicide when exposed to gas is considered to be highly poisonous to fungi. One of the most commonly used germicides for this purpose is methylene and ethylene dibromine where traces amounts may be found in the wheat flour and bread (Das, 1969). Therefore, Das (1969) reported that the bromine content of the bread may be from the gassed germicides wheat flour containing more bromine residue or due to the natural bromine of the flour or to that of salt used. Additionally, Das (1969) further reported that washing does not remove the high content of bromine added by the gassing. Despite this, all studies reviewed reported a low level of $KBrO_3$ in wheat flour than in bread products, though a significant decrease is expected in the baked bread due to the effect of temperature, pH and period of fermentation on the compound. This may signify that potassium bromate was intentionally added during the bread formulation process.

ii. Other adulterated improvers: Although, many brands of bread improvers are produced and marketed as an alternative to potassium bromate, these improvers had not been subjected to safety studies particularly to ascertain their purity if not adulterated with potassium bromate. An exception to this is a study conducted by Abduljalil *et al.,* (2021) which evaluated the potassium bromate content in five (5) brands of bread improvers produced and packaged by different manufacturers. The study revealed the presence of a high concentration of potassium bromate in the entire brands of improvers analyzed. All the studies that evaluated the level of potassium bromate in wheat flour reported a low level of the compound in flour than in bread products, despite the effect of the baking condition such as temperature, pH and period of fermentation on $KBrO_3$. This is an indication that other bread ingredients like improvers may be adulterated with potassium bromate by their respective manufacturers for economic gain if not deliberately added by the bakers during bread preparation for cost effectiveness.

iii. Brominated flour: Different types of food additives are used by flour millers during the milling process. A good number of reports have revealed the use of flour improvers during the flour milling process which enhances the nutritive value and attractiveness of the flour and consequently the overall quality of bread (Aboaba and Bakare, 2001). Aboaba and Bakare, (2001) stated that these additives range from vitamins to bleaching and maturing agents which amongst which were potassium bromate, chlorine oxide, ascorbic acid and benzyl peroxide. Some wheat flours are enriched with oxidizing improvers such as potassium bromate and ascorbic acid in order to stimulate the development of gluten in the dough and this type of flour provides dependable results (making the dough stronger and elastic) required by the majority of bakers. The use of $KBrO_3$ among flour millers is becoming common all over the world due to its low-price and its ability to oxidize sulphydryl groups of the gluten protein of the flour into disulphide bridges, thereby becoming less extensible and highly elastic such that it can retain the carbon dioxide gas produced by the yeast (Johnson *et al.,* 2013). The report showed that some flours are brominated in order to make bread rise in the oven, increasing the volume of a loaf and its texture so as to attract high patronage from the bakers whose demands are to produce products that are pleasing to the public (Nakamura *et al.,* 2006; Emeje *et al.,* 2010). Aboaba and Bakare (2001) reported that potassium bromate is normally diluted with other chemicals such as magnesium carbonate or calcium sulphate to make a 0.20g/kg concentration in the flour. Addition of potassium bromate to newly milled flour increases its shelf life (Ogah *et al.,* 2021), its flavour, colour, texture, appearance and stability of the flour and dough (Freer, 1999). Both Ndoni (2009) and Emeje *et al.,* (2010) reported that the majority of flour milling industries in Nigeria include bromate during the milling process in order to improve the quality of their products so as to get high patronage from bakers. No doubt the above factors may contribute to the presence of potassium bromate in bread products, although all the studies carried out on the determination of the compound in bread and wheat flour revealed a high concentration of $KBrO_3$ in bread than in wheat flour which may justify the compound is intentionally added by the bakers. Nigeria bread bakers have a dubious way of including potassium bromate into their bread formulation. These ways include crushing of potassium bromate tablet into powder for easy mixing and homogeneity with wheat flour or simple dissolution in water.

