Sudan University of Science & Technology
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Determination of Residual Potassium Bromate in Bread in Khartoum state

Graduation Project in Partial Fulfillment of the Requirements of the B.Sc. Degree (Honors)

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بسم الله الرحمن الرحيم

الآيــة

قال تعالى:

الرَّحْمَنُ (1) عَلَّمَ الْقُرْآنَ (2) خَلَقَ الْإِنسَانَ (3) عَلَّمَهُ الْبَيَانَ (4)

صدق الله العظيم

سورة الرحمن

Dedication

To my honorable parents, and my beloved brothers and sisters, to my dear teachers and my best friends

Acknowledgements

First I thank Alamity Allah for granting me the strength to do this study.

I would like to express my immense graduate and appreciation to my supervisor ***Dr. Elfatih Ahmed Hassan*** *for his help, suggestions and close supervision throughout the study.*

Finally all my thanks go to everyone who supported and helped me to accomplish this study.

Abstract

This work was carried out in the Khartoum state to investigate the presence of residual potassium bromate in bread .

20 different sample of bread were sampled from Khartoum and Omdurman Localities . The samples were analyzed using standard method for analysis . A freshly prepared 1% potassium iodide in 0.1N hydrochloric acid was used to develop the color. The result represented eleven samples (A,C,E,H,I,J,K,M,O,P,R) show no presence of potassium bromate .

A pure potassium bromate sample was used to generate a calibration curve for use colorimtric analysis . The absorbance of the sample were then converted to concentration using calibration curve . the least Quantity of potassium bromate detected was 5 mg/kg and the maximum quantity was 40 mg/kg .Which for with the permissible limits .

المستخلص

تم تقدير نسبة متبقي برومات البوتاسيوم في الخبز باستخدام جهاز الكلروميتر في 20 عينة مختلفة من أمدرمان والخرطوم وتم الكشف عنها نوعيا باستخدام 2 مولاري من حمض الهيدوركلوريك و1% من يوديد البوتاسيوم المحضر حديثا ووجد ان 11 عينة (A,C,E,H,I,J,K,M,O,P,R) لا تحتوي على برومات ا لبوتاسيوم وعند الكشف كمياً عن العينات التي تحتوي على برومات البوتاسيوم وجد أن اقل تركيز 5 مل جرام / كلجم وأعلى تركيز 40 مل جرام / كلجم و هذه النسب توجد فى المدى المسموح به.

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Chapter One

1.1.1 Bread:

Bread is food baked from mixed yeast leavened dough obtained from flour and bromate and Phosphate flour or their combination in the presence of sacharomyces cerevisae yeast and 1% bread bean flour . the bean flour activates the whitening of the dough improves the quality of the bread . and increase lipoxy genase which produces hydroperoxide an oxidizing agent

Ascorbic acid dissolve in water is also added to the dough mixture to improve the gluten content ***(Alisa and Linden 1999)***

1.1.2Chemistry of bread making:

Basic dough is made by kneading flour and water , when flour is mixed with water the protein present in flour ,_glutenin and gliadin forms (gluten)

The glutenin forms strands of long thin chain like molecules while the shorter gliadin forms bridges between the strands glutenin " the result net works

Glutenin , gliadin + water → gluten

(proteins in flour) (Elastic mass of Molecules)

When the dough is allowed to rest the gluten bonds relax making it easier to give shape to the dough yeast . the leavening agent in bread making . feed on sugar and get activated ,yeast metabolizes sugar to produce CO2 and ethanol

Enzymes in flour

yeast act on

↑

Starch molecules → break down → simple sugara

↓

And yeast act on

CO_2 + ethanol

The by product ″ethanol″ is an alcohol that contributes to the breads flavor ***(www.Constant system .com)***

Potassium bromated is a strong oxidizing agent . Its action in bread is formation of disulphide bond between gluten and strip the hydrogen atom form the sulf - hydral linkage so make more sulfur available for the gluten strengthened disulphide bond increase gas retaining capacity.

$$Br\bar{O}3 \quad + \quad 6RSH \rightarrow \bar{Br} + 3RSSR + 3H2\,O$$

(Bromate) (protein thiol) (protein disulfide) ***(Omer,2008)***

1.2 Food additives :-

Food additive are defined as chemical substances ,deliberately ,added to foods ,directly or indirectly , I known and regulated quantities ,for purposes of assisting in the processing and preservation of foods ; or in improving the flavor , texture , or appearance of foods . Additives may be reactive or nonreactive , nutritive or nonnutritive , but they should be neither toxic nor hazardous

Certain Additives are classified as generate recognized as safe (GRAS) when they have been 00used for long periods of time without apparent harm (e.g normal salt , baking soda) . Currently over 3000 intentional additives are allowed ***(Guthrie , Picciano, M.F, 1995)***

Food additives including sodium bicarbonate , sodium benzoate , ammonium bicarbonate and potassium bromate were subjected to the Ames spot forward mutation assay using Escherichia coil . The mutant stains of the organism were examined in the presence or absence of rat liver metabolizing enzyme . The results showed that sodium bicarbonate , sodium benzoate and ammonium bicarbonate produced no mutant strain of the organism and no alteration in the phenotype characteristic of the organism as compared with potassium bromate ***(Akintonwa,2007)***

1.3 Potassium Bromate:-

1.3.1 Potassium bromate Historical Background:-

Potassium bromate (KBrO3) is one of the food additives that have been used in limited ways and amounts by the baking industry for almost a century with no known health concern . It has been used in baking since at least 1914 when a patent was issued by the united states patent office**.** ***(ABA,AIB,2008)***

