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Implications of Potassium bromate in our day- to- day usage of commercial products.

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ABSTRACT

The increased fear of the presence of chemicals in food has received much attention in recent years. This paper aims to examine the use of one such chemical in food. The use of potassium bromate in food items, especially bakery products, due to its effectiveness in enhancing gluten strength and improving dough texture, has been a subject of controversy for over a few decades. The health risks of potassium bromate are beyond doubt, with many studies having highlighted the concerns about the chemical's ability to induce tumors in rats, caused by the chemical's carcinogenic and mutagenic properties. Despite this, many countries have reported the presence of potassium bromate in their bakery items. This research work investigates the presence of potassium bromate in bakery products located in Chennai, India, by implementing qualitative and quantitative analyses. The results indicated the use of potassium bromate in a few commonly used brands with increased concentrations. Additionally, for a multifaceted approach, we integrated insights from zebrafish (Danio rerio) since they share a similarity of 70% genes with humans. The outcome demonstrated cellular damage to the embryos. The results of this study shed light to the presence of potassium bromate in bakery products in the perimeter of Chennai, India,

indicating that residents could be exposed to significant health risks associated with potassium bromate.

Key words- Potassium bromate, carcinogenic, baking, dangers, toxicity, public health, food additives.

INTRODUCTION

Potassium bromate ($KBrO_3$) stands as a compound of significant interest and controversy within the realms of chemistry, the food industry, and public health. It is colorless, odorless, tasteless, and crystalline, serving as a potent oxidizing agent. Initially employed in chemical synthesis and analytical chemistry, its use later extended to the food industry, primarily as an oxidizing agent in various reactions (Yuji Kurokawa *et al.*,1983).

Potassium bromate was discovered by French chemist Antoine Ballard in 1875 during his studies on halogens (Gill Hyssop *et al*.,2019). He identified it as a strong oxidizing agent while investigating compounds formed with bromine and hydroxides. Initially used in chemical synthesis, it was patented for bakery use in 1914 due to its effectiveness as a dough conditioner. Potassium bromate became popular in the 1960s and 1970s for enhancing dough strength and bread texture. However, studies in the 1980s and 1990s revealed its carcinogenic properties, leading to DNA damage and tumor formation in laboratory animals. Consequently, health and regulatory agencies took action, with the European Union leading the ban in the 2000s, followed by countries including Argentina, Brazil, Canada, Nigeria, South Korea, Peru, and India (banned in 2016).

Potassium bromate is produced by passing bromine through a hot potassium hydroxide solution, forming unstable potassium hypobromite that quickly converts into bromide and bromate. Additionally, electrolysis of potassium bromide solutions yields bromate. Due to its lower solubility potassium bromate precipitates out when the solution is cooled to 0°C, allowing for its separation from potassium bromide.

Potassium bromate enhances bread structure by reducing crumbling and strengthening gluten networks. In its absence, alternatives like oil, eggs, or xanthan gum are used, but some bakers combine these with potassium bromate to improve texture. It boosts gluten strength in yeast-containing dough, aiding gas bubble retention and contributing to a crisp crust. In yeast-free dough, potassium bromate supports gluten formation, which, along with baking powder or soda, helps create a desirable texture and crispness in the final product (Desrosier 1977).

Potassium bromate has been associated with tumor formation in rats, particularly affecting the kidneys and thyroid glands, which raises concerns about its carcinogenic potential in humans. Direct human studies are unethical, making animal models, especially rats, essential for understanding health risks. Rats, which metabolize potassium bromate in a manner similar to humans, are used to explore its carcinogenic mechanisms and long-term effects. These studies provide insights into how potassium bromate induces DNA damage and cancer, helping to establish safe exposure limits for humans. Despite species differences, the findings from rat studies are critical for regulatory agencies in formulating safety guidelines and regulations for food additives (Keep *et al*.,2008).

Due to potassium bromate's hazardous nature and its impact on dough strength and texture, its use in baking has raised significant health concerns. This study aims to assess its toxicity by exposing zebrafish (Danio rerio) embryos to potassium bromate. These are chosen for their genetic similarity to humans, transparent embryos, and rapid development, which facilitate the observation of developmental effects and toxicity.

METHODOLOGY

Sample Collection

Samples of flour, white bread, pasta sheets, and pizza bases were collected from small convenience stores and commercial bakeries. After baking to remove moisture, the samples were ground into a fine powder. Brand selection was based on a questionnaire about frequently used products, with shops chosen for their accessibility and convenience.