The bread bakers may intentionally add potassium bromate due to the following factors:

i. Economic gain: Potassium bromate ($KBrO_3$) is a strong oxidizing agent, generally used to make the bread alluring to the consumers (Dada *et al.,* 2017) and more lucrative to the bakers (Uwah and Ikwebe, 2020). The use of potassium bromate reduces the fermentation period; hence baked bread can be obtained in a shorter period than if not used. Potassium bromate is considered to be inexpensive and readily available compared to other additives; hence using it will significantly reduce the cost of bread production. The use of KBrO3 gives a superior end product, makes the bread to be stronger, and increases its texture as well as its volume (Gandikota and MacRitchie, 2005). This will no doubt attract the consumers which will consequently increase the profit and the general turnover because the more the quantity, the more pleasing is the bread to the buyers. Recently, Mahmud *et al.,* (2021) confirmed that bakeries and confectionaries were using $KBrO_3$ in their products for economic benefit, this may be the reason why 92% of Nigerian bread samples contained potassium bromate as reported by Emeje *et al.,* (2010). The continued usage of potassium bromate by the baker was attributed to its cheapness.

ii. High Cost of Ingredients: High cost of bread ingredients can make most bakers opt for low-cost ingredients such as potassium bromate as alternatives. Though there is a high demand for bread in Nigeria, the majority of the country's population are in extreme poverty, therefore cannot afford to purchase bread at high prices, this created an enabling environment for the bakers to use the substandard ingredient to lower the production cost which in turn will increase their profit. This condition also created a supporting base to which many inferior and local bakeries prosper, who are not properly enlightened with the health hazard of banned ingredients like potassium bromate.

iii. Poor enlightenment and enforcement: Many bakers are not fully informed on the health consequence of potassium bromate. Despite the fact that researchers periodically evaluate the bakers' compliance level but their findings have been disturbing which may be attributed to ignorance. The Nigerian illiteracy rate was estimated to be around 62.02% in 2018 (Statista, 2022a) and this can significantly contribute to the inappropriate use of the compound by the bakers without knowing its hazardous effects. Poor enforcement of the stipulated regulation by the concerned regulatory bodies can also contribute to the continued use of the compound by the bakers.

What do regulatory bodies need to do?: In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) is the Agency saddled with the responsibility of regulating all food-related products including bread by section 5 and 30 of the National Agency for Food and Drug Administration and Control Act Cap NI laws of the Federation of Nigeria (LFN) 2004 (NAFDAC, 2022a). The Agency has the sole mandate of ensuring bread products and other confectionaries are produced under Good Hygienic Practice (GHP) and using standard and approved ingredients. The formerly Food and Drug Administration (FDA) now NAFDAC was established by Decree No. 15 of 1993 as amended by Decree 19 of 1999 and presently Act Cap N1 Laws of the Federation of Nigeria, 2004 as an Agency under the Federal Ministry of Health. In 1993, the Agency banned the use of potassium bromate in bread and is violation of decree 20 of 1999 and NAFDAC Decree 15 of 1993 (Akunyili, 2004) and requested the support of all Nigerians especially bread bakers to desist from use of $KBrO_3$ as bread enhancer. Akunyili (2004) also reported that in addition to the ban, the Agency stipulated stringent regulations and penalties on the use of the compound. In spite of the Agency's ban and regulations, yet available information on the Agency's revised 2019 tariff showed no specific administrative penalty for the use of potassium bromate, unlike other offences and violations (NAFDAC, 2019a). In 2017 NAFDAC introduced an international activity code named "Opson" which is an INTERPOL-EUROPOL platform for fighting counterfeit food and beverages (NAFDAC, 2017). But since when the OPSON VI activity was launched in Abuja and Lagos on 17th March, 2017 and 21st March, 2017 respectively, but no available details to prove the commencement and effectiveness of the activity in other states of the country. This activity (OPSON) if properly and effectively coordinated will drastically reduce the importation of potassium bromate. Furthermore, NAFDAC need to introduce a mechanism to checkmate the bakers' compliance with a view to prevent the Nigerian populace against the toxic effects of potassium bromate. The USFDA had recently launched a food compliance programs which offers instructions and guidelines for their staff to evaluate bakers' compliance with the laws specified by FDA (USFDA, 2022). The USFDA also established the Food Safety Modernization Act, aimed at preventing food related problem before they happen. This FDA's Act was introduced to identify food hazards that requires urgent address or minimized through various food sampling for gathering data and information. Therefore, NAFDAC need to introduce and meritoriously coordinate similar programs and regulations in order to specifically evaluate and monitor the bakers' compliance. This is because, years after the ban on the use of potassium bromate, but available data showed that Nigerian bakers have not complied with the ban. Therefore, the following grey areas if properly addressed by the Agency will drastically reduce if not eradicate the use of $KBrO_3$ as bread improver by the Nigerian bakers.