Available information indicate that potassium bromate is produced in Argentina , Brazial , China , Germany ,India , Israel ,Italy ,Japan and Spain ***.(Chemical Information Services,1995)***

Bromate salts have been used as a food ingredient , being added to beer and cheese , and also used as a neutralizing agent for permanent wave hair styling products. ***(Dupuis ,1997)***

Potassium bromate (KBrO3) was approved in the United States by the Food and Drug Administration (FDA), for use in bromate flour at 50 ppm .potassium bromate (KBrO3) is prior-sanctioned for use in baked goods and flour under provisions of 21 code of Federal Regulation (CFR) parts 136 (Bakery products) and 137 (Cereal flour and related product). ***(Mack,1988)***

The amounts of potassium bromate used in baking may subsequently prove to be of little or no concern ; however the baking industry still need to take the necessary steps to reduce any potential of bromate residues in finished products to safe levels.

The American Bakers Association (ABA) and American institute of baking International (AIB) for several years have been working with United States Food and Drug Administration (FDA) and with the Japanese baking industry to improve testing and baking technology to permit continued use of bromate as a functional ingredient in baking in a manner that is safe and reliable .To this effect ,and in consultation with the FDA , the wholesale baking industry has progressively reduced the amount of potassium bromate***.(ABA,AIB ,2008)***

According to WHO,(2005) there is a potential risk to human from exposure to bromate via drinking water. Although Bromate is not typically found in drinking water ;however ;the bromate ion can form as a disinfection by product from the ozonation disinfection process.

Potassium Bromate ($KBrO_3$) classified by the International Agency for Research on Cancer (IARC) as category 2B carcinogen. Long – term toxicological studies based on drinking water for rats have established Potassium bromate as a renal carcinogen. Quantitative risk analysis indicated that residues in the finished bread above 20 ppb in baked goods would lead to a potentially significant level of risk.

Many studies also indicated that bromated is harmful when used in high doses, In addition a very low dose of bromate showed cancer in experimental animals .And showed that bromate may cause changes in gene or chromosome (mutagenic) .

The toxicological symptoms of bromate characterized by loss of reflexes, problems with the central nervous system and anemia.In long term exposure kidney failure may occur and death may occur if medical treatment is not successful ***(IARC,1986)***. Potassium Bromate ($KBrO_3$) is harmful when ingested, causing irritation to the gastrointestinal tract. Symptoms may include nausea, vomiting and diarrhea. It May cause abdominal pain, reduced urinary output, low blood pressure, methemoglobinemia, convulsions, liver and kidney damage, and coma. Cyanosis may occur as a later Symptom. Death may occur from renal failure, within one to two weeks.

Estimated lethal dose is 4 grams ***(www. Jtbaker. com,2011)*.**

The joint ***(WHO / FAO)*** Expert Committee on Food Additives (JECFA), however, has concluded that potassium bromate is not appropriate for use as a flour- treatment agent due to the potential carcinogenicity in experimental animals. Potassium bromate ($KBrO_3$) is used primarily as a maturing agent for flour and as a dough conditioner **.** It is also used as a laboratory are reagent and oxidizing agent , in permanent – wave compounds ,as a food additive and in explosives ***(Budavari,1969)***

Recent change in world legislation prohibited usage of (KBrO3) in food industry , especially for improving dough for bread making in bakeries.

Accordingly , KBrO3 has been banned from use in food products in Europe , United Kingdom in 1990 ***(Edwards,2007),*** Canada in 1994***(www.cspinet.org,2010)*** **,**Nigeria in 1993***(Ekop,A.S.et al.2008),*** Brazil , Peru and recently in sri Lanka ,China ,and Sudan in 2004

The history of potassium bromate in Sudan began before 60 years when it was first introduced as food additive to Bread (70ppm) to be an improve ,providing strength and stability .

Internationally , its usage as food additive in food industries is prohibited in so many countries as well as in Sudan since 2004 when the toxicity reports were evident . Hence prohibition was adopted in the country . Illegal usage of potassium bromated was still reported in some bakeries in Sudan ***(BU,2003)***

1.3.2 Properties of potassium bromate:-

1.3.2.1 Physical properties:-

Potassium bromated is a white solid (powder or crystalline) with a melting point of 350 C (approx) and a decomposition temperature (Boiling point) of 370 C . It has a density of 3.27 g/Cm^3 (at 17.5 C).

And is very soluble in water and slightly soluble in alcohol ***(Shaban,2007)***

1.3.2.2 Chemical properties:-

It has a molecular weight of 167.01 g/mol and the molecular formula $KBrO_3$. It is otherwise known as "Bormic acid . potassium salt" . The molecular shape is most likely a trigonal Pyramid ***(Cotton,Willkinson and Guas,1987)***

With the three oxygen atoms each at a vertex fanning away from the bromine atom and the potassium cation loosely bound to the bromine atom at an orientation of approximately 180 from the negatively changed bromate anion (Figure1)

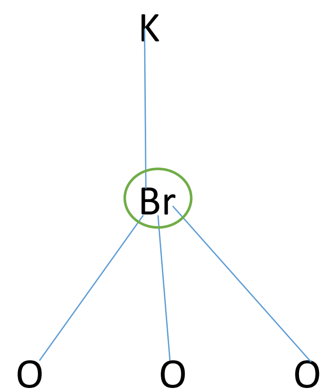


Figure1.1: Probably structure of the potassium bromate molecule

Potassium bromated is an ionic compound with extremely unequal sharing of electrons between bromine and potassium . Most of the charge resides with bromine . Each oxygen atom shares its six electrons with two from bromine (per oxygen). Bromine has 7 electrons and completes its octet with one from Potassium **(P.Patinaik,1992)**

Potassium bromate is a strong oxidizer , reacting in water as follows :

$KBrO_3 + 3H_2O + 6e \rightarrow K^+ + Br^- + 6OH^-$ E = +0.61 V

Or in an acidic environment as follows :

$6H^+ + KBrO_3 + 5e^- \rightarrow \frac{1}{2}Br_2 + 3H_2O + K$ E = +1.52 V

The positive value of the electronegative potential for these reactions indicates oxidizing strength . therefore ,$KBrO_3$ is a stronger oxidizer inacid than in neutral pH media .

