# ASSESSMENT OF BROMATE AND HEAVY METAL LEVELS IN SELECTED BREAD SAMPLES MARKETED IN KEFFI, NASARAWA STATE

**BY**

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## DECLARATION

I hereby declare that this dissertation has been written by me and it is a report of my research work. It has not been presented in any previous publication for the award of Master of Science (Industrial Chemistry). All quotations are indicated and sources of information specifically acknowledged by means of reference.

_____ _____

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## CERTIFICATION

This dissertation titled "Assessment of the Bromate and Heavy Metal Levels in Selected Bread Samples Marketed in Keffi, Nasarawa State" meets the regulations governing the award of Master Degree in Industrial Chemistry (M.Sc.) of the School of Postgraduate Studies, Nasarawa State University, Keffi and is approved for contribution to knowledge.

| | |
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## DEDICATION

This research work is dedicated to GOD Almighty**.**

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## ABSTRACT

Bread is a staple food in many countries around the world and it is usually prepared from flour and food additives; one of which is potassium bromate, which promotes its various properties such as appearance, texture, shape and size. This work investigated the levels of potassium bromate and heavy metals in ten different bread samples produced and marketed in Keffi-Nasarawa. Bromate was determined using a UV-Visible Spectrophotometer and heavy metals with an Atomic Absorption Spectrophotometer. The results indicated that potassium bromate was present in all the samples with concentrations ranging from 2.82±0.375 - 24.3±0.525mg/kg which were higher than the WHO permissible limit of 0.02 mg/kg. The results of the heavy metals analysis also revealed that some bread samples contained high concentration of cadmium (cd), iron (fe), chromium (cr), and zinc (zn) while lead (pb), nickel (ni), copper (cu) were within the WHO permissible limits. The presence of bromate in all the bread sample use in this research shows that bakers seems not comply with the ban placed on the use of potassium bromate by NAFDAC, thus, bread consumers and bakers are at risk of exposure to potassium bromate and some heavy metal with health implications.

# CHAPTER ONE

# INTRODUCTION

## 1.2 Background to the Study

Bread is an important staple food in many countries of the world especially the African countries and South Eastern part of Asia (Owen, 1997). Statistical analysis in Nigeria showed that bread is one of the most consumed food types in homes, restaurants and hotels with predominant consumption among the poor and young ones who constitute more than 70% of the over 150 million people in Nigerian (Akinyele *et al*., 2004). Bread is made from carbohydrate flour sources. Some of the basic ingredients, apart from flour, are sugars, table salt, flavors, and at least, a flour improver such as potassium bromate (Vicki, 1997). Potassium bromate as a flour improver has been in use for more than eighty years (Gandikota *et al*., 2005). The use of potassium bromate as a food additive has been a common choice among bakers and flour miller throughout the world because it is probably the most efficient oxidizing agent and also cheap (Osuji, 2006). It acts as a dough conditioner and maturing agent by oxidizing the sulfhydryl groups of the gluten protein in flour into disulphide bridges making more elastic and less extensible, this will make the dough viscoelastic such that it can retain the carbondioxide gas produced by the yeast.

The overall role of bromate in bread is to make it rise in the oven by increasing the loaf size and texture (Nakamura *et al*., 2006). However, from health perspective, toxicological studies have shown that consumption of potassium bromate can lead to degrading of vitamins A1, A2, B1, B2, and E commonly found in bread (Kurokawa *et al*., 1990). Moreover, literature reveals that consumption of potassium bromate can also lead to the development of sore throat, cough, abdominal pain, nausea, diarrhea, vomiting, hearing

impairment, kidney failure bronchial and ocular problems, haemolysis, extreme irritation and injury to tissues especially those of the central nervous system and kidneys when inhaled (Artkin,1993). Another study conducted by Kurokawa *et al.*,(1986) indicated that potassium bromate have the potential to cause cancer in both experimental animals and humans by inducing oxidative stress in tissues. In California, the American Bakers Association (ABA) and American Institute of Baking International (AIBI) adopted a limit of 0.02 mg/kg on bromated level in finished products (ABA/AIBI, 2008). In Nigeria, the use of bromate in flour milling and baking was banned in 2003 (Oyekunle *et al*., 2014), but despite of the ban, investigations by some researchers have shown that high levels of bromate in bread and other wheat flour food products were still found (Nakamura *et al*., 2006; Emeje *et al*., 2010; Magomya *et al*., 2013; Oyekunle *et al*., 2014).

Unlike bromate, heavy metals are not bread additives but they may get into bread from the wheat flour used in bread production or from some other sources during production such as the water and salt additions to flour, the fuel used for baking, contact with metal surface during mixing and kneading dough, packaging materials (Ahmed & Fadel, 2012; Magomya *et al*., 2013). Above a certain concentrations, some metals which are classified as essential elements are toxic to humans causing various diseases. Depending on the type of heavy metal, some of the health effect include gastrointestinal disorders, tremor, diarrhea, paralysis, vomiting, convulsion, diabetes, anaemia and cancer, Heavy metals disrupt the physiological functions of the body by accumulating in vital organs and glands such as the brain, heart, kidney, liver and bone (Goyer, 2001). Some studies have also confirmed the presence of higher-than-the-permissible levels of heavy metals in bread loaves produced in some parts of Nigeria (Nakamura *et al*., 2006).

## 1.2 Statement of the Problem

Potassium bromate and heavy metals are bread contaminants and pose serious challenges to human health. While potassium bromate is an additive used by bakers as an enhancer in bread production, heavy metals find their way into the bread during processing. Potassium bromate usage in bakeries despite the banned in Nigeria by the National Agency for Food Drug and Administration Control in 2004 on account of its health effect and carcinogenicity, is still being employed by the bakers. Thus, there is need to evaluate the levels of potassium bromate and heavy metal contamination of bread samples produced and consumed commercially in keffi Nasarawa State central Nigeria where there is no evidence of such a study.

## 1.3 Aim and Objectives of the Study

The aim of this research work is to assess the levels of potassium bromate and some heavy metals in bread samples produced from ten different bakeries in Keffi Local Government Nasarawa State.

The objectives of the study includes, to evaluate the levels of

(i) Potassium bromate in the bread samples

(ii) Lead, Cadmium, Copper, Zinc, Nickel, Lead, Iron and Chromium level in the bread samples

## 1.4 Significance of the Study

The results of this research will be used to create awareness on whether the commercial bread samples produced and commonly consumed in Keffi-Nasarawa State are free from potassium bromate and heavy metal contaminants or not, so as to ascertain its safety for human consumption . It will also inform the regulatory body like the National Agency for Food, Drug Administration and Control (NAFDAC) on the compliance of the Bakers to the ban placed on the use of potassium bromate thereby enabling more surveillance.

## 1.5 Scope of the Study

This study covered only the assessment of bromate and heavy metals such as lead, cadmium, copper, zinc, nickel, iron and chromium in ten (10) commonly consumed commercial bread samples produced in Keffi-Nasarawa State.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Bread Flour

The baking of bread requires precision and specific ingredients, and one of the most integral components to baking bread is bread flour**.** Bread flour is a high-protein flour that typically contains between 12 and 14% protein and is designed for baking yeasted breads (Johnson *et al.,* 2015). The high protein content means that bread flour has more gluten in it, which makes the dough more elastic and light and results in a chewy and airy texture when baked. Bread flour is also set apart from other types of flour because it requires significant kneading to create a good gluten structure, which results in the airy and chewy texture (Johnson *et al.,* 2015). Bread flour is sometimes called strong flour because the flour is made from hard wheat varieties, unlike other types of flour. The difference between bread flour and all-purpose flour is in their protein content and uses. Bread flour usually contains between 12 and 14% protein compared to an average of 8 to 11% in all-purpose varieties. Investigation reveals that, with a lower protein content, all purpose flour isn't able to create enough gluten to give the dough the light and elastic texture that you need to create good bread dough (Emeje *et al.,* 2010).

## 2.2 Bread Production

The production of bread involves four basic ingredients flour, yeast, salt and water. During the process of production, these ingredients are mixed properly to form dough and then allowed to rest. The yeast then grows utilizing the carbohydrates by breaking them down into simple sugars to produce alcohol and $CO_2$ gas which is trapped in the dough,

forming pores which result in the rising of the dough. The risen dough is transferred to the oven where the heat penetrates it and the gas expand the size of the dough increasing it and giving the dough its size and shape. Caramelization of the sugar then takes place giving the bread its taste and smell. To make a good bread the dough must have elastic nature, ability to hold gases produced by yeast and the property to retain its shape. All this is done by the Gluten protein that is naturally found in the flour, bonding between the gluten molecules is the result of oxidation. In the olden days, flour was aged by exposing it to the open air for oxidation for days to weeks and then added to dough, later chemical oxidizing agents have come into use that have reduced the ageing time, Potassium bromate been one of these oxidizing agent is the most preferred among bakers (Preethi, 2016).Table 2.1 shows some examples of bread improvers including potassium bromate and its alternative such as ascorbic acid, a non-toxic oxidizing agent and also enzymes such as hemicellulase, cysteine and carbohydrase.

**Table 2.1: Bread Improvers**

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

(khaniki *et al.,* 2005)

## 2.3 Potassium Bromate

Potassium bromate ($KBrO_3$) is a chemical compound represented by the formula $KBrO_3$, it has a molecular weight 167.00g/mol and density of 3.27g/$cm^3$. It exists as white crystalline powder or granules, highly soluble in ethanol but almost insoluble in acetone, it is very stable when dissolved in water at room temperature, it melts at 350$^oC$ and decomposes at 370 $^oc$ (Sax& Lewis, 1989).The structural formula of Potassium bromate is shown in Figure 2.1.