i. Post-Marketing Surveillance (PMS): Postmarketing surveillance is a series of activities conducted by regulatory bodies for the evaluation and collection of information regarding food and drugs after they have been approved for use in a population (Douglas, 2019). Also, the PMS helps to ensure that foods and drugs are safe and well-performing and to certify that actions are commenced if their risk outweighs the benefits (WHO, 2021). The post marketing surveillance otherwise called post-market surveillance will help in monitoring the safety of not only bread production but including its ingredients after they have been released on the market for consumers. In Nigeria, the post-marketing surveillance of food product is overseen by NAFDAC which placed a suitable PMS system to effectively monitor the quality and safety of products and directed all producers of food products (bread inclusive) to comply with their standard requirements to ensure quality food items are sold in the country (NAFDAC, 2019b). Although the Agency has adopted the PMS system in foods, there is a need for effective and efficient utilization of the system particularly on bread products and their ingredients because the system seems to be more active on drugs and medical devices. This will enable the Agency to evaluate the safety of bread and other ingredients particularly flours and improvers at a market level which will guarantee the actual contents of the products. Additionally, the Agency should mandate the manufacturers of bread and its enhancers as well as wheat flour and other ingredients to provide a robust system to receive a feedback from consumers on their products on possible health effects.

ii. On-the-Spot Analysis: This is a simple qualitative analysis technique for determining the presence of potassium bromate in bread and its ingredients. At the course of post-marketing surveillance, there is need for NAFDAC to adopt on the-spot-analysis of bread and its ingredients which will ascertain the actual formulation of the products. An instant systematic, subjective and semiquantitative method of analysis should be established and sustained by NAFDAC in order to evaluate the bread products and ingredients in relation to the standard specified by the Agency. This analysis is to serve as a preliminary qualitative test which is considered simple that can be done at the spot and requires simple analytical reagents. This method involves addition of 2ml of 0.01M promethazine and 0.6ml of 12M hydrochloric acid on a portion of bread sample where a pink colour change indicate the presence of $KBrO_3$ as reported by Alli *et al.,* (2013). This method is scientifically effective and its adoption will mandate the bakers to fully comply with the Agency's regulation, if they believed that their products released in the markets are being properly and effectively monitored and evaluated. Therefore, with the bakers' dubious means of adding the compound into bread formulation, on-the-spot analysis of bread will help the Agency to evaluate the bakers' compliance which will ultimately guarantee wholesome bread products for human consumption.

iii. Effective Monitoring and Enforcement: There is a need for the National Agency for Foods, Drugs Administration and Control (NAFDAC) and Standards Organization of Nigeria (SON) to ensure effective monitoring of bakeries in order to ascertain their compliance with the Agencies' stipulated standards of Good Manufacturing Practice (GMP), Good Hygienic Practice (GHP) and Hazard Analysis Critical Control Points (HACCP) guidelines and requirements. Effective impromptu monitoring and enforcement of the Agencies' regulations will significantly stop the use of banned ingredients like potassium bromate by bread bakers and flour millers.

iv. Stringent regulation: NAFDAC is solely responsible for regulating and controlling the manufacture, sale and use of foods products and to conduct appropriate tests and ensuring compliance with the standard specification for effective control of the quality of food as enshrined by the Agency's law (Food and Drug Act Cap F. 32 LFN) (NAFDAC, 2022b). Furthermore, the Agency is mandated to establish relevant quality assurance systems, including certification of the production sites and of the regulated products as provided by Food and Drug Act Cap NI LFN as contained under functions and power of the Agency (part II; item c) (NAFDAC, 2022a). Therefore, there is need for the Agency to establish stringent regulation for the production and marketing of bread products and their basic ingredients particularly flour, and bread improvers so as to ascertain their compliance with banned additives.

v. Sustained Routine and Surveillance inspections: There is a strong need for relevant regulatory bodies in Nigeria to establish a mechanism for effective and efficient regular and impromptu inspections of both registered and unregistered bakeries. This is to enable investigative inspections and prompt responses to consumer complaints and alerts.