It reacts with strong acids , such as gastric acid n, to produce hydrogen bromate , an irritant . On heating ,it decomposes explosively ,producing oxygen . It also reacts violently with bother substances of an organic combustible , or otherwise oxidizable nature . The reaction with hydrides of calcium or strontium with bromates causes explosion ; mixing with concentrated mineral acids , lead acetate , or phosphonium iodide results in ignition ; and the reaction of finely divided bromates Cbe explosive if heat or frication is applied ***(Bushuk ,Hlynka , Cereal chem.,1960)*** Heating to decomposition produce toxic fumes Br^- and K2O***(Lee,Tkachuk, Cereal chem ,1960)***

1.3.3 Production of potassium bromate :

Bromate salts are produced intentionally for some commercial uses, and the bromate ion occurs as an unwanted by- product of certain drinking water disinfection processes .Both the potassium and sodium salts of bromated are currently in commerce worldwide .

Sodium bromate has been used in neutralizer solution for permanent wave and hair straightening products ***(WEEL, 2007)***

While the use of the potassium salt in these hair care preparation has signification decreased ***(Mack,1988)*** they remain permitted as commercial ingredients .with maximum limits as to their concentrations within the cosmetic product ***(FDA,2006)***

The drinking water disinfection processes of ozonation , and to a lesser extent , chlorination , can yield the bromate ion as an unintentional by-product of the disinfection reaction ***(DeAngelo et al .,1998)***

Ozonation has the desirable advantage of being able to control cryptosporidium parvum***(Amy et al ,2000).*** Cryptosporidium is a zoonotic parasitic protozoan , and It is oocysts are refractory to most disinfectant chemicals. The acid form ,bromic acid , is stable only in water . Many bromate salts are possible , but potassium bromate and sodium bromate are the most common ***(FAD,2006)***

The formation of bromate ion in bromide ion containing waters via ozonation is through a series of oxidation processes . the direct ozonation pathway is described by the following mechanism:-

$Br^- + O3 \rightarrow OBr^- + O2$ ❶

$OBr^- + H^+ \rightarrow HOBr$ ❷

$OBr^- + O3 \rightarrow BrO2 + O2$ ❸

$OBr^- + O3 \rightarrow Br^- + 2O2$ ❹

$BrO^-2 + O3 \rightarrow BrO3^- + O2$ ❺

The mechanism shows that the initial reaction of ozone and bromide ion forms hypobromite ion and oxygen . The hypobromite ion can react react with acid to form hypobromous acid

,as shown in Equation ❷.The hypobromite ion can also react with ozone to form bromite ion or bromide ion ,as shown in Equation ❸and ❹.The formation of bromite ion can occurs when hypobromite ion reacts with ozone to form bromite ion ,Which reacts with ozone to form bromate ion .The reactions also form oxygen, Which is a dcompostion product of ozone. ***(Haag and Hoigne, 1983)***

1.3.4 Uses of potassium bromate:

The use of potassium bromate ($KBrO3$)result in a pleasing quality product that is popular with consumer (ABA,AIB,2008). potassium bromate ($KBrO3$) is a slow acting oxidizing agent that works during fermentation, proofing and baking. The primary food use for potassium bromate is that of a maturing agent in flour and as a dough conditioner and texture improver in bakery products such as bread, rolls, and blueberry muffins ***(IARC,1999)*** The oxidation process affects the dough structure and rheology. It improve dough handing properties contributing to loaf volume, grain and texture .potassium bromate is used to help bread rise in the oven and to create a good texture. Currently potassium bromate is used in a targeted way by bakeries for certain products .when used properly, potassium bromate does turn to harmless bromide in the finished baked product. Nevertheles ,it is important to recognize that potassium bromate is an ingredient that should be handled carefully . Form the time concerns about potassium bromate were raised, the American baking industry has been working with suppliers to limit its use and to develop substitutes .

In 1990 AIB did a study of potassium bromate substitutes and found limited application. Subsequently, AIB has conducted courses in potassium bromate and newer enzyme technologies to better trained bakers in the use of oxidant. Substitutes are still being developed however, They still do not meet all the needs of the baking industry ***(ABA,AIB,2008).***

Potassium bromate ($KBrO3$) has also been used in the malting process of barley for the brewing of beer and the production of distilled spirits ***(IPCS,2006).***

1.3.5 Effects of baking on potassium bromate-treated flour:-

Bushuk and Hlynka (1960) reported when potassium bromate was present in flour at levels of 5-80 mg/kg, no residual bromate was detectable in bread prepared from the flour by a bulk-fermentation process after 20-25 minutes baking.