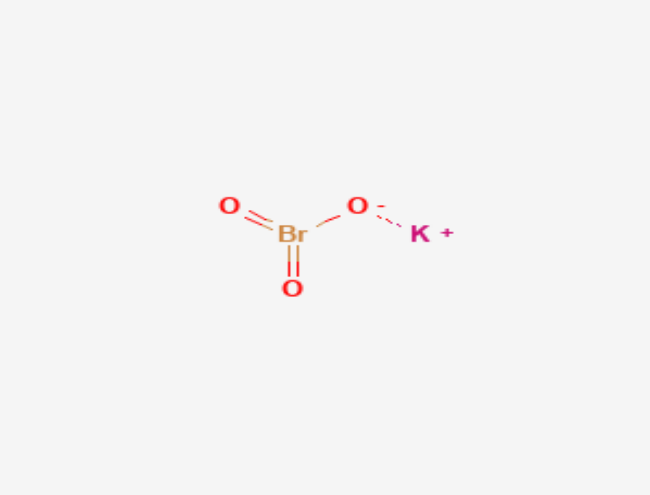



Figure 2.1: Structural Formula of Potassium Bromate

Potassium bromate ($KBrO_3$) is produced by passing bromine gas into a hot solution of potassium hydroxide as shown in equation (1). An industrial electrolytic process is used for large scale production, it can also be produced as a by – product of potassium bromide production by absorption of bromine from ocean water into potassium carbonate (IARC, 1986).

$6KOH+3Br_2 \rightarrow KBrO +5KBr+3H_2O$…….…. ……. (1)

Potassium bromate is an oxidizing agent used mainly as a food additive and it has no medicinal uses. It is used in flour milling, beer malting, cheese making, production of fish paste and fermented beverages (IARC, 1986). It is also used as a primary standard and brominating agent in analytical chemistry and in the production of explosives (Devries *et al*., 1997). Bromate may be found in some drinking water samples as by-product of ozone disinfection (Ueno *et al*., 2000). It is currently regulated in treated drinking water at maximum contaminant level of 10µg/l in USA and Europe (EPA, 2001). Potassium bromate under the right conditions will be completely used up in the baking bread. However, if too much is used or the bread is not cooked long enough or at high enough temperature, then residual amount will remain (WFE, 2006). This residual amount of potassium bromate is undesirable. Bromate being an oxidizing improver acts slowly and throughout the dough fermentation and has two primary effects, it enables loaves with high volume qualities to be produced from the low protein wheat and also help produce bread with fine crumb structure.

### 2.1.1 Toxic Effect of Potassium Bromate

Potassium bromate is a toxic substance and has various health effects. However, literature reveals that Potassium bromate causes abdominal pain, diarrhoea, nausea, vomiting, kidney failure, oligonuria, anuria, deafness, vertigo, and hypotension, depression of the central nervous system, thrombocytopenia with other related health problems (Johnson *et al.*, 2015). Emeje *et al*. (2010) reported that Potassium bromate also affects the nutritional quality of bread by degrading the vitamins and essential fatty acid contents of flour and degrades vitamins A2, B1, B2, E and niacin which are commonly found in bread.

Moreover, International Agency for Research on Cancer (IARC) has classified it as a possible carcinogen to humans. It has acute and chronic health effect on man.

The acute (short term) health effects may occur immediately or shortly after exposure to potassium bromate. Contact can irritate and burn the skin and eye, breathing potassium bromate can irritate the nose, throat and lungs causing coughing, wheezing and or shortness of breath. Chronic (long term) effect of exposure to potassium bromate can last for months or year, Studies has shown kidney, thyroid and gastrointestinal cancer in animals fed with bread containing potassium bromate (Kurokawa *et al.,* 1990). Many scientists believe there is no safe level of exposure to carcinogens and that such a substance may also have the potential for causing reproductive damage to human (Kurokawa *et al.,* 1990).

## 2.2 Heavy Metal

Heavy metal can be defined as any metallic element that has a relatively high density when compared to its size (Singh *et al*., 2011). They are potential environmental contaminants with the capability of finding their ways into the human food and causing human health problems. They are given special attention throughout the world due to their common existence 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 (Duffus, 2002). Heavy metals include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium(Cr), copper (Cu), selenium (Se), nickel (Ni), silver (Ag) and zinc (Zn). The less common metallic contaminants include aluminium (Al), cesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), strontium (Sr), and uranium(U) (Mcintyre, 2003), why the metalloids are arsenic, silicon, boron, germanium,

antimony and tellurium that are able to induce toxicity at low level of exposure (Singh et al., 2011). Cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Duffus, 2002)

### 2.2.1 Heavy Metal Toxicity

Heavy metals are toxic as they are not metabolized by the body and as such accumulate in the tissues. Compounds accumulate in living things any time they are taken up and stored faster than they are metabolized or excreted (Mcintyre, 2003). The level of toxicity of these substances which is the extent of adverse effects caused by the substances, varies according to the concentration, duration and location of exposure to the metal, as well as the specific response of the organism (Hague, 2008). The concentration in a human system which causes unhealthy effects depends on several factors including the route of entry of the metal whether it was inhaled in form of dust, ingested or got in contact with the human skin.

Heavy metals such as copper, nickel, chromium, manganese, zinc and iron are essential in very low concentrations for the survival of all forms of life. But when present in large quantities these cause metabolic anomalies. In contrast, heavy metals such as Lead (Pb) and Cadmium (Cd) are well known as toxic when their intake through ingestion or inhalation is excessive. The deficiencies, excesses or imbalances in the supply of inorganic elements from dietary sources can have destructive influence on human health (Oforka et al., 2014). Since 1973, As, Cu, Cd, Fe, Hg, Pb and Zn have been considered by the Joint FAO/WHO Codex Alimentarius Commission to be potentially toxic for human diet (USEPA, 1984).

#### 2.2.1.1 Cadmium

Cadmium and its compound are classified as Group 1 carcinogens for humans by the International Agency for Research on Cancer (1999). Cadmium can cause both acute and chronic intoxication (Chakraborty *et al*., 2013). Cadmium is highly toxic to the kidney and it accumulates in the proximal tubular cells in higher concentration. On very long exposure time at lower concentration, it can become deposited in the kidney and finally lead to kidney disease, fragile bones and lung damage (Henson & Chedrese, 2004). The permissible level of Cadmium in food is 0.05 mg/kg (FSA, 2009).

#### 2.2.1.2 Zinc

Zinc is the most abundant trace element in the cytoplasm of humans, but 90 percent of the mineral is located in the muscle, bone and liver (Khaniki, 2005). It makes strong exchangeable complexes with organic molecules, proteins, nucleic acids and membranes. Zinc requirements are highest during periods of greatest growth such as pregnancy, infancy and early childhood (Prasad, 1984). Mahindru (2004) reported that Zinc toxicity may result from excessive ingestion of the element in food or drink although the margin of safety is large. The tolerable level of Zinc in food is 50mg/kg (Alimentarius, 2003).

#### 2.2.1.3 Copper

Copper is an essential trace metal, vitally important for both physical and mental development in human, usually found in many food types particularly vegetarian foods such as nuts, seeds and grains. It is important for energy production in cells and is required for women's fertility in relation to estrogen metabolism amongst others. However, copper accumulates easily in the body, hence, high level intakes of heavy

metals have damaging effects on human beings and animals, since there is no good mechanism for their elimination (Mahindru, 2004). Conditions associated with increased copper body burden are arthritis, fatigue, insomnia, scoliosis, osteoporosis, heart disease, cancer, migraine, heart seizures, gum diseases, skin and hair problems. Reported also are mentaland emotional disorders such as depression mood swings, fears, anxiety, panic attacks, violence and memory loss (Singh *et al.*, 2011). Permissible level of copper in food is 10 mg/kg body weight (Alimentarius, 2003).

#### 2.2.1.4 Nickel

Nickel is also an essential trace element whose role in the body is not yet fully known, it is contained in many body tissues. Ordinarily, nickel is not known to be toxic to the body as the body does not readily absorb it (Ahmed & Fadel, 2012). However, Nickel can combine with carbon monoxide (CO) to form a complex, Nickel tetracarbonyl ($Ni(CO)_4$), which is extremely poisonous, even more than CO. Elevated dietary intake of the metal is associated with increased thyroid problems, cancer and heart disease (Duffus, 2002). The WHO (1994) recommended 0.1-0.3 mg/kg body weight of Nickel for daily intake (Alimentarius, 2003)

#### 2.2.1.5 Lead

Lead serves no useful purpose in the body and its toxicity affects virtually every organ in the human body. Its ability to mimic or inhibit calcium affects the actions of calcium-dependent or related processes. Lead also interacts with proteins such as sulfhydryl, amine, phosphate and carboxyl groups. The toxic metal primarily affects the peripheral and central nervous systems, renal functions, blood cells, metabolism of vitamins D. It

is associated with hypertension, reproductive toxicity, developmental effects and neurological disorders, some of which may be irreversible (Duffus, 2002). Permissible limit of lead in food ranges from 0.2-2.5 mg/kg (FAO, 2007)

#### 2.2.1.6 Chromium

Chromium exists in two states trivalent and hexavalent (Sakar, 2006).Trivalent chromium typically found in foods and supplements has low toxicity, hexavalent chromium is known toxin, Long term exposure to hexavalent chromium may lead to skin problems, nasal problems and lung cancer (Sarkar, 2005).Although chromium has been suggested for many condition regarding its effectiveness .Chromium is available as a supplement in several forms such as chromium yeast and chromium picolinate, chromium has known benefit for people with either high and low blood sugar. The permissible limit in food is 0.05-0.2 mg/kg in adult and 0.02-0.0 6mg/kg in children (WHO, 2007).

#### 2.2.1.7 Iron

Iron constitutes 5% of the earth crust. It ranks fourth in abundance after oxygen, silicon and aluminum (underwood, 1977). Iron is the most important transition metal in the animal body, where it occurs almost entirely in elaborate co-ordination compounds based on the porphyrin nucleus, notably the haem pigments, which carry oxygen (Coultate, 1994) . A deficiency of iron is manifested as anaemia, while excess iron intake may lead to cirrhosis of liver and haemochromatosis (Fifield & Haines, 1995). The recommended daily dietary allowance for iron is 18mg for adults and 10 – 15mg for children (WHO, 2007).

### 2.2.2 Possible Contamination Sources during bread Production

The possible contamination sources along the processing line is represented in Figure 2.2 (Khaniki *et al.*, 2015).