2.10 Heavy Metals

Heavy metals are ubiquitous and can be found anywhere in the environment; in water, in foods, in the soil and in the air. Contamination of the environment with these heavy metals has been associated mainly with anthropogenic activities. Such activities include discharge of effluents and emissions from mines and illegal mining activities ("galamsey" operations), smelters, agricultural runoffs, and improper disposal of domestic waste. Some of the waste materials such as paint wastes, electronic goods, and used batteries increase the quantity of heavy metals found at dumpsites, and invariably, in the atmosphere (Cui *et al.,* 2005). Slow leaching of these metals into the acidic environment could result in leachates with high metal concentrations such as arsenic, cadmium, mercury, lead, and nickel, among others (Bortey-Sam *et al.,* 2015; Polat, 2015). The leachates usually end up in water bodies, and in the soils and pollute our fish and vegetation in the long run. Food consumption has been identified as the major pathway of human exposure to heavy metals, compared to other ways of exposure such as inhalation and dermal contact (Monferran, *et al.,* 2016; Zhuang, *et. al*., 2009). In Nigeria, there has been a surge in industrialization, especially in the Accra-Tema metropolis, including aluminum processing plants, oil industries, plastic manufacturing industries and agro chemical industries, which pose as a source of threat to inhabitants residing within the metropolis.

2.10.1 Health effects of heavy metals

Heavy metals such as lead, cadmium, mercury, arsenic, chromium can have deleterious effect on the health of humans. They may produce neurotoxic, nephrotoxic, as well as carcinogenic effects. In addition, heavy metals result in the poor functioning of the various systems in a person's body. Their effects include the penetration of the blood-placenta barrier, which could have dire effects on an unborn child. Other effects include damage to the cardiovascular system, the brain and also the bone structure, thus causing osteoporosis (Ekpo *et al.,* 2008). Cadmium may be found accumulated in the cortical tissues of the liver and kidneys, while methyl-mercury compounds could be mostly deposited in the cerebral tissue, and lead would likely be found hidden in bone tissues. Clinical symptoms of their toxic effect at little exposure are usually not immediately noticed, however, many months, years or generations after, their effect may be finally noticed (Rudy, 2009). Chromium, another heavy metal, can cause skin ulcers when it comes into contact with the skin thus eliciting severe redness and swelling of the skin. The effect of chromium also includes irritation to the lining of the nose, cough, asthma, and wheezing (Griswold and Sabine, 2009; Abbas and Rahman, 2012). Report has showed that some diseases are associated with heavy metal pollution in the city of Asa dam in Ilorin caused by the tanning industry. The most prevalent diseases included Asthma, Acute (upper) respiratory infections, Diarrhea/dysentery among children under five years as well as Typhoid, Hypertension, Dermatitis, Neuro-psychiatric diseases, Nephritis and prolonged cough, all resulting from the inhalation of polluted air, contaminated soil dust, and drinking polluted water (Abbas and Rahman, 2012). Furthermore, Honda *et al.,* (2015) also confirmed Abbas and Rahman's study, by showing that a high concentration of ambient Zn was seen to have been associated with increases in cases of asthma among children under 14 years. Again, proven by Honda et.al was the fact that hexavalent chromium was a major cause of lung cancers (Honda *et al.,* 2015).

2.10.2 Some heavy metals in bread and other food samples

Aquatic organisms such as fishes possess the capacity to accumulate heavy metals in their tissues. These fishes accumulate these heavy metals by absorbing them into their gills and also in their body tissues. When bioaccumulation occurs, it creates a much higher concentration of the metal than what may be found in the environment. This poses a threat to higher organisms up the food chain as organisms like human beings might end up feeding on these fishes that have bio-accumulated these metals (Atobatele and Olutona, 2015).