Potassium bromate ($KBrO3$) present on flour at30 mg/kg was quantitatively converted to bromide in bread prepared from the flour by a bulk-fermentation process ***(Lee and Tkachuk,960).***

Bread was made by bulk fermentation and also by mechanical development from flour doughs containing 0-20 mg/kg potassium bromate and the amount of residual bromate in the bread was determined. When the potassium bromate was 50 mg/kg or less, no residual potassium bromate could detected; at higher levels of addition, increasing amount of residual potassium bromate were detected, bulk fermentation giving higher residual levels than mechanical development ***(Thewlis,1974).***

1.3.6 Other sources:

Cosmetic use sodium and potassium bromate in neutralizer solution for home permanent wave cosmetic products ***(WHO, 2004).***

According to the U.S. FDA ***(FDA,2006)*** , the Consumer Product Safety Commission responded to a number of cases of accidental ingestion by children of bromate neutralizer solutions by requiring in 1990 that permanent wave neutralizers, in liquid form, containing in a single container more than 600 mg of sodium bromate or more than 50 mg of potassium bromate be packaged in child- resistant packaging.

The Cosmetic Ingredient Review (CIR) Expert Panel describes potassium and sodium bromate as ingredient found safe with qualifications in each case being that the concentration be less than or equal to 10.17% of the product (calculated as sodium bromate) **(CIR,2006).**

1.3.7 Environmental Occurrence and Human Exposure:

1.3.7.1 Air:

According to WHO, (2004, 2006) they are not aware of environmental scenario in which bromate enters the ambient air in significant quantities, although if it were present in dusts, it could become air brome. The physical properties of bromate salts (negligibly small vapor pressure and decomposition at the melting point) are such that they will not volatilize into the atmosphere.

1.3.7.2 Soil:

Bromate only slightly adsorbs to soil and its properties as a strong oxidant most likely lead to reactions with organic matter to form the bromide (Br-) ion ***(WHO, 2004, 2006).***Bromide similarly would only slightly adsorb to soil or sediment ***(Health Canada, 1999).***

1.3.7.3 Water:

Bromate is not commonly found in water, but it may be formed as a byproduct of ozone disinfection of drinking water and also as a contaminant introduced from treatment of water with concentrated hypochlorite ***(Haag and Holgne ,1983)*.**Thus, ozonation treatment of drinking water represents an important potential pathway of bromate formation. In such conditions, drinking water is the primary route of exposure to bromate. In the Netherlands, the exposure of humans to bromate in drinking water, relative to other pathways of exposure, has been reported as approaching 100***% (Van Dijk-Looijaard and Van Genderen,2000).***

Amy and Associates (2000).observed bromate formation in water under various ozonation conditions simulated and modeled to inactivate *Cryptosporidium.* They reported that bromate formation is affected by such water quality conditions as bromide concentration, PH, temperature, carbonate alkalinity, ultraviolet light (UVA), disinfectant concentration and time (mg/L-min) and transferred ozone dose, among other factors. They noted that even waters with lower concentrations of bromide can approach the U.S.EPA standard of 10µg/l for bromate (U.S. EPA, 2006) at sufficient ozone concentration and disinfection times. Bromate was recently discovered at relatively high levels in to Los Angeles reservoirs used to store water already treated by chlorination ***(Kemsley, 2008).***

Water official speculated that sunlight may have interacted with residual chlorine to oxidize bromide to bromate. The bromate levels in Silver Lake and Elysian reservoirs were reported as 68 and 106 ppb, respectively. High bromate levels were reportedly also found in two other reservoirs in San Diego County. ***(Bouland et al 2005)*** described introduction of bromate into drinking water as a contaminant of sodium hypochlorite, which may be used in the disinfection process.

Weinberg et al. (2006) examined the finished waters from forty treatment plants that use hypochlorite for disinfection, throughout the United States. They found bromate levels ranging from 0.02 to 3.19µg/L in the post-treatment water (median 0.49µg/L, with respective 25^{th} and 75^{th} percentile levels of 0.25 and 1.0µg/L). Authors related bromate levels to the amount of bromate contamination in the hypochlorite feedstocks before treatment use ***(Weinberg et al 2003).***

Reported that the annual mean concentration of bromate in finished drinking water from surface water sources in the united states was 2.9Mg/L with a range of < 0.2 -25 Mg/L . Bottled water is regulated as a food product in the United State *(By FDA)* rather than as an environmental issue. however , it is useful to look at some historic high levels of bromate from bottled water sources .In the United States , recently as 2006 , the FDA recalled certain brands of bottled water having bromate concentrations of about 27 ppb ,exceeding the FDA standard of 10 ppb

(Mercer,2006) .this follows a widely publicized incident in 2003 in United Kingdom in which a popular brand of bottle water was reported to have bromate levels of approximately 20ppb ***(U.K.FSA,2002)***

1.3.8 Metabolism of potassium bromate in animal and human:-

Following oral administration $KBrO_3$ is rapidly absorbed from gastrointestinal tract .administered rats with 100 mg/Kg body weight . Potassium bromate was detected in the plasma within 15 minutes . Bromate ion is very stable in the body and only small amount reduced to bromide by glutathione processes in the liver and kidney . It is excreted in urine either as bromated or bromide ***(Tkachuk, Hlynka , Cereal chem ,1961)***

1.3.9 Toxicity and different Biological effect of potassium bromate:-

The research on toxicological potentials of some food additive has received great attention as people expressed concerns about the mutagenic and carcinogenic potentials of food additive worldwide **(Anderson,1996)**

There have been a lot of controversies in the use of potassium bromate in so many parts of world due to the toxicological effects ***(Michael,1999)***

The historical uses and acute toxicity of potassium bromate have been well characterized (FAO/WHO) A very strong oxidizing agent potassium bromate represents a severs hazard in mesothelium of the male rat , the kidney of the female F344 rat . the both acute and chronic toxicity. It is a complete carcinogen .Potassium bromate reportedly produced cancerous lesions in the kidney , thyroid and peritoneal kidney of male Syrian Golden hamsters , the kidney and small intestine of B6C3F1 male mice ,and the liver of male mice .