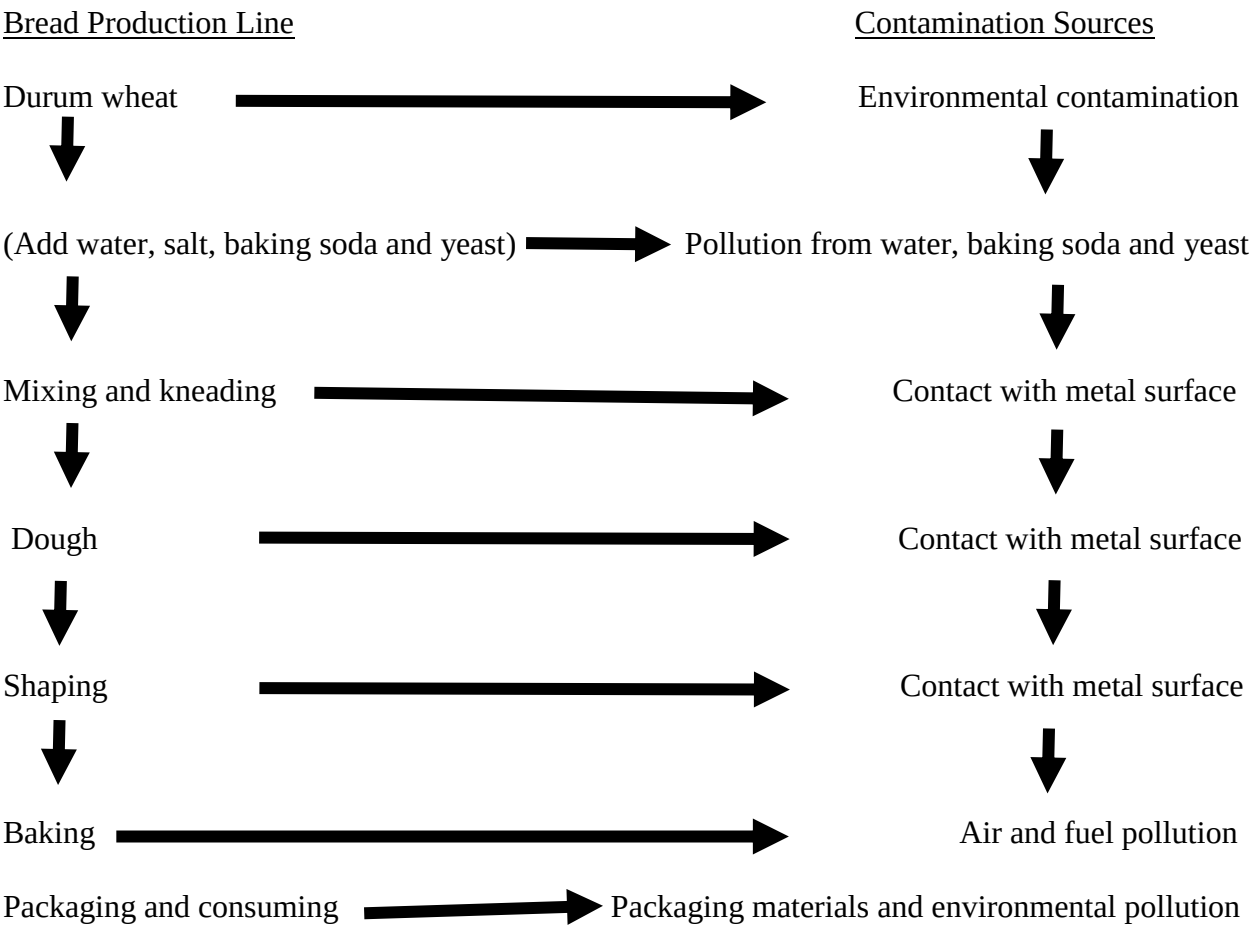



Figure 2.2: Production line and sources of Contaminants during Bread Production

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Area

The study area was Keffi, local government area of Nasarawa State, Nigeria. Geographically, Keffi lies within located 8$^{o}$ 85$^{'}$N latitude and 7$^{o}$ 87$^{'}$N and 6$^{o}$23$^{'}$E and 6$^{o}$ 41$^{'}$E longitude. It is situated elevation is 321meters above sea level with an area of 138km$^{2}$ as shown in Figure 3.1.It has a population of 92,664 at the 2006 census (Nagis, 2019)

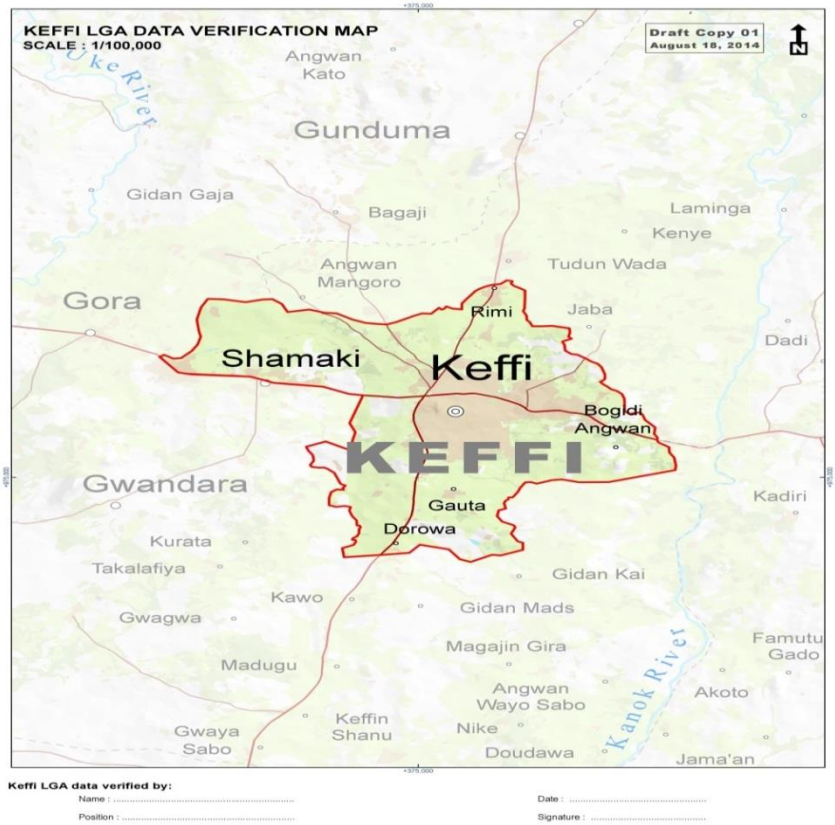



Figure 3.1: Map of Keffi and its Environs

## 3.2 Materials

The materials and reagents used in this research include; colorimeter, atomic absorption spectrophotometer, oven, weighing balance, volumetric flask, filter paper, beakers, hot plate, spatula, filter paper, sample bottles, funnel, nose mask, hand gloves, agate mortar and pestle, hydrochloric acid, perchloric acid, potassium iodide, potassium bromate and samples of bread from different bakeries.

## 3.3 Methods

### 3.3.1 Sample Collection

Freshly prepared bread samples were purchased directly from ten different bakeries in Keffi - Nasarawa State and were taken to the laboratory. All samples were coded with letters A-J to avoid bias. Table 3.1 shows the description of the bread samples purchased from the bakeries.

**Table 3.1: Description of the Bread Samples**

| Code | Regulatory NAFDAC Status | Loaf type | Manufacturer's Specification |
|---|---|---|---|
| A | Not Registered | Unsliced | Bromate free |
| B | Registered | Unsliced | Bromate free |
| C | Registered | Unsliced | Bromate free |
| D | Registered | Unsliced | Bromate free |
| E | Registered | Unsliced | Bromate free |
| F | Registered | Unsliced | Bromate free |
| G | Registered | Unsliced | Bromate free |
| H | Registered | Unsliced | Bromate free |
| I | Registered | Unsliced | Bromate free |
| J | Registered | Unsliced | Bromate free |

### 3.3.2 Sample Preparation

The bread sample was sliced into pieces with a clean table knife and 20 g portion was accurately weighed and dried at constant temperature in an oven at 55 $^{o}$C for 24 hrs. The crust was grounded to a fine powder using agate mortar and pestle, and then stored in air tight containers prior to analysis (Johnson *et al.*, 2015). The same preparation procedure was applied for all the ten bread samples.

### 3.3.3 Preparation of Potassium Bromate Stock Solution

A stock solution of 1000 mg/L of standard solution of potassium bromate was prepared using the method reported by Emeje *et al*. (2010). 1.0 g of Potassium bromate was weighed and poured into 1000 $cm^3$ volumetric flask, then mixed with distilled water up to mark. The working a liquots of 1, 2, 3, 4 and 5mg/L from the stock solution of the potassium bromate were prepared using serial dilution and were used for the construction of a calibration curve for the determination of potassium bromate in the bread samples.

### 3.3.4 Digestion of Sample

The powdered sample of the bread sample (1.0g) was weighed and placed in a 100 $cm^3$ beaker which was labeled according to the sample number. Concentrated $HNO_3$ ($15cm_3$) was added and the contents were mixed gently and placed on a thermostated heating mantle at 100 $^0C$ for about 1hr and then 5 $cm^3$ of 0.1M HCl added to the mixture and digested further for about 30 mins, until a clear colorless digest was obtained. The mixture was removed from the heating mantle and allowed to cool and filtered into a 100 $cm^3$ volumetric flask using a whatman filter paper and made up to mark with distilled water, prior to heavy metal analysis. The same experimental procedure was applied to each of the ten bread samples.

### 3.3.5 Determination of potassium bromate in bread sample

The method reported by Emeje *et al.* (2010) was adopted for the determination of potassium bromate in the bread samples. Fine powder of bread sample (1.0 g) was weighed and placed into a test tube and 10 $cm^3$ of distilled water was added into the test tube. The mixture was then shaken vigorously and allowed to stand for 20 mins, at a

temperature of 28 ± 10ºC. The resulting solution (5 $cm^3$) was decanted into another test tube with the addition 5$cm^3$ of 0.5% potassium iodide solution in 0.1M hydrochloric acid. A color change from yellow to purple indicated the presence of bromate. The quantitative determination of potassium bromate was done by generating a calibration curve using aliquots of potassium bromate solution of varying concentrations. Thus, the absorbance characteristics of the colour developed were determined at 620 nm on the UV-VIS spectrophotometer (2550 shimadzu). The absorbance values were used to estimate the corresponding concentrations of the potassium bromate in the bread sample using calibration curve. All the experiments were carried out in triplicates.