2.10.3 Nickel

Nickel, another widespread heavy metal can exist in several different oxidation states, with the most prevalent state being Ni^{2+}. As compared to most metal pollutants Ni is less toxic and more soluble in water. It is a transition metal that shows a wide range of both redox behavior and complex formation. Humans may be exposed to nickel by breathing polluted air, drinking contaminated water, eating food or smoking cigarettes. Skin contact with nickel-contaminated soil or water may also result in nickel exposure. Nickel has been enlisted by the National Toxicology Program (NTP) as being carcinogenic. Furthermore, the International Agency for Research on Cancer (IARC) has listed some nickel compounds as being in Group 1 indicating that "there is sufficient evidence for carcinogenicity in humans" and other nickel compounds as Group 2B, which also connotes its carcinogenicity in humans (Gordon *et al.,* 2011).

High nickel concentration in sandy soils can cause a damaging effect in plants, as well as stop the growth rates of algae (Abbas and Rahman, 2012). When this occurs, it could reduce the amount of feed that becomes available to the fish. Being exposed to Ni could result in nausea, vomiting, abdominal discomfort, diarrhea, headache, cough and shortness of breath.

2.10.4 Mercury

In water bodies, soils, the air, and general earth surface, Mercury poses as one of the most toxic heavy metals present in the environment. Mercury can be found in three states; in the mercurous, mercuric, and the methyl mercury states. However, the most toxic form of mercury is the methyl mercury. This form of mercury causes health effects to both organisms and the environment. Most of the mercury pollution found in the environments is caused by anthropogenic activities. The contamination chain of Hg follows, closely, the cyclic order: industry, atmosphere, soil, water, phytoplankton, zooplankton, fish and human (Kadar *et al.,* 2000). Activities such as mining, combustion, urban discharges, and the current e-waste activities in Nigeria are a major cause of mercury pollution in the environment. Humans are mostly exposed to mercury pollution through two sources. One of these is through eating sea foods such as fish, lobsters, and, crabs, shrimps, and the likes that have methyl mercury in their tissues. The second most common is through the release of mercury from dental amalgam. This may dissolve in saliva and be ingested, causing mercury poisoning (Castro-González and Méndez-Armenta, 2008).

A study conducted by Obodai *et al.,* (2011) from the Benya and Nakwa Lagoons in the Central region of Nigeria revealed that the concentration of mercury in the black chin tilapia (Sarotherodon melanotheron) was 0.33 mg/kg. This far exceeds the EPA standards for mercury (0.001 mg/kg) contamination. These high levels of mercury concentration could cause kidney failure to the inhabitants whose source of fish is mainly from these rivers (Obodai, 2011).

2.10.5 Lead

Lead is a metal, which occurs naturally in the earth's crust. It may be found in minute quantities, and is often found as a complex with other elements such as silver, zinc, or copper. Lead could be found in certain products such as paint pigments, batteries, cosmetic products as well as dietary supplements. The effects of lead poisoning in the human body cannot be underestimated. Lead poisoning may result in the malfunction of the tubular structures of the kidneys, and thus lead to chronic renal diseases. Lead exposure could also lead to enormous repercussions on the reproductive health of humans. In women, lead poisoning could lead to miscarriages, still births, as well as sterility, with men also exhibiting signs of reduced semen quality (Juberg *et al.,* 2000).

Exposure to lead bullets can also have adverse effects on humans. This occurs when these bullets are used in game hunting. The lead bullets, more importantly those of shell burst type, could serve as the secondary source of lead in meats, and could become an associated risk to human health as reported by Jarzyńska and Falandysz (2011).

Tabari *et al.,* 2010 documented that lead may be found in the selected fish in the Gorgan coast. However, the results indicated that fishes from the Capsian Sea at the Gorgan coast were below the FAO standards, of 0.1- 0.2 mg/L thus may not cause hazardous effects and was wholesome for consumption (Tabari *et al.,* 2010).