Sample Preparation

Atta, wheat flour, and maida were used directly for quantitative analysis. Crusts were removed from bread, and both bread and pizza base samples were dried at 85°C for one hour before grinding. The dried, powdered samples, including the pasta sample, were stored in airtight containers.

QUALITATIVE ANALYSIS

Weigh 1 gram of the sample and transfer it to a test tube. Add 10 ml of distilled water to the test tube and shake well to ensure thorough mixing and prevent lump formation. Decant 5 ml of this mixture into a separate container. Mix this decanted sample with freshly prepared 1% potassium iodide solution in 0.1N hydrochloric acid. The appearance of a purple color indicates the presence of potassium bromate (Magomya *et al*., 2020).

QUANTITATIVE ANALYSIS

After positive qualitative results, four bakery samples—white bread, wheat flour, Pasta, and Pizza Base were subjected to quantitative analysis. One gram of each sample was dissolved in 10 ml of distilled water and incubated at 36°C for 30 minutes. After homogenization, 5 ml of each sample was transferred to new test tubes. To these, 5 ml of potassium bromate and 5 ml of 1% potassium iodide solution in 0.1N hydrochloric acid were added and mixed.

The solutions were analyzed using UV-Visible spectroscopy at 620 nm to measure iodine absorbance. The absorbance values were compared to a calibration curve from standard potassium bromate solutions to determine the concentration of potassium bromate in the samples (M. O. Emeje *et al*., 2010).

Standard Calibration Curve

A calibration curve establishes the relationship between potassium bromate concentration and absorbance at 620 nm. To create it, add 2, 4, 6, 8, and 10 ml of potassium bromate standard to separate test tubes, then dilute to 10 ml with distilled water. Add 5 ml of 1% potassium iodide in 0.1N hydrochloric acid to each, including a blank. Measure absorbance spectroscopically at 620nm to determine potassium bromate levels in bakery samples.

Fish Embryo Acute Toxicity Test

To assess the toxicity of potassium bromate, zebrafish (Danio rerio) embryos were used as test subjects. The study utilized two methods: an LDH (Lactate Dehydrogenase) test to evaluate cytotoxicity and a Nitric Oxide assay to examine vascular effects like vasodilation and vasoconstriction. These tests aimed to provide insights into the potential risks associated with potassium bromate exposure (Roland Nagel, 2002).

Preparation of Zebrafish (Danio rerio) for Assays

The embryos were placed in six beakers with water, and potassium bromate solutions of varying concentrations (100, 250, 500, 750, and 1000 µg) were added. A control group with no potassium bromate was also prepared. Over 72 hours, embryos were observed under a microscope for signs of toxicity, such as somite formation failure or tail-bud attachment issues. After observation, the embryos were transferred to Eppendorf tubes and homogenized with Tris-HCl buffer (pH 7.4) to prepare them for further analysis, and stored at 20°C overnight.

Nitric Oxide (NO) Assay

To perform the nitric oxide (NO) assay, 50 µL of each sample is added to Eppendorf tubes, followed by 500 µL of Griess reagent. The mixture is homogenized and placed in a UV-Visible spectrophotometer to measure absorbance at 420 nm. The absorbance readings provide quantitative data on nitric oxide concentration in the samples (Badreldin H Ali *et al.*,2018).

LDH (Lactate Dehydrogenase) Assay

The LDH assay begins by transferring 100 µl of the sample into a test tube, followed by the addition of 1 mL of glycine buffer. The sample is incubated at 37°C for 15 minutes, then the pH is adjusted to 10 by adding 1 mL of 0.1N NaOH, followed by another 15-minute incubation. Afterward, 1 mL of 2,4- dinitrophenyl hydrazine is added and incubated for 15 minutes. Finally, 1 mL of NAD is added, and the sample undergoes a final incubation before being analyzed using UV-Visible spectroscopy at 420 nm (Asghar Ghasemi *et al.*, 2007).