### 3.3.6 Heavy Metal Measurement

The absorbance of the blank, standards and the digested sample solutions were measured on Atomic Absorption Spectrophotometer (Model AA 6800 shimadzu japan) using an air-acetylene flame. The instrument was then standardized using the standard solutions and the blanks, the concentrations of the sample solution were read on the instrument and the concentrations of each element in the sample were then calculated using

$$[M] = \frac{CxV}{W} \quad \text{----------} \quad (2)$$

where:

[M] is the concentration (mg/kg) of the analyte in the sample,

C is the concentration of analyte in the analytical sample in mg/l,

V is the volume of the analytical sample in ml and

W is the weight of the sample in g.

# CHAPTER FOUR

# DATA PRESENTATION AND ANALYSIS

## 4.1 Data Presentation

The results of the bromate levels in the bread samples is presented in Table 4.1, while Table 4.2 is the reference table of bromate levels in state/country. Table 4.3 shows the concentrations of heavy metal levels in bread samples, while Table 4.4 is the reference.

Table 4.1: Potassium bromate levels in bread samples produced in Keffi Nasarawa State

| Bread Samples | $KBrO_3$ (mg/kg) |
|---|---|
| A | 24.3±0.5 |
| B | 7.40±0.6 |
| C | 7.72±0.6 |
| D | 2.82±0.4 |
| E | 6.85±0.7 |
| F | 7.38±0.3 |
| G | 10.68±0.3 |
| H | 3.30±0.1 |
| I | 7.03±0.0 |
| J | 6.25±0.5 |

Letter A to J represents the code for different loaves of bread samples analyzed. Values represent mean ± SD of 3 replicate determinations

Table 4.2: A Reference Table of Potassium Bromate levels (mg/kg) in bread in states/countries

| Town/City | Potassium Bromate levels (mg/kg) | Source |
|---|---|---|
| Ile –Ife, Nigeria | 4.025-41.336 | Oyekunle *et al*., 2011. |
| Zaria, Nigeria | 2.46-16.46 | Magomya *et al.,* 2013. |
| Portharcourt, Nigeria | 0.45-6.40 | Obunwo & konne, 2014. |
| Gwagalada (Abuja), Nigeria | 3.60- 9.20 | Alli *et al.,* 2013. |
| Nsukka (Enugu), Nigeria | 2.36-12.660 | Emeje *et al.,* 2015. |
| Addis-Abbaba, Ethopia | 5.615-9.974 | Ergetie & Hymete, 2012. |
| Present Study (Keffi) Nigeria | 2.82-24.3 | |

Table 4.3: Concentrations of Some Heavy Metals in Bread Sample (mg/kg)

| Bread sample | Pb | Cd | Fe | Cu | Cr | Ni | Zn |
|---|---|---|---|---|---|---|---|
| **A** | ND | ND | 35.83±0.0 | 0.52±0.0 | ND | 1.074±0.0 | 1.89±0.0 |
| **B** | ND | 0.13±0.0 | 29.10±0.0 | 0.06±0.0 | ND | 0.691±0.0 | 3.31±0.0 |
| **C** | ND | 0.14±0.0 | 50.74±0.0 | ND | 4.83±0.0 | 1.196±0.0 | 3.31±0.0 |
| **D** | ND | 0.22±0.0 | 12.65±0.0 | ND | 5.70±0.0 | 0.798±0.0 | 2.41±0.0 |
| **E** | 0.066±0.0 | 0.23±0.0 | 37.18±0.0 | 0.8±0.0 | 3.86±0.0 | 1.037±0.0 | 1.13±0.0 |
| **F** | 0.100±0.0 | 0.32±0.0 | 175.68±0.00 | 0.5±0.0 | 6.78±0.0 | 0.209±0.0 | 1.50±0.0 |
| **G** | 1.20±0.1 | 0.08±0.0 | 1156.9±0.0 | ND | 0.81±0.0 | 0.403±0.0 | 1.75±0.0 |
| **H** | 1.80±0.0 | ND | 8.76±0.0 | ND | ND | 0.956±0.0 | 2.35±0.0 |
| **I** | 1.30±0.0 | 0.20±0.0 | ND | 0.06±0.0 | 2.49±0.0 | 0.647±0.0 | 2.75±0.0 |
| **J** | 1.60±0.0 | 0.31±0.0 | 31.03±0.0 | ND | 7.41±0.0 | 0.699±0.0 | 2.40±0.0 |

ND - Not Detected.

Table 4.4 A Reference Table of Metal Concentration (mg/kg) in bread in Town/City

| Town/City | Pb | Cd | Ni | Fe | Cr | Cu | Zn | Source |
|---|---|---|---|---|---|---|---|---|
| Ile ife/Nigeria | 0.03-0.10 | _ | _ | _ | _ | 0.23-0.46 | 2.23-6.63 | Oyekunle *et al.*,2011 |
| Zaria/Nigeria | 0.34-3.13 | 0.013-0.098 | _ | 0.53-8.45 | | 0.13-0.66 | 0.24-2.11 | Magomya *et al.*,2013 |
| Lagos/Nigeria | 0.00-1.26 | 0.01- 0.10 | _ | _ | _ | _ | 1.18-3.12 | Dada *et al.*,2017 |
| Isfahan/Iran | 0.375-2.89 | 0.006-0.019 | 0.00625-0.2125 | 19.31-47.45 | | 1.98-3.25 | 1.56-6.1 | Feyzi *et al.*, 2017 |
| Present Study | 0.66-1.6 | 0.08-0.32 | 0.691-1.074 | 12.65-1156 | 0.81-7.41 | 0.06-0.8 | 1.13-3.31 | |

## 4.2 Data Analysis

The results in Table 4.1 show that all the investigated bread samples contained potassium bromate. The concentration of bromate obtained in all the bread samples was higher than the 0.02 mg/kg permissible limit of bromate in bread according to the US Food and Drug administration FDA (Ekpo *et al*., 2008). These findings were in agreement with that of Oyekunle *et al.* (2011) who reported bromates concentrations of 4.025-41.336 mg/kg in some analysed bread samples in Ile-Ife, in the southern part of Nigeria. Other researchers; Magmoya *et al.* (2013) who analysed some bread samples in Zaria metropolis Kaduna State reported the values of 2.46-16.46 mg/kg of bromates concentrations, Alli *et al.* (2013) reported bromates concentrations in bread samples within Gwagalada area of FCT ranged from 3.6-9.2 mg/kg and in the Eastern part of Nigeria, Obunwo & Konne (2014) reported values within 0.45-6.40 mg/kg in bread samples. It was observed from Table 4.1 that the bromate concentrations (2.81-24.5 mg/kg) obtained in this study were higher than the values reported by various authors (Magogya et al, 2013, Dda et al, 2017 and Fayzi et

al, 2017), except for the concentrations reported for Ile-Ife (4.025-41.336 mg/kg). as reported by Oyekunle *et al*; 2011.

The variations in the bromate concentrations obtained could be attributed mainly to the concentration of bromate applied in the bread during the course of production by the different bakers. Also, the high level of bromate found in the analysed bread samples implied that there is non-compliance to the ban placed on the use of bromate in bread making by NAFDAC in Nigeria. This is worrisome considering the health challenges potassium bromate poses on human health and the fact that bread is a staple food that is mostly consumed by people of different socio-economic status and that many work in this bakeries.

The results of heavy metal concentration obtained from the analysed bread samples are presented in Table 4.2.

Lead in samples E, F, G, H, I, and J, had concentrations ranging from 0.66 mg/kg in sample E to 1.80 mg/kg in sample H. This implies that 60 % of the bread samples contained lead. However, the lead concentration detected in 60 % of the bread samples is within the permissible limit of 0.2 – 2.5 mg/kg (FAO, 2007). This implies that all the bread samples were relatively free from lead associated ailments such as cancer, interference with Vitamin D metabolism and poor mental development in infants (Singh *et al.*, 2011).

The values obtained for cadmium ranged from 0.08 mg/kg in sample G to 0.32 mg/kg in sample F. The result also shows that only samples A and H contained no cadmium which represents only 20 % of the bread samples. However, samples B, C, D, E, F, G, I and J contained Cadmium which was found to be a bit higher than the permissible limit in food

(FSA, 2009). This could be attributed to the contamination sources such as water, raw materials and contact with metal during bread production process (Khaniki *et al.,* 2005).

The result shows that 90 % of the bread samples (A, B, C, D, E, F, G, H and J) contained iron with concentrations ranging from 12 mg/kg in sample D to 1156.9 mg/kg in sample G, except for sample I which iron was not detected. The values obtained in this study were found to be higher than the permissible limit (10-18mg) in food (FAO, 2007) and also higher than the values reported by Magmoyma *et al.*(2013) in Zaria metropolis (0.53 – 8.45 mg/kg) and Feyzi *et al.*(2017) in Iran (19.31 – 47.45 mg/kg).The high level of Iron recorded might be due to contact of the bread with metal and water source used in the course of bread production(Khaniki *et al.,* 2005). However, high concentrations of iron can cause stomach and intestinal side effects such as nausea and vomiting. It may also cause high levels of hemoglobin in the blood (Manhindru, 2004). This suggests that high consumption of most of the bread samples may likely cause some of these health challenges. The result for copper shows that samples A, B, E, F and I contained copper level which ranged from 0.06 mg/kg in sample B to 0.80 mg/kg in sample E. These values obtained were below the permissible limit for copper in food (Alimentarius, 1994). This result is in agreement with that obtained by Oyekunle *et al.* (2011).