2.10.6 Arsenic

Arsenic is a widely distributed metalloid which is naturally found in rock, soil, water and air. Arsenic may occur in either organic or inorganic forms, and more often than not, the inorganic form poses a greater threat to life whereas the organic form represents a lesser hazard. Its inorganic form exists in groundwater, while the organic forms such as arsenobetaine are primarily found in fish. “As” is mostly found in large quantities in the environment (air, water and soil) as a result of anthropogenic activities. Some of these activities include smelting of non-ferrous metals and the production of energy from fossil fuel, contamination of soils by mine-tailings as well as the manufacture and use of arsenical pesticides and wood preservatives (Saha *et al.,* 2016). Arsenic exposure and toxicity to human life may occur through inhalation, absorption through dermal contact, and predominantly, through ingestion of contaminated food and water, whose sources may be polluted by man’s activities (Olmedo *et al.,* 2013). The inorganic form of arsenic is toxic and when ingested in large quantities, it results in gastrointestinal symptoms, disturbances of the cardiovascular and central nervous systems, and eventually death. The WHO in 2004 stated that arsenic exposure via drinking water is causally related to cancer in the lungs, kidney, bladder and skin. Victims of A poisoning who survive the effect tend to live with bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy. Ingestion of inorganic arsenic may induce peripheral vascular disease, which in its extreme form leads to gangrenous changes (Raissy *et al.,* 2011).

2.10.7 Cadmium

Cadmium, another heavy metal is one of the equally lethal elements that affect mankind and animals alike. It can have adverse effects on respiratory, neurological, gastrointestinal and cardiovascular systems. Workers who are susceptible to cadmium exposure include workers at incinerators, those who deal in electroplating, those at landfills, and also those who deal in the recycling of electronic parts, as well as that of plastics (Chahid *et al.,* 2014). Having Nigeria as the hub of e-waste recycling in West Africa could mean an increase in the cadmium concentration in soils, water bodies close to the e-waste site, as well as in the air. Cadmium's lethal property is heightened mainly due to the bio-accumulative property it possesses. Research has shown that cadmium level in the body can be even recorded above permissible levels when it stays for long in its elemental forms. Another property of Cadmium is its long half-life period, which ranges between ten and thirty years (Rudy, 2009).

There are several biomarkers that could be used in detecting cadmium accumulation in the organs of the body. Blood and urine are two of the biomarkers that are used in this exercise. Blood is able to give an indication of the amount of Cadmium in the body. Likewise, urine is able to give an indication of the cadmium concentration in the body. In order to account for variation of dilution in urine, the cadmium levels are divided by urinary creatinine or specific gravity determined in the same urine sample (Nordberg, 2010).

Heavy metal concentrations that tend to occur above permissible limits do not only have repercussions on the animal or fish but it could also pose deleterious effects on human health especially when consumption is regular and, in enough quantity, (Ihedioha and Okoye, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Preparation of Samples

five (5) bread brands were purchased at several stores in Ilorin, Kwara State, Nigeria. Only bread brands produced and commonly consumed in Ilorin were used in this study. Several bread samples of six different brands obtained from different stores were stored in polyethylene bags and transported to the laboratory for analysis. Each bread brand was oven-dried to constant weight, pulverized to a fine powder using an agate mortar and pestle and homogenized to form composite samples of the same brand.

3.2. Materials and chemicals

All reagents used are of analytical grade purchased from recognized chemical companies. Hydrochloric acid (Sigma chemical Co, St Louis USA), Potassium iodide (BDH, England), Potassium Bromate (Sigma Chemical Co, St Louis, USA). The laboratory apparatus used includes, beaker, test tube, measuring cylinder, hotplate, oven and test tube rag.

3.3 Qualitative analysis of potassium bromate in bread

One gramme (1 g) from each bread brand was measured out into different test tubes. Water was added to wet the samples. 0.5 mL 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 (Ufuoma *et al.,* 2020).

3.4 Quantitative analysis of potassium bromate in bread

The approach reported by Emeje *et al.,* (2009) was adopted with slight modification. One gram (1 g) of each of the 6-bread sample was weighed and transferred into a test tube, 10 mL of distilled water was added into each test tube, shaken vigorously and allowed to stand for 20 min at a temperature of 28 ± 10ºC. 5 mL of the solution was decanted into another test tube via a Whattman No 1 filter paper; then 5 mL of 0.5% prepared potassium iodide solution in 0.1N HCl was added to the solution. Any color change was noted. The presence of potassium bromate was indicated by change in color from light yellow to purple. The absorbance of the sample was taken at 620 nm in a UV-vis spectrophotometer (UV 1200, Japan). Absorbance of the sample was converted to concentration with reference to Beer's calibration curve previously constructed for potassium bromate using the pure sample. Values reported here are mean of three replicate determinations. The other bread samples were similarly treated.