Several toxic effects of $KBrO_3$ mainly carcinogenic and mutagenic effects have been reported in experimental animals ***(Fisher,1979)***

Lethal oral doses of bromated in humans have been estimated as 154-385mg Kg-1 body weight while serous poisoning results at doses of 46-92 mg /Kg body wt .Oral doses of 185-385 mg/Kg body wt result in irreversible toxic effects like renal failure and deafness in humans ,while lower doses are associated with vomiting , diarrhea , nausea and abdominal pain ***(Mark,1988)***

Due to the harmful effect of $KBrO_3$ The joint Expert committee on /food additives , JECFA has recommended that when bromate is used in food processing no residues should be left ***(FAO/WHO,1998)***

However , we recently reported toxic bromate residues in Nigerian bread***(Okolie ,1984)***

Which shows that human are still being exposed to this toxin not withstanding several existing legislations out lawing its use .

Despite the plethora of evidence on toxicity of KBrO3 toxicity on some rabbit ocular tissues , there is a dearth of information on its effect on ocular function . it is known that bromate includes oxidative stress in tissues indeed oxidative DNA damage appears to be the basis of bromate –induced kidney carcinogenesis ***(Taylor,1993)***

Joint FAO/WHO (1992) Committee's initial recommendation of acceptable level of 0 to 60 mg/Kg flour was withdrawn because long term toxicity and carcinogenicity studies in vitro and in vivo revealed renal cell tumors in hamsters . Also , toxicity studies showed that potassium bromate affects the nutritional quality of bread by degrading vitamin A, B1,B2 ,E and niacin ,the main vitamin in bread ***(PCHRD,2000)*** The presence of bromate in bread may cause renal failure , respiratory depression , hearing loss , break down of vitamins and cancer to humans***(IPCS,1994)***

A number of case studies of acute human intoxication with potassium bromated have been reported following accidental ingestion or attempted suicide . In autopsy case , degeneration of kidney tubules and liver parenchymal cells ,and acute myocarditis were the principle pathological change observed ***(Paul,1966)***

1.3.10 Effect of potassium bromate on liver and blood Constituents of Wister Albino Rate:-

Twenty –four Wister albino rats were divided into four groups and treated orally with potassium bromate at doses of 0 , 50 ,100 and 200mg/Kg body wt for 21 days . Rats received 200 mg/Kg body wt died within 18 days . A significant reduction in Hb ,PCV and MCHC values were observed in animals received 200 mg/Kg body wt in the second week while no change occurred in the groups treated with 50 and 100 mg/Kg body wt .

The activity of Alanine Transaminase (ALT) was significantly increased in rats received 100 and 200 mg/Kg body wt of potassium bromate from the first week , while total protein and albumin were significantly decreased from the first week in animals treated with 200mg/Kg body wt and the second week at the dose 100mg/Kg body wt .Histologically liver degradation and haemorrhage was evident in the groups treated with 100 and 200 mg/Kg body wt . the dose of 50 mg/Kg body wt did not cause any changes compared to the control ***(Omer,2008)***

1.3.11Observations in man :-

A number of case studies of acute human intoxication with potassium bromate have been reported following accidental ingestion or attempted suicide . In autopsy cases , degeneration of kidney tubes and liver parenchymal cells , and acute myocarditis were the principle pathological change observed ***(Paul,1966)***

1.3.12The Mutagenic Potentials of potassium Bromate :-

This revealed that potassium bromate is mutagenic bacteria and could be said posses carcinogenic potential ***(Akintonwa,2007)***

The work of kotsonis showed that any substance whose dietary concentration is up to 1 ppm should undergo an extensive toxicological assay .Thus, the ability of these chemicals to cause changes in the genetic materials in the nucleus of cells ways that allow the changes to be transmitted during cell division is known as mutagenesis . The potassium bromate is mutagenic in bacteria ,Escherichia coli and could be said to possess carcinogenic potentials ***(Kotsonis,1996)***

Potassium bromate has been determined to be clastogenic in chromosome aberration assays .Chinese hamster lung cells treated with 0.0625 - 0.25 mg/dm^3 potassium bromate showed that 10 – 100% structural aberrations in chromosomes (M.Ishidate,1980)In the absence of exogenous metabolic activation .

Potassium bromate has been evaluated for acceptable level of treatment for flour to be consumed by man . It also used in treating barely in beer making and improvement of the quality of fish-paste products in Japan ***(FAO/WHO)***

1.4Colorimeter Instrument :-

A Colorimeter is a device used in colorimetry . In scientific fields the word generally refers to the device that measures the absorbance of particular wavelengths of light by a specific solution . This device is most commonly used to determine the concentration of a unknown solute in a given solution by the application of the Beer-Lambert law , which states that the concentration of a solute is proportional to the absorbance .

1.4.1Description :-

Louis Jules Duboscq , a French instrument maker , made a colorimeter for a professor of industrial chemistry at the Commercial College in Paris in 1854 . Duboscq's was not the colorimeter on the market but it was , he would later claim , the first that allowed for the simultaneous color comparision of two Duboscq described an improved version to the French Academy of scinces in 1868 , noting that it was suitable for the analysis of colored materials for Commerical purposes . That instrument had twov glass tubes , one for a standard solution and the other for the sample to be analyzed . Light was reflected by mirror at the bottom up through the tubes , refracted by a set of prism , and viewed through an eyepiece .