The chromium levels detected in samples C, D, E, F, G, I and J ranged from 0.81 mg/kg in sample G to 6.78 mg/kg in sample J. This also implies that 70 % of the bread samples contained chromium, whereas 30 % of the bread samples (A, B and H) contained no chromium. The values obtained for the samples, C, D, E, F, G, I and J, were found to be higher than the maximum permissible limit (FAO, 2003). This suggests that these samples are not safe for consumption. High level of chromium have been linked to cancer

and neurological disorder (SON, 2007). The result for zinc shows that all the bread samples contained traces of zinc which ranged from 1.13 mg/kg in sample E to 3.31 mg/kg in sample B; and were found to be within the permissible limit in foods (USDA, 2003), but higher than the level of 0.03 – 0.10 mg/kg reported by Oyekunle *et al.* (2011) in bread sample within Ile Ife, Nigeria, and 1.18 – 3.12 mg/kg as reported by Dada *et al.* (2017). The differences in the values obtained might be due to both experimental and environmental factors.

The result equally shows that all the bread samples contained nickel which ranged from 0.691-1.074 mg/kg, sample B having the maximum concentration of 1.074 mg/kg and sample A having the minimum concentration of 0.691 mg/kg. These concentrations are higher when compared with 0.1–0.3 mg/kg recommended by World Health Organization (1994).

# CHAPTER FIVE

# SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 5.1 Summary

The levels of potassium bromate and heavy metals were determined in ten bread samples produced in Keffi, Nasarawa state to ascertain the safety of its consumption and the extent of bakers compliance to the ban placed on potassium bromate due to the adverse effect it has on human health. The bread samples were freshly purchased directly from ten different bakeries in Keffi-Nasarawa State for analysis. The bromate concentrations were determined using UV-VIS spectrophotometer (2550Model). Some portion of the samples were also digested and subjected to Atomic Absorption Spectrophotometer (Model AA 6800 shimadzu japan) analysis for heavy metal concentrations. The results indicate that the ten bread samples produced investigated contained potassium Bromate and some heavy metals above permissible limit, this worrisome considering the fact that bread is considered a staple food consumed by most people and the adverse effect potassium bromate and heavy metals pose on human health.

## 5.2 Conclusion

The result of this study revealed the presence of potassium bromate which was above the safe limit (2.82-24.3 mg/kg) for human consumption in all the bread samples. Heavy metals were present in some of the bread samples. The level of lead detected in the bread sample was within the WHO permissible limit (0.66- 1.80 mg/kg), however, the detected level in cadmium (0.08-0.32mg/kg) was above the permissible limit in sample B, C, D, E, F, G, I. Most of the bread samples contained iron, copper, chromium, nickel and zinc within tolerable level for human. Thus, bread consumers and bakers are at risk of exposure to potassium bromate and some heavy metals with health implications.

## 5.3 Recommendations

This research work recommends the following:

i. There should be a continuous surveillance and enforcement of the ban on the use of potassium bromate in bakeries by relevant agencies especially NAFDAC,

ii. Bakers should be mandated to comply with set standards of Good Manufacturing Practice and Hazard Analysis Critical Control guidelines acceptable worldwide, penalty should be enforced on defaulters.

iii. The use of alternative bread improvers such as ascorbic acid, a non-toxic oxidizing agent and also enzymes such as hemicellulases (volume enchasing), gluthathione oxidase (protein strengthening) and exo-peptidase (improves color and flavor) should also be encouraged as a replacement to potassium bromate in bakeries.

iv. The availability of a quality control laboratory with simple and low cost procedures for detecting bromate and heavy metals should be designated for NAFDAC approved for bakery.

v. Government at all level should also ensure its citizens are informed through mass enlightenment campaign on the dangers of production and consumption of bromated bread. Flour manufacturers and bakers should also avoid practices that may increase metal concentration.

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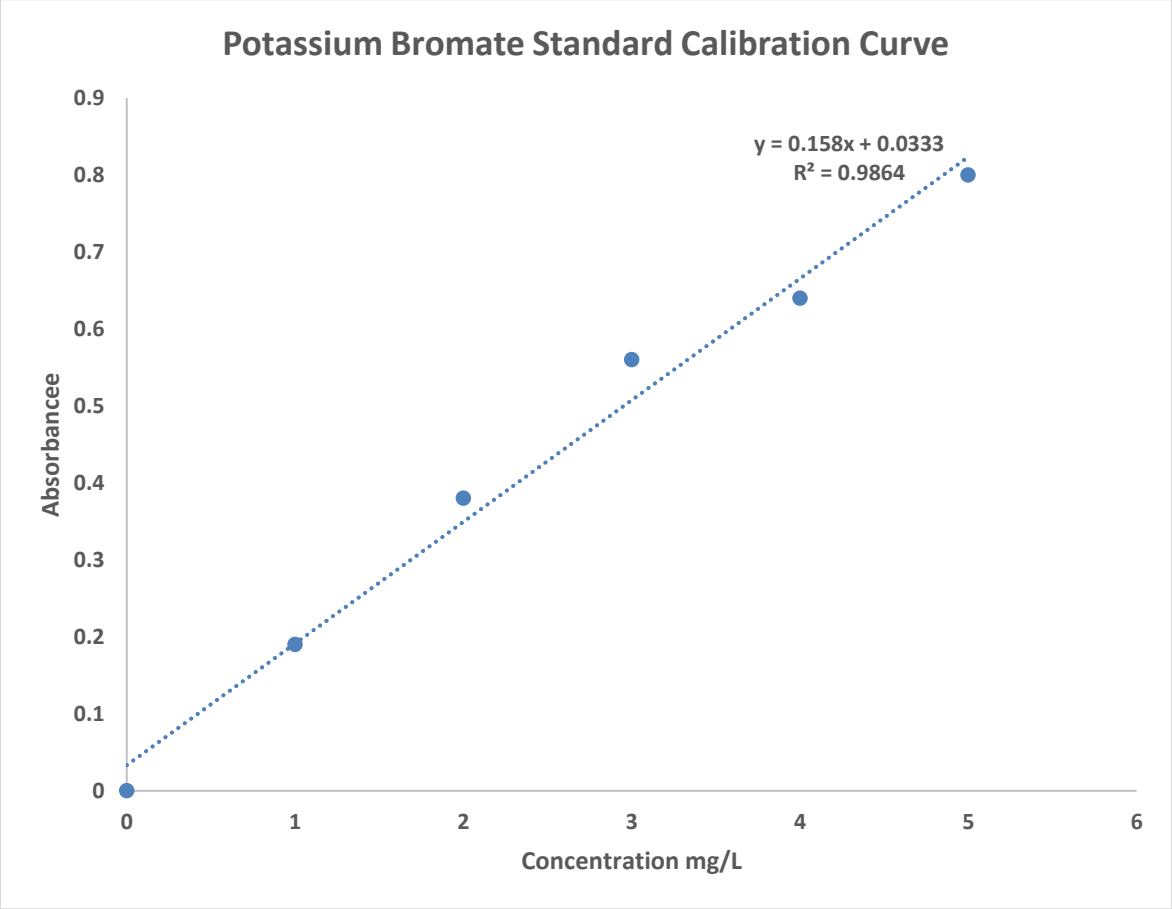
# APPENDICES

## APPENDIX $A_1$

### Concentration and absorbance of potassium Bromate standard solution

| CONCENTRATION(ppm) | ABSORBANCE |
|---|---|
| **0** | 0 |
| **1** | 0.19 |
| **2** | 0.38 |
| **3** | 0.56 |
| **4** | 0.64 |
| **5** | 0.8 |

## APPENDIX $A_2$


Potassium Bromate Standard Calibration Curve
y = 0.158x + 0.0333
R² = 0.9864
Absorbancee
Concentration mg/L
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6

## APPENDIX B

## ABSORBANCE OF BREAD SAMPLES

| Sample | $A_1$ | $A_2$ | $A_3$ | Ă±S.D |
|---|---|---|---|---|
| **A** | 0.822 | 0.789 | 0.805 | 0.805±0.017 |
| **B** | 0.277 | 0.246 | 0.278 | 0.278±0.018 |
| **C** | 0.295 | 0.279 | 0.256 | 0.256±0.032 |
| **D** | 0.110 | 0.122 | 0.134 | 0.122±0.040 |
| **E** | 0.247 | 0.288 | 0.271 | 0.228±0.012 |
| **F** | 0.256 | 0.267 | 0.276 | 0.298±0.044 |
| **G** | 0.368 | 0.380 | 0.364 | 0.380±0.079 |
| **H** | 0.130 | 0.136 | 0.141 | 0.136±0.006 |
| **I** | 0.254 | 0.255 | 0.256 | 0.255±0.001 |
| **J** | 0.215 | 0.231 | 0.246 | 0.231±0.016 |

Calculating the concentration of each bread sample using equation y=mx+c

Where y=Absorbance

m is the slope

x is the concentration of bromate in the bread sample

c is the intercept

From the Potassium Bromate calibration curve plotted y=0.158x+0.0333

## APPENDIX C

Concentration of potassium Bromate in Bread Samples (mg/L)

| **Sample** | **$C_1$** | **$C_2$** | **$C_3$** | **$\hat{C}$±S.D** |
|---|---|---|---|---|
| A | 4.99 | 4.78 | 4.89 | 4.90±0.105 |
| B | 1.54 | 1.35 | 1.55 | 1.48±0.113 |
| C | 1.66 | 1.56 | 1.41 | 1.54±0.126 |
| D | 0.49 | 0.56 | 0.64 | 0.56±0.075 |
| E | 1.37 | 1.23 | 1.51 | 1.37±0.14 |
| F | 1.41 | 1.48 | 1.54 | 1.48±0.065 |
| G | 2.12 | 2.20 | 2.09 | 2.13±0.057 |
| H | 0.61 | 0.65 | 0.68 | 0.65±0.035 |
| I | 1.40 | 1.41 | 1.41 | 1.40±0.006 |
| J | 1.15 | 1.25 | 1.35 | 1.25±0.10 |

Converting mg/l to mg/kg

$$M=\frac{\text{Concentration of potassium bromate in mg/L} * \text{Volume of Sample in ml}}{\text{Sample weight (Kg)} * 1000}$$