The calibration curve was prepared by weighing out 1.0 g of potassium bromate using a weighing balance, and dissolved in 1000 ml of distilled water. Different concentrations were made by solving for original volume (V1) using the formula V1 = C2V2 /C1. With Required volume (V2) and original concentration (C1) constant (10 and 1000 ml, respectively), different values were obtained for the original volume (V1) with each of the values obtained for V1 corresponding to the varying required concentration (C2). They were all made up to 10 ml with 0.5 and 10 ml having the lowest and highest concentration, respectively. A 5 ml quantity of freshly prepared 1 g of potassium iodide solution in 0.1N HCl was added to each pure sample. The absorbance of the sample was taken at 620 nm 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; 0.2, 0.4, 0.6, 0.8, 1.0 µg/g (ppm) (Fig. 2).

3.5 Determination of heavy metals

Levels of heavy metals in the bread sample were also determined by weighing 1.0g of the ground sample into a digestion tube and digesting it with 10 ml of a mixture of concentrated HNO_3 and concentrated HCl (in 3:1 ratio) on a hot plate. On cooling, the digested sample was filtered into a 50 ml volumetric flask and made up to the mark with distilled water. The filtrate was then aspirated into the Atomic Absorption Spectrophotometer (AAS) and the levels of the metals (Pb and Fe) determined.

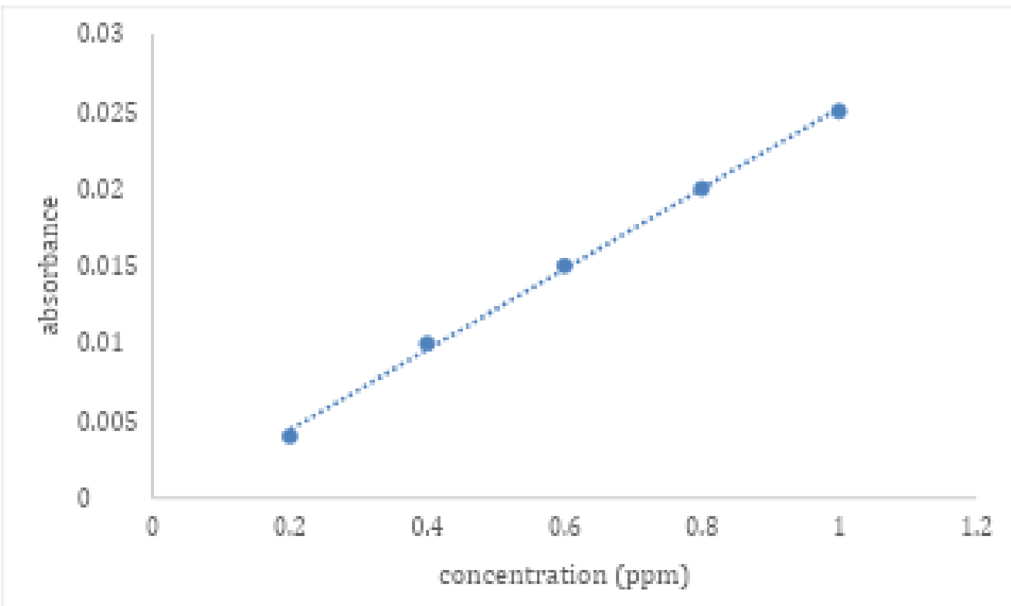

Fig.2: calibration curve for potassium bromate

3.6 Statistical Analysis

Statistical analysis was done using excel software and the results were presented as mean ± SEM (Standard error of mean) for triplicate measurements where necessary.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Table 1: Potassium Bromate and Metal Concentrations in Some Bread Samples in Ilorin