Identical glass plungers in each tube allowed the operator toadjust the height of the column of the liquid s unitil the intensities appeared the same .

The Duboscq colorimeter become much more important after Duboscq's death in 1886 than it everwas during his lifetime , especially among biochemists who found that , in conjunction with suitable reagents , it officient and effective technique for identifying foreign substances in bodily fluids .

Several French and German firms were making Duboscq and modified Duboscq colorimeters by the early years of the 20th century and Americans followed suit when World War limited the supply of European goods coming into United States.

Bausch and Lomb's first Duboscq colorimeter , introduced in 1920 , followed the French optical design but the mechanical element were changed : the base and frame were made of heavy castings ; the rack and pinion were so arranged that the operating heads were always in a fixed position ; the cups were made of ground glass cylinders and plates and encased in heavy metal; and there were adjustable verniers .

Bausch and Lomb later described their colorimeters as instruments of precision , which will meet the most exacting requirements of the analyst "instruments of precision , which will meet the most exacting requirements of the analyst" and noted that they would serve " where speed and accuracy are essential "

This example is marked "BAUSCH AND LOMB OPTICAL CO.ROCHESTER , N.Y. U.S.A. NO.4860 " It is of the 50 mm size (that is , the glass tubes are 50 mm high),and may date from the 1930S.

The National Bureau of standards transferred it to the Smithsonian in 1965 ***.(www.americanhistory.si.edu/com)***

1.4.2Construction :-

Wavelength selection , Printer button , Concentration factor adjustment , UV mode selector (Deuterium lamp) , Readout , sample compartment , Zero control (100% T) , Sensitivity switch , ON / OFF switch

The essential parts of colorimeter are :

- A light source (often an ordinary low – voltage filament lamp)
- An adjustable aperture
- A set of colored filters
- A cuvette to hold the working solution
- A detector (usually a photoresistor) to measure the transmitted light

- A meter to display the output from the detector

In addition , there may be :

- A voltage regulator , to protect the instrument from fluctuations in mains voltage
- A second light path , cuvette and detector . This enables comparison between the working solution and a “blank” , consisting of pure solvent , to improve accuracy

There are many commercialized colorimeters as well as open source versions with construction documentation for education and for research.

1.4.2.1Filters :-

Changeable optics are used in the colorimeter to select the wavelength of light which the solute absorbs the most , in order to maximize accuracy . The usual wavelength range is from 400 to 700 nanometers (nm) . If it is necessary to operate in the ultraviolet range (below 400 nm) then some modifications to the colorimeters are needed . In modern colorimeter the filament lamp and filters may be replaced by several light-emitting diodes of different colors ()

1.4.2.2Cuvettes :-

In a manual colorimeter the cuvettes are inserted and removed by hand . An automated colorimeter (as used in an autoAnalyzer) is fitted with a flowcell through which solution flows continuously .

1.4.2.3Output :-

The output from a colorimeter may be displayed by an analogue or digital meter and may be shown as transmittance (a linear scale from 0-100%) or as absorbance (a logarithmic scale from zero to infinity). The useful range of the absorbance scale is from 0-2 but it is desirable to keep within the range 0-1 becouse ,above 1, the results become unreliable due to scattering of light .

In addition , the output may be sent to a chart recorder , data logger , or computer.

1.4.3Colorimeter Assays :-

Colorimetric assays use reagents that undergo a measurable color change in the presence of the analyte . They are widely used in biochemistry to test for the presence of enzymes , specific compounds , antibodies ,hormones and many more analytes . for example ,

- Para-Nitrophenylphosphate is convert ed into a yellow product by alkaline phosphate enzyme

- Coomassie blue once binding to proteins elicits a spectrum shift , allowing quantitative dosage . A similar colorimetric assays ,the Bicinchoninc acid assays, uses a chemical reaction to determine protein concentration .
- Enzyme linked immunoassays use enzyme –complexed-antibodies to detect antigens . Binding of the antibody is often inferred from the color change of reagents such as TMB.

1.4.4.Colorimeter types:-

1.4.4.1Tristimulus Colorimeter :-

Tristimulus colorimeters are often used in the application of digital imaging . The tristimulus colorimeter measures color from light sources such as lamps , monitors and screens . By taking multiple wideband spectral energy readings along the visible spectrum , this colorimeter can profile and calibrate specific output devices . The measured quantities can approximate tristimulus values , which are the three primary colors needed to match a test color .

1.4.4.2 Densitometer :-

A densitometer measures the density of ligh passing through a given frame . Density can be characterized as the level of darkness in film or print . When an image is printed , the ink pigments block light naturally when deposited by the printing process . Graphics industry professionals use densitometers yo help control color in the various steps of the printing process.

1.4.4.3Spectroradiometer :-

Spectroradiometer quantify the spectral power distribution emitted from a given light source . In other words , the spectroradiometer measures the intensity of color. Characteristically similar to spectroradiometers are used to evaluate lighting for sales within manufacturing and for quality control purposes . Other applications include confirming a customer's light source specifications and calibrating liquid crystal displays for tetevisions and laptops .

1.4.4.4 A spectrophotometer :-

spectrophotometer is an analytical tool that measures the reflection and transmittance properties of a color sample . Using functions of light wavelengths , the spectrophotometer passes a beam of light through the sample to record both absorbance and transmittance . The instrument does not require human interpretation and is much more complex than a standard colorimeter . Common applications for the spectrophotometer include color formulation and industry research and development .(www.ehow.com)

1.4.5 Advantage and disadvantage of colorimeter :-

1.4.5.1 Advantage:-

- Can be specific to one chemical species
- Good for process quality control for non-chemistry personnel
- Can be inexpensive per analysis.(www.ehow.co.uk/list-6857) A

1.4.5.2 Disadvantage :-

- Similar colors from interfering substances can produce errors in results
- More precise analysis can require tighter wavelength band width (more expensive)
- Matrix interferences can produce bad results in uncontrolled situation.