Where volume of sample =5ml

Sample weight=1g

M is the concentration of potassium bromate in mg/kg

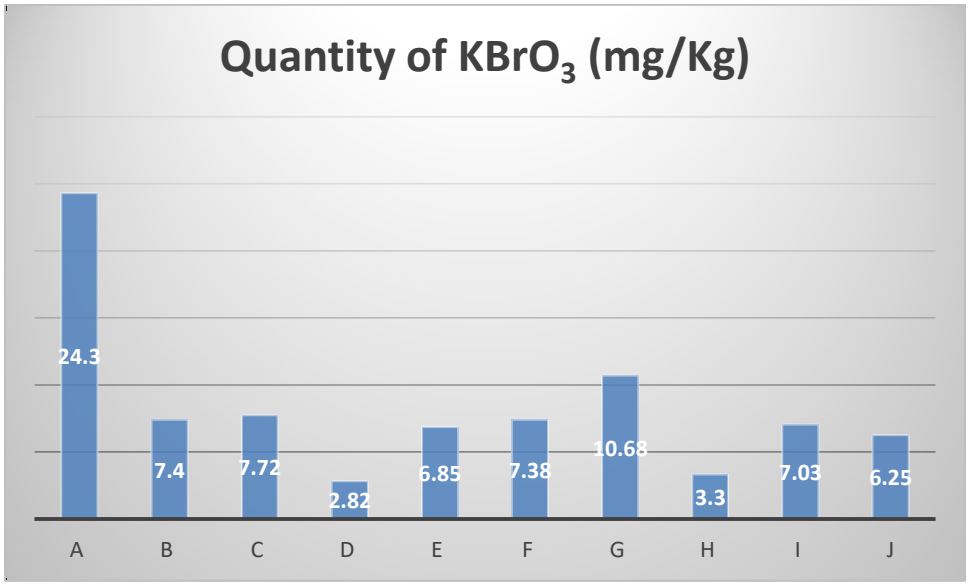
## APPENDIX D

Concentration of potassium Bromate in Bread Samples (mg/kg)

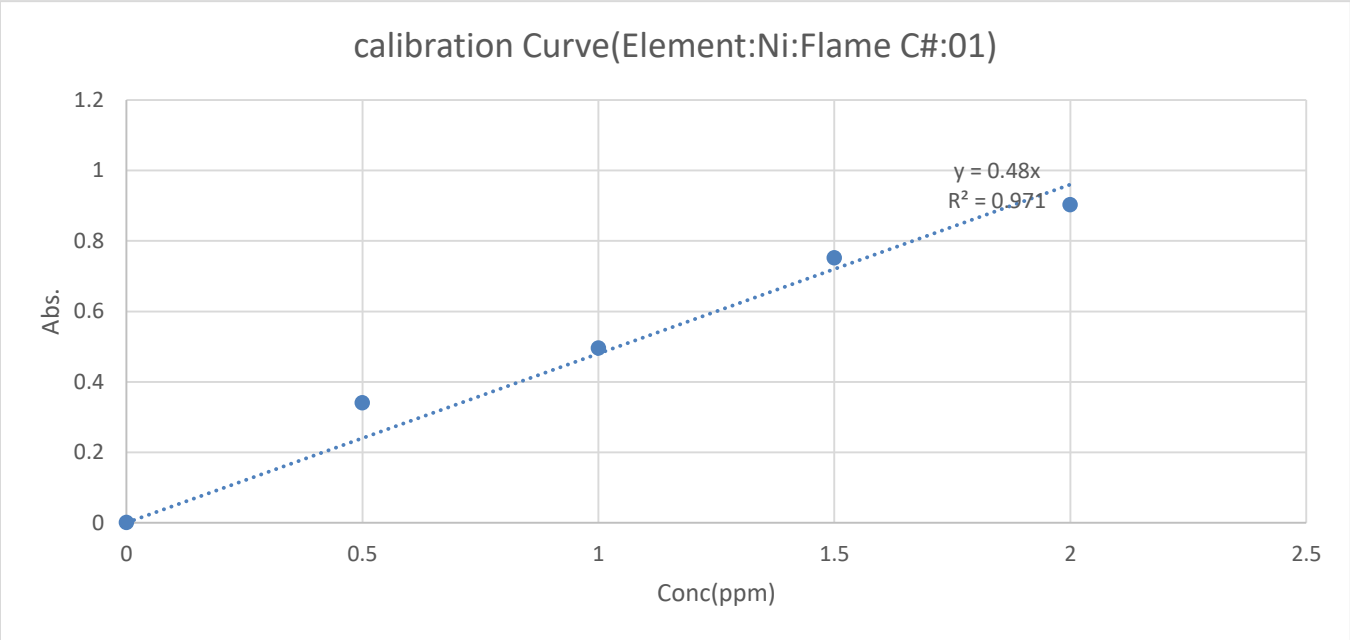
| **Sample** | **$C_1$** | **$C_2$** | **$C_3$** | **$\hat{C}$±S.D** |
|---|---|---|---|---|
| A | 24.95 | 23.90 | 24.45 | 24.3±0.525 |
| B | 7.70 | 6.75 | 7.75 | 7.40±0.563 |
| C | 8.30 | 7.80 | 7.05 | 7.72±0.629 |
| D | 2.45 | 2.80 | 3.20 | 2.82±0.375 |
| E | 6.85 | 6.15 | 7.55 | 6.85±0.70 |
| F | 7.05 | 7.40 | 7.70 | 7.38±0.325 |
| G | 10.6 | 11.0 | 10.45 | 10.68±0.284 |
| H | 3.25 | 3.25 | 3.40 | 3.30±0.087 |
| I | 7.00 | 7.05 | 7.05 | 7.03±0.0289 |
| J | 5.75 | 6.25 | 6.75 | 6.25±0.50 |

## APPENDIX E

Concentration of Bromate for Bread Sample A-J



## APPENDIX $F_1$



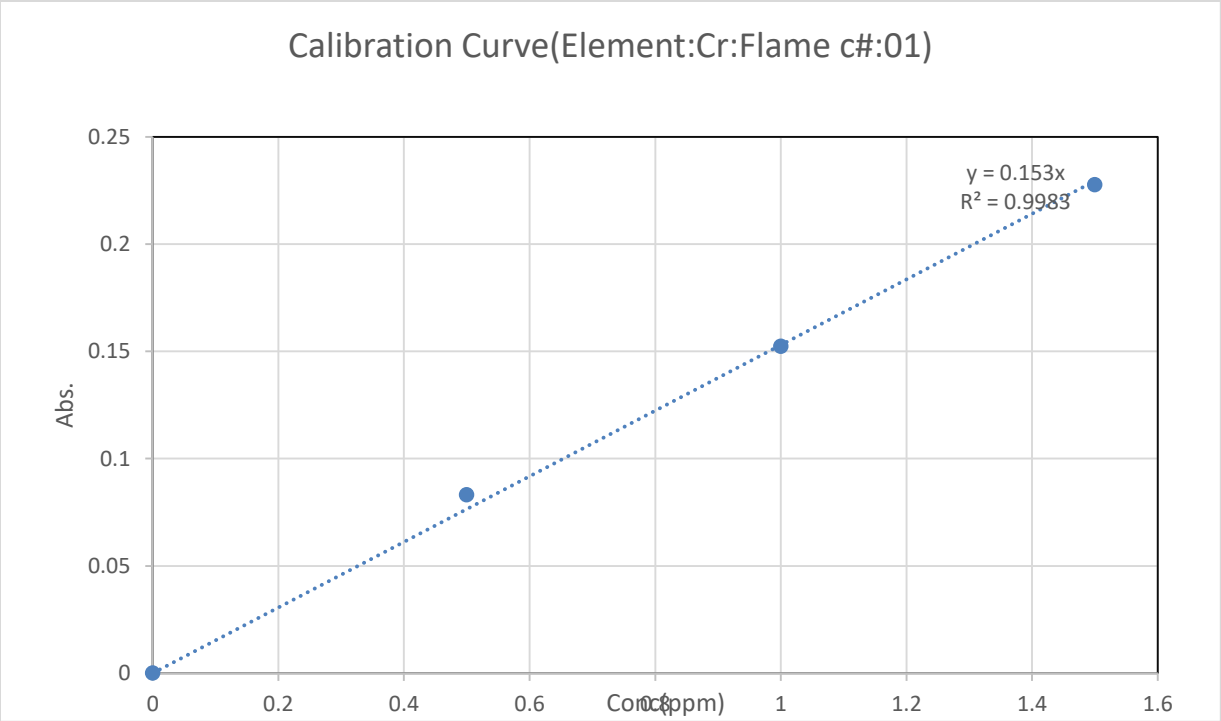

| CONC | ABS |
|---|---|
| 0.5 | 0.34 |
| 1 | 0.496 |
| 1.5 | 0.752 |
| 2 | 0.903 |
| 2.5 | 1.071 |

## APPENDIX $F_2$

CONCENTRATION OF NICKEL IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_1$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|---|
| **A** | 1.0745 | 1.0742 | 1.0739 | 1.0742±0.03 |
| **B** | 0.607 | 0.609 | 0.602 | 0.606±0.04 |
| **C** | 1.1975 | 1.193 | 1.1945 | 1.195±0.002 |
| **D** | 0.7984 | 0.789 | 0.7902 | 0.79253±0.005 |
| **E** | 1.0374 | 1.0364 | 1.037 | 1.03693±0.0005 |
| **F** | 0.2086 | 0.205 | 0.2061 | 0.20657±0.018 |
| **G** | 0.4206 | 0.4107 | 0.418 | 0.41643±0.005 |
| **H** | 0.9558 | 0.955 | 0.9549 | 0.95523±0.0005 |
| **I** | 0.645 | 0.646 | 0.648 | 0.64633±0.002 |
| **J** | 0.6996 | 0.6998 | 0.6999 | 0.69977±0.002 |