Sample bread	$KBrO_3$ Concentration (mg/kg)	Lead (Pb) Concentration (mg/kg)	Iron (Fe) Concentration (mg/kg)
KW/POLY	3.16 ± 1.13	2.13 ± 1.77	4.41 ± 2.38
SOFTY	5.32 ± 2.72	5.15 ± 1.2	5.52 ± 2.63
50/50	2.96 ± 0.64	2.34 ± 0.70	1.21 ± 1.40
GGC	7.28 ± 2.14	3.60 ± 1.14	6.40 ± 2.20
D CHOICE	6.42 ± 1.57	4.24 ± 1.43	1.22 ± 0.45

The results of the study show that the mean bromate levels in the five samples ranged between 0.12 ±0.08mg/kg and 7.28 ±2.14mg/kg (Table 1). The highest level was recorded in Alaba, located in one of the numerous slums in the city. Lead (Pb) concentrations in the samples ranged between 0.23±0.10 mg/kg and 5.15±1.20 mg/kg.

The concentrations of Iron (Fe) in the samples also ranged between 0.45±0.10mg/kg and 6.40± 2.20 mg/kg.

All the bread samples obtained from the six bakeries recorded, in varying degrees, the presence of potassium bromate. We also observed that the concentrations of bromate in the all samples exceeded the permissible limits of 0.02 mg/kg set out by the National Agency for Food and Drug Administration and Control (NAFDAC).

Although the bromate levels in the class of bread analyzed were quite high and thus constituted a danger for the consumption of such loaves of bread, it could be said that the bakers added the bromate for purely economic reasons. Potassium bromate is cheap and is probably the most efficient oxidizing agent (Akunyili, 2005). The addition of bromate also gives more bulkiness to the dough development resulting in more loaves of bread being cut out (Abubakar *et al.,* 2008). However, the health effects far outweigh the economic benefits. Potassium bromate is extremely irritating and injurious to tissues especially those of the central nervous system and the kidneys (Oloyede and Sunnmonu, 2009). Unfortunately, the consumers of these cheap loaves of bread are generally children and the low- income earners who are ignorant of the health risks associated with bro mate-laden bread samples. Studies in some other cities in Nigeria (Abubakar *et al.,* 2008; Oloyede and Sunnmonu, 2009; Alli *et al.,* 2013 and Magomya *et al.,* 2013) have also recorded the presence of potassium bromate in loaves of bread, despite its ban by the regulatory body, NAFDAC.

The levels of some heavy metals (Pb and Fe) studied in the bread samples were as follows: Mean Pb levels ranged between 0.23± 0.10 mg/kg and 5.15± 1.20 mg/kg. The mean Fe levels, on the other hand, ranged between 0.45± 0.10 mg/kg and 6.40± 2.20 mg/kg. Permissible levels of Pb and Fe in food substances are in the ranges of 0.2 – 2.5mg/kg and 2.5 – 5.0 mg/kg respectively (Magomya *et al.,* 2013). From the results obtained, samples Softy, D choice and GGC had higher levels of Pb than the permissible concentration required of 0.025 mg/kg by NAFDAC. Similarly, samples Softy and GGC recorded higher levels of Fe than the recommended permissible limit in food substances. These results thus indicate that bakeries producing bread samples Softy, Alaba and Fresh are operating at sub - standard levels.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results indicate that bread produced by these bakeries in Ilorin contain potassium bromate above the permissible level. A significant proportion of them also introduce higher Pb and Fe levels in the loaves than the permissible levels in food substances by the regulatory agencies. As earlier indicated, bread is considered a staple food in Nigeria. It becomes thus worrisome that toxic chemicals and metals are unwittingly introduced into the menu of majority of the citizens. The health effects arising from the introduction of these chemicals may be far- reaching and devastating, especially to the unsuspecting public.

5.2 Recommendation

After the conclusion of the present research the following recommendation can be proffered:

i. There is need for the regulatory agencies to carry out regular and systematic monitoring of bakeries, particularly the unregistered ones that operate in obscure places.
ii. Ascorbic acid should be adopted for all bakeries. It is safe and can be consumed and used instead of potassium bromate
iii. It is suggested, among others, that the environment be kept clear of contamination, to ensure healthy food production and consumption.

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