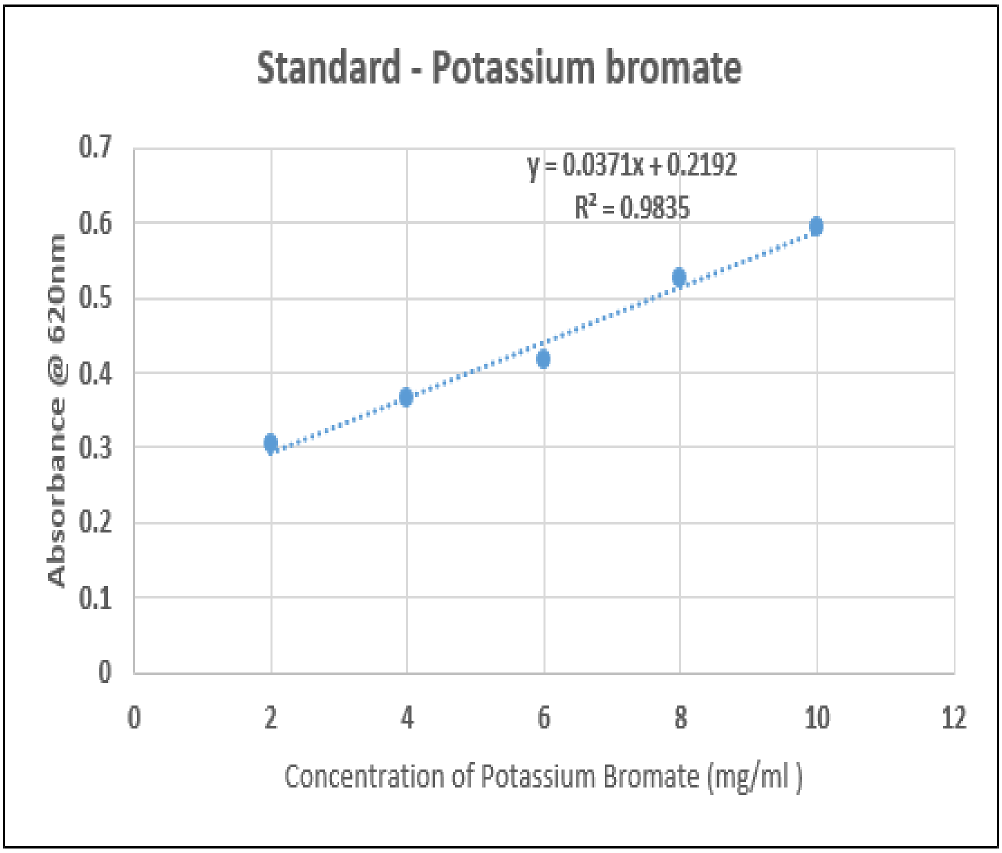
RESULTS

Qualitative Analysis of Potassium Bromate in Bakery Items

The qualitative analysis revealed a significantly higher concentration of potassium bromate in the pizza base, indicated by a strong reaction to the indicator solution. Pasta sheets also showed an immediate color shift, confirming the presence of potassium bromate. Additionally, detectable levels were found in white bread and flour, underscoring the need for thorough evaluation of raw materials used in food production.

Standard Calibration Curve

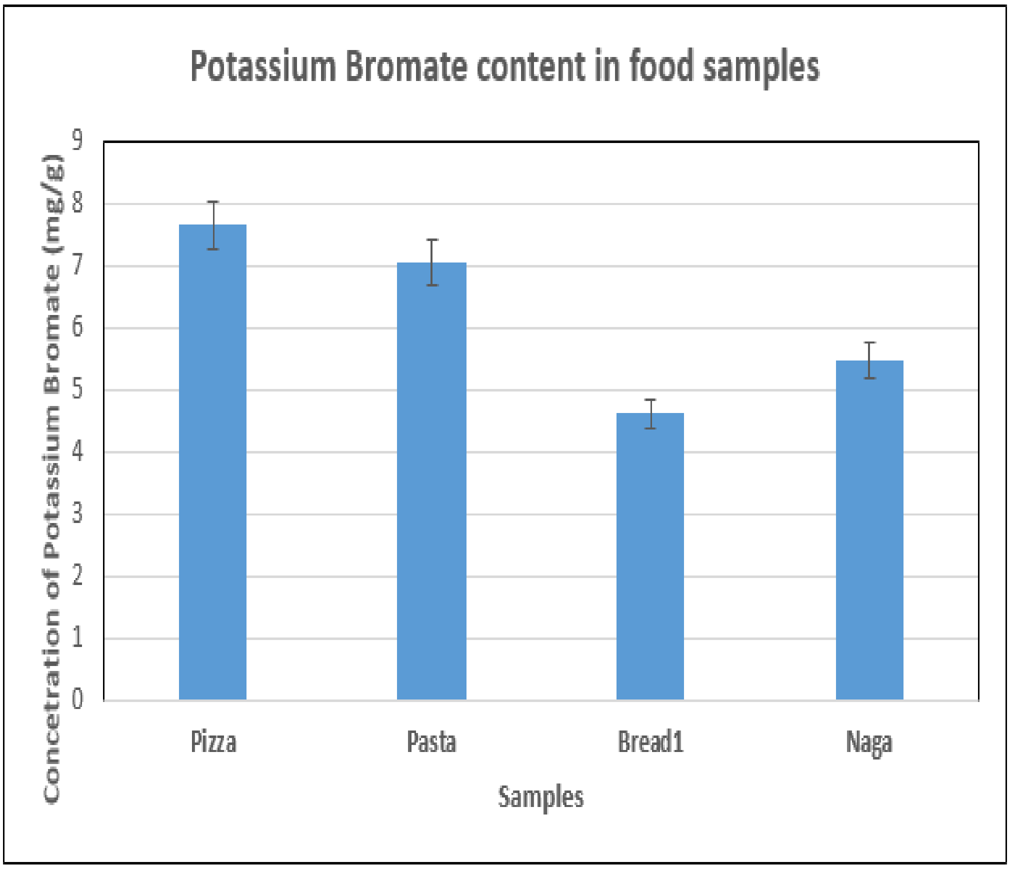
A calibration curve establishes the relationship between potassium bromate concentration and absorbance at 620 nm.