## APPENDIX $G_1$



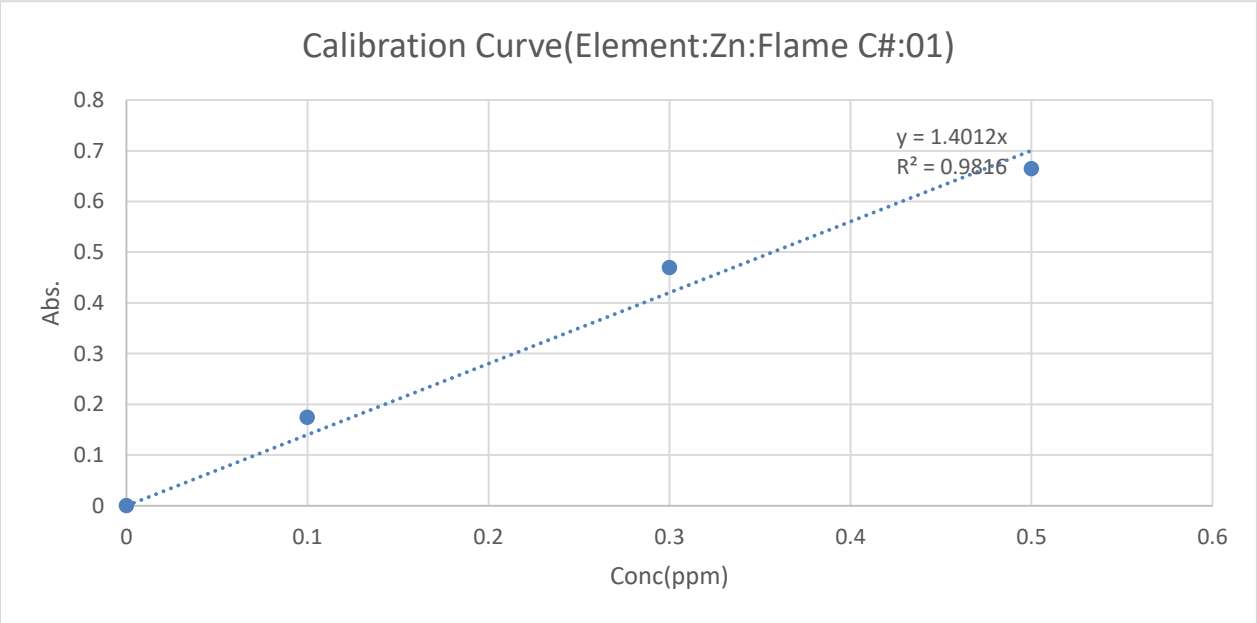

| CONC | ABS |
|---|---|
| 0.500 | 0.0831 |
| 1.00 | 0.1523 |
| 1.500 | 0.277 |

## APPENDIX $G_2$

CONCENTRATION OF CHROMIUM IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_1$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|---|
| **A** | ND | ND | ND | ND |
| **B** | ND | ND | ND | ND |
| **C** | 0.048 | 0.0486 | 0.0484 | 0.0483±0.0003 |
| **D** | 0.0563 | 0.0565 | 0.0582 | 0.057±0.001 |
| **E** | 0.0398 | 0.379 | 0.0381 | 0.0386±0.001 |
| **F** | 0.067 | 0.0675 | 0.0689 | 0.0678±0.001 |
| **G** | 0.008 | 0.0079 | 0.0084 | 0.0081±0.0003 |
| **H** | ND | ND | ND | ND |
| **I** | 0.0252 | 0.0245 | 0.025 | 0.0249±0.0004 |
| **J** | 0.0741 | 0.074 | 0.0742 | 0.0741±0.0001 |

## APPENDIX $H_1$



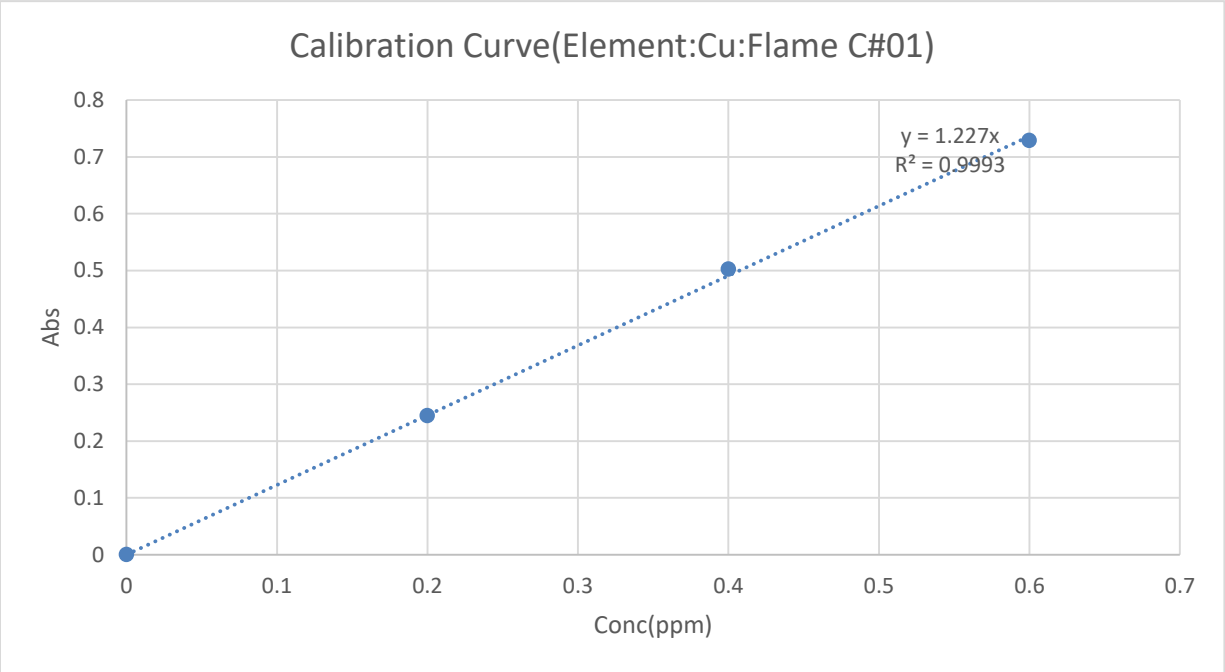

| CONC | ABS |
|---|---|
| 0.1 | 0.1741 |
| 0.3 | 0.4694 |
| 0.5 | 0.6644 |

## APPENDIX $H_2$

CONCENTRATION OF ZINC IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_1$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|---|
| **A** | 0.0187 | 0.0185 | 0.0195 | 0.0189±0.0005 |
| **B** | 0.0329 | 0.033 | 0.0334 | 0.0331±0.0003 |
| **C** | 0.0328 | 0.033 | 0.0334 | 0.033067±0.0003 |
| **D** | 0.0238 | 0.0239 | 0.0246 | 0.0241±0.0004 |
| **E** | 0.0113 | 0.0112 | 0.0114 | 0.0113±0.0001 |
| **F** | 0.0149 | 0.015 | 0.0151 | 0.015±0.0001 |
| **G** | 0.0173 | 0.0176 | 0.0176 | 0.0175±0.0002 |
| **H** | 0.0232 | 0.0235 | 0.0238 | 0.0235±0.0003 |
| **I** | 0.027 | 0.0274 | 0.0275 | 0.0273±0.0003 |
| **J** | 0.0238 | 0.024 | 0.0241 | 0.023967±0.002 |

## APPENDIX $I_1$



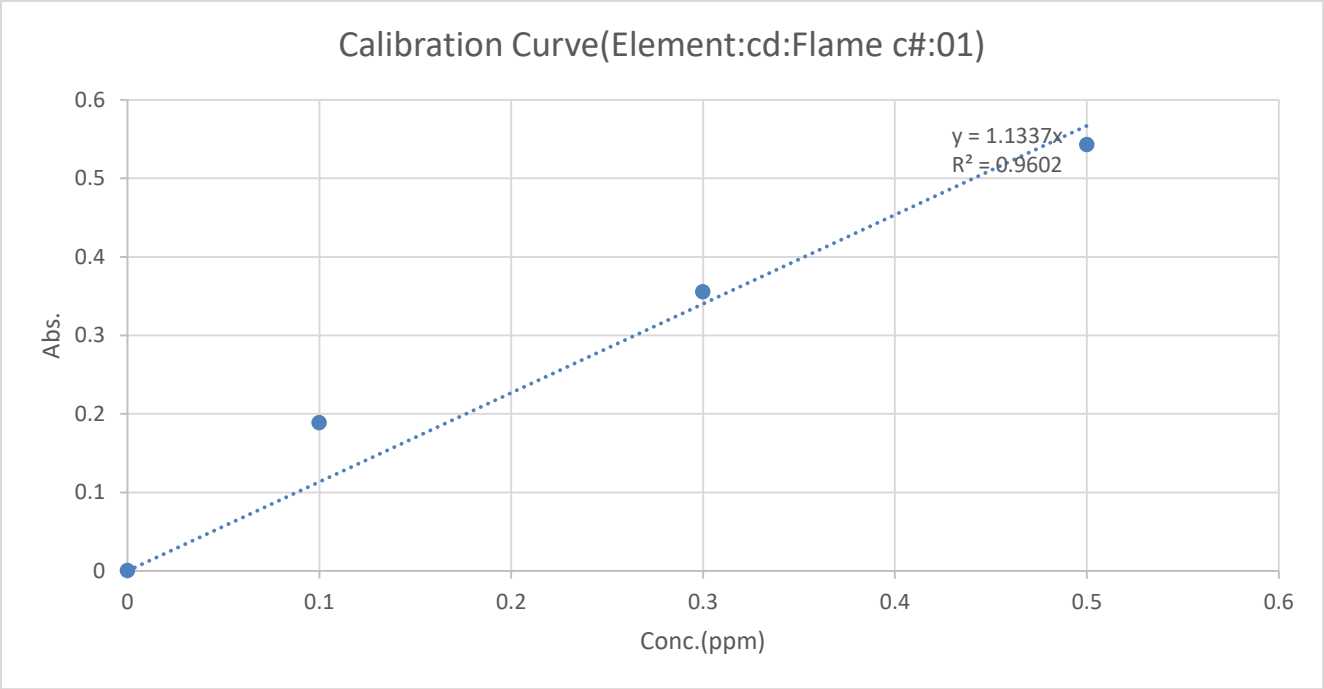

| CONC | ABS |
|---|---|
| 0.2000 | 0.2447 |
| 0.4000 | 0.5025 |
| 0.600 | 0.7286 |

## APPENDIX $I_2$

CONCENTRATION OF COPPER IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_2$ | $C_2$ | $C_3$ | C±S.D |
|---|---|---|---|---|
| **A** | 0.0049 | 0.0052 | 0.0052 | 0.0051±0.0002 |
| **B** | 0.00058 | 0.0006 | 0.00062 | 0.0006±0.00002 |
| **C** | ND | ND | ND | ND |
| **D** | ND | ND | ND | ND |
| **E** | 0.0079 | 0.0081 | 0.0081 | 0.008033±0.00012 |
| **F** | 0.0049 | 0.0051 | 0.0052 | 0.005067±0.0002 |
| **G** | ND | ND | ND | ND |
| **H** | ND | ND | ND | ND |
| **I** | 0.0059 | 0.0057 | 0.00064 | 0.00408±0.003 |
| **J** | ND | ND | ND | ND |