Chapter Two

2.0 Materials and methods :-

2.1 Materials:-

2.1.1 Equipments:

20 breads samples

Test tubes

Beakers

Spatula

Colorimeter

Pipette

Volumrtic flask

Rack

Measuring cylinder

2.1.2 Reagents:

Potassium bromate

Distilled water

Potassium iodide

Hydrochloric acid

2.2 Methods:

2.2.1 Sample collection and preparation:

Bread samples were purchased from bakeries and open markets at a local Omdurman and Khartoum capital of Sudan. A total of 20 different brands of breads were used to study.

Table 2.1: Bread samples and Location

Omdurman:

Sample	Manufacture location
A	Alssahabi
B	Almuntasirp
C	Albashayr
D	Alttaqwi
E	Alththawra
F	Aldduktur
G	Janay
H	Abu talal
I	Aljamiea
J	Eayni eali syqa

Khartoum:

Sample	Manufacture location
K	Alssuq Alshshaebi
L	Abu Raqya
M	Eishi
N	Jabrat murabbae5
O	Sihr aleuyun
P	Qawarrir
Q	Samuria
R	Al'amal
S	Yathrib
T	Big-Mart

2.2.2 Reagent preparation:

0.1M potassium bromate (KBrO3) 0.25g of potassium bromate crystal was weighed and dissolved in 250 ml of distilled water.

0.1N of hydrochloric acid

This was prepared by using the formular :

$$\frac{\text{Molarity} * \text{Molar Mass} * 100}{\text{Specific gravity} * \text{Percentage purity}}$$

Where;

Mole = 0.1M

Molar Mass = 36.465g/mole

Specific gravity = 118

% Purity = 36 %

2.2.3 Standard curve Preparation:

Required concentration of pure potassium bromate for blanking were prepared in the range 0,50,100, 150,200,250,500,600,700, 800 and 1000. From the above value, ten replicate determination were used to calibrate the colorimetry curve. The original values were calculated by using the formular:

$C_\circ V_\circ = C_r V_r$,$V_\circ = C_r V_r$,$C_\circ$

Where $C_\circ$ = original concenteration (1000 ml)

$V_\circ$ = original volume

C_r =Required concentration (0-1000 ml)

V_r = required volume (50 ml)

By the calibration curve can by calculation of unknown concentration by use the following relationship:-

y = mx + b

Where :-

y = absorbance

m = intercept

x= unknown concentration

b = slope

2.3 Qualitative analysis of potassium bromate in bread:

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

2.4 Quantitative analysis of potassium Bromate in bread :

A 1.0 g quantity was weighed out from the crumb part of each bread sample using an electronic weighing balance. This was transferred into different test tubes. 10ml of distilled water was added ; the mixture was shaken and allowed to stand for 20 minutes at 28±10 C°. A 5ml volume was decanted from the test tube 5ml quantity of freshly prepared 1% potassium Iodide solution in 0.1N hydrochloric acid was added. Color change was observed from milky to light purple with variations in the degree of color change. The absorbance of the sample was taken at 620nm in a colorimeter and converted to concentration using a calibration curve constructed for Potassium bromate using the pure sample ***(David 1976).***

The pure sample for the calibration curve was prepared by weighing out 0.25g of Potassium bromate using a weighing balance, and dissolved in 250 ml of distilled water. Different concentrations were made by solving for+6

+ original volume ($V_{\circ}$) using formular $V_{\circ} = C_r V_r$ with required volume (V_r). and original concentration ($C_{\circ}$) constant , different values were obtained the original volume(2.5, 5, 7.5, 10, 12.5, 25, 30, 35,40 and 50ml) with each corresponding to the varying required concentration (C_r). They were all made up to 50ml with 2.5ml and 50ml having the lowest and highest concentration respectively.

A 50ml quantity of freshly prepared 1% of potassium Iodide solution in 0.1N HCl was added to each pure sample. The absorbance of the sample was taken at 620nm in a colorimeter. The result was used to plot a graph of absorbance against concentration. Hence, a calibration curve for potassium bromate was constructed using the concentration; 50,100, 150,200, 250, 500, 600**,** 700 ,800 and 1000 ppm.

Chapter Three

3.1 Results:-

3.1.1 Qualitative analysis:-

Table 3.1:- Result of Qualitative analysis

Sample	Black spots
A	_
B	+
C	_
D	+
E	_
F	+
G	+
H	_
I	_
J	_
K	_
L	+
M	_
N	+
O	_
P	_
Q	+
R	_
S	+
T	+