Standard calibration curve of potassium bromate

Quantitative Analysis of Potassium Bromate in Bakery Items

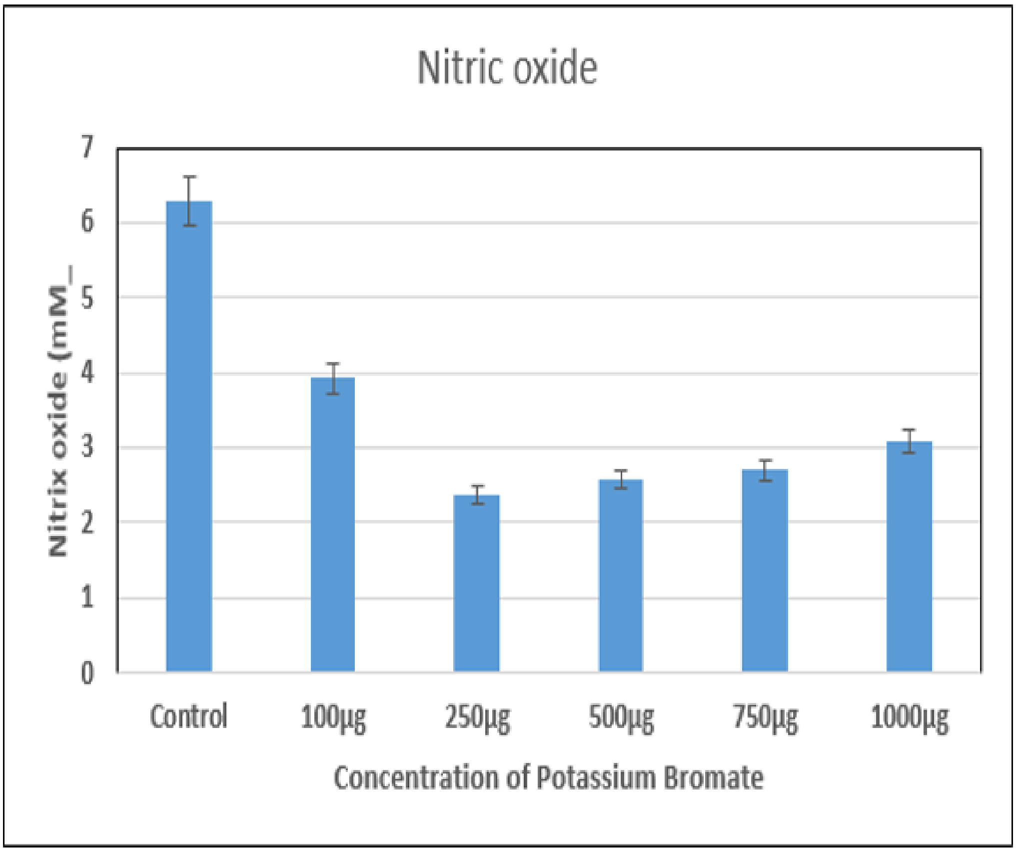
The quantitative analysis confirmed the successful measurement of potassium bromate concentrations using UV-Visible spectroscopy and a calibration curve. Varying levels were detected in white bread, wheat flour, Pasta, and Pizza Base. Accurate absorbance measurements at 620 nm enabled precise determination