## APPENDIX $J_1$

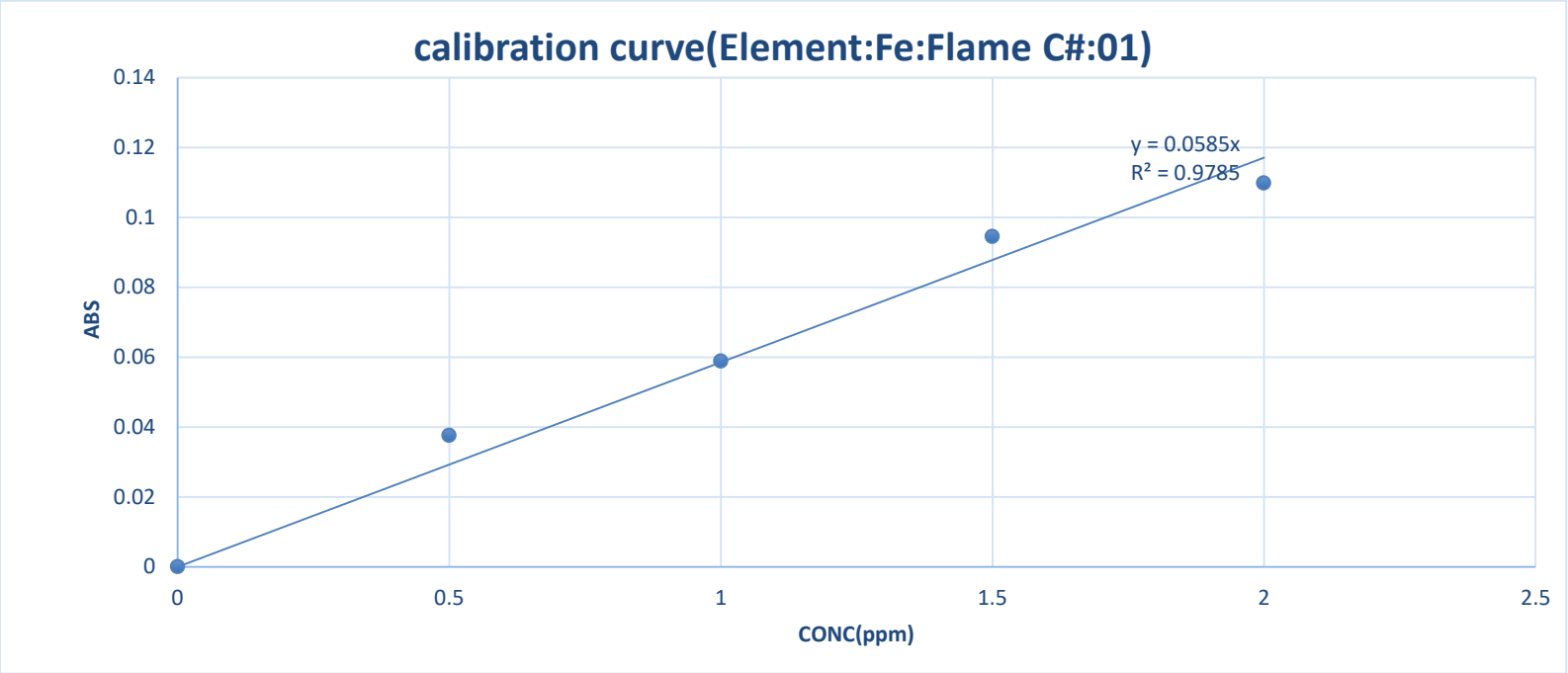

Calibration Curve(Element:cd:Flame c#:01)
y = 1.1337x
R² = 0.9602
Abs.
Conc.(ppm)
0
0.1
0.2
0.3
0.4
0.5
0.6

## APPENDIX $J_2$

CONCENTRATION OF CADMIUM IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_I$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|---|
| A | ND | ND | ND | ND |
| B | 0.0013 | 0.0012 | 0.0014 | 0.0013±0.0001 |
| C | 0.0013 | 0.0014 | 0.0015 | 0.0014±0.0001 |
| D | 0.0022 | 0.002 | 0.0024 | 0.0022±0.0002 |
| E | 0.0022 | 0.0024 | 0.0025 | 0.002367±0.0002 |
| F | 0.003 | 0.0033 | 0.0034 | 0.003233±0.0002 |
| G | 0.0079 | 0.0079 | 0.0082 | 0.008±0.0002 |
| H | ND | ND | ND | ND |
| I | 0.0018 | 0.002 | 0.0022 | 0.002±0.0002 |
| J | 0.0029 | 0.0031 | 0.0032 | 0.003067±0.0002 |

## APPENDIX $K_1$



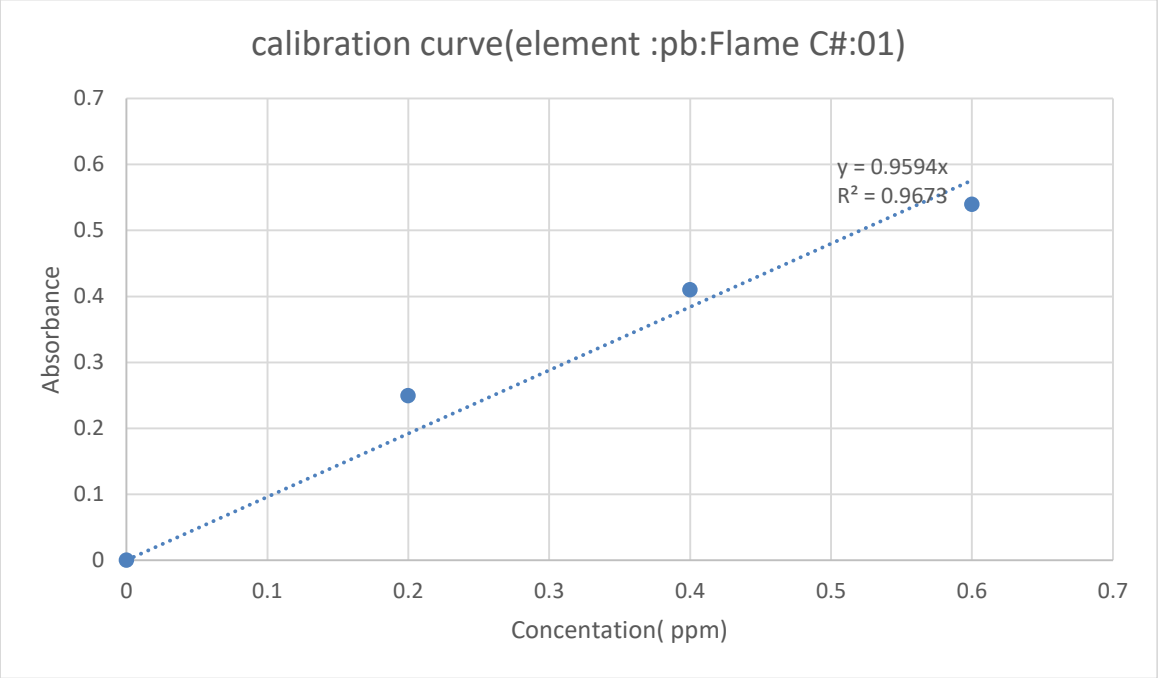

| | |
|---|---|
| 1 | 0.0589 |
| 1.5 | 0.0945 |
| 2 | 0.1089 |
| 2.5 | 0.1489 |

## APPENDIX $K_2$

CONCENTRATION OF IRON IN BREAD SAMPLES (mg/l)

| SAMPLE | $C_I$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|---|
| **A** | 0.358 | 0.3584 | 0.3585 | 0.3583±0.0003 |
| **B** | 0.2908 | 0.2911 | 0.2912 | 0.29103±0.0002 |
| **C** | 0.5073 | 0.5074 | 0.5076 | 0.50743±0.0002 |
| **D** | 0.1264 | 0.1266 | 0.1266 | 0.12653±0.0001 |
| **E** | 0.3714 | 0.3719 | 0.3721 | 0.3718±0.0004 |
| **F** | 1.7559 | 1.7561 | 1.7563 | 1.7561±0.0002 |
| **G** | 11.573 | 11.5697 | 11.567 | 11.5699±0.003 |
| **H** | 0.0874 | 0.0877 | 0.0877 | 0.0876±0.0002 |
| **I** | ND | ND | ND | ND |
| **J** | 0.31 | 0.31 | 0.311 | 0.31033±0.0006 |

## APPENDIX $L_1$




| CONC | ABS |
|---|---|
| 0.2 | 0.2491 |
| 0.4 | 0.4100 |
| 0.6 | 0.5391 |

## APPENDIX $L_2$

CONCENTRATION OF LEAD IN BREAD SAMPLES (mg/l)

| $C_I$ | $C_2$ | $C_3$ | $\hat{C}$±S.D |
|---|---|---|---|
| **ND** | ND | ND | ND |
| **ND** | ND | ND | ND |
| **ND** | ND | ND | ND |
| **ND** | ND | ND | ND |
| **0.0066** | 0.0068 | 0.0068 | 0.006733±0.0001 |
| **0.01156** | 0.01164 | 0.01178 | 0.01166±0.0001 |
| **0.01296** | 0.01299 | 0.1299 | 0.05195±0.07 |
| **0.0182** | 0.0182 | 0.0189 | 0.018433±0.0004 |
| **0.0139** | 0.014 | 0.0141 | 0.014±0.0001 |