3.1.2 Quantitative analysis:-

3.1.2.1 Colormetric reading standard curve :-

Table 3.2 :-

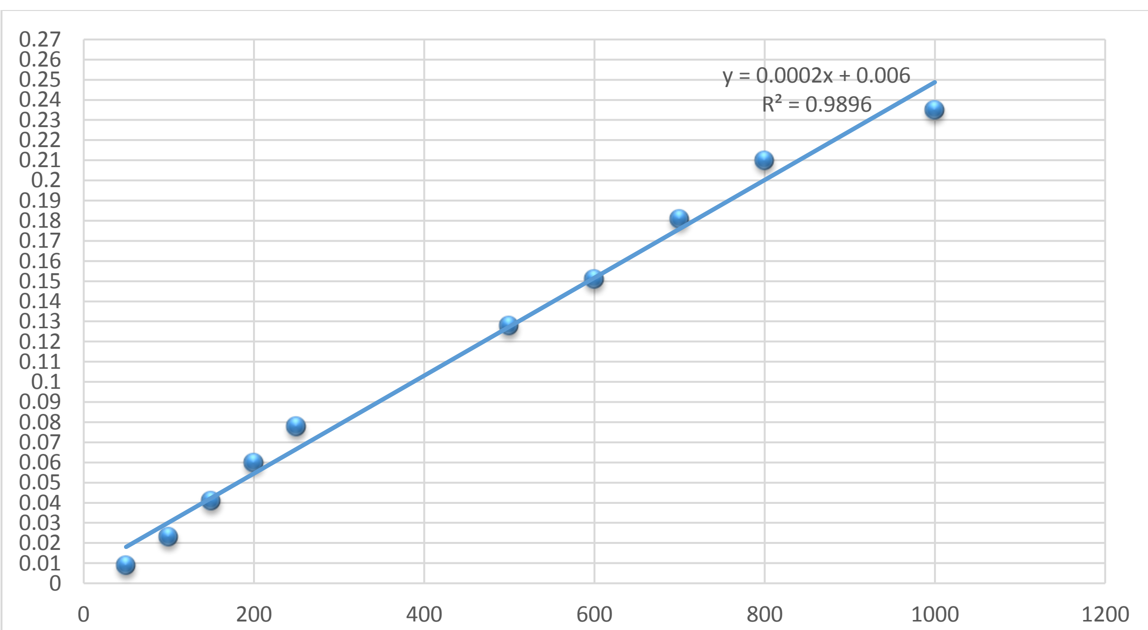
Concentration	Absorbance
50 ppm	0.009
100 ppm	0.023
150 ppm	0.041
200 ppm	0.060
250 ppm	0.078
500 ppm	0.128
600 ppm	0.151
700 ppm	0.181
800 pm	0.210
1000 ppm	0.224

3.1.2.2 Colormetric reading sample:-

Table 3.3 :-

sample	Sample color reaction with KI	Quantity of $KBrO_3$ found	Quantity of $KBrO_3$ (mg/kg) X=(y-b)/m
B	Light purple	0.011	25
D	Light Purple	0.009	15
F	purple	0.014	40
Q	No Visible color	0.007	5
L	purple	0.013	35
N	Light purple	0.012	30
Q	No visible color	0.008	10
S	Light purple	0.010	20
T	No visible color	0.007	5

Fig 3.1: The digram shows relation ship between concentration/ppm agains absorbance/abs :-



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3.2 Discussion :-

Potassium bromate complexed with potassium iodide gave up a purple coloration . The color change ranged from very light purple to purple with increase in concentration . The degree of color change correlates with the level of potassium bromate present. A result showing the color identification of potassium bromate in the 20 bread samples is represented in the table 3.1 eleven of bread samples (A,C, E, H,I, J,K,M,O,P,R) did not show any visible color change when treated with potassium iodide. It is they are free of potassium bromate or that potassium bromate was present in the samples in residual amount that could not be detected by the reagent . All the other samples indicated positive result for the presence of potassium bromate ***(David, 1976)***

Potassium bromate is a flour improver that acts as a maturing agent. According to USDA , potassium bromate improves dough processing properties and interval crumb quality . During the preparation of the dough, a network of protein molecules linked together by disulphide bond is formed . The strength and elasticity of the network which gives the dough its characteristic properties is best when the network comprises of long chain proteins such as gluten . Short chain peptides such as glutathione which is present as well , react with gluten molecules breaking down the dough structure .

This structural breakdown can be prevented by the addition of oxidizing agents such as potassium bromate . In the presence of any of this oxidizing agent, the glutathione is oxidized to glutathione disulphide and therefore cannot interfere with disulphide links of the gluten molecules . ***(IARC,19886)***

Table 3.1 also shows the presence of black spot on the samples when treated with 0.5N potassium iodide solution in 2M of hydrochloric acid . Black spot was observed on the samples indicating the presence of potassium bromate . From the results represented in table 3.1 below , eleven (A,C,E,H,I,J,K,M,O,P,R) did not show any visible black spot .

This also implies that the above may be free of potassium bromate or that potassium bromate was present in the samples in residual amount that could not be detected by the reagent . All the other bread samples had black spot which indicates positive result for the presence of potassium bromate in varying degrees **.**

The quantitative amount of potassium bromate found in each bread sample was respresented in table 3.2 the absorbance of the sample was taken at 620 nm in colorimeter.

A pure potassium bromate sample was used to generate a calibration curve . The absorbance of the samples were then converted to concentration (ppm) using the calibration curve . The least Quantity of potassium bromate detected was 5 mg/kg and the maximum quantity was 40 mg/kg.

3.3 conclusion :-

The concentration of residual potassium bromate in bread samples (B,D,f,G,L,N,Q,S,T) was found to be in the range (5mg/kg- 40mg/kg) and which fall with the permissible limites.

3.4 Recommendation :-

Potassium bromate is an oxidizing agent that has been used as a food additive , mainly in the bread-making process. Potassium bromate if consumed has the capacity to cause such diseases as cancer , Kidney failure and several other related diseases . It also negative effect on the nutritional quality of bread; it degrades vitamins A_2 , B_1 , B_2 , E and niacin , which are the main vitamins available in bread . (IARC,1986)

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

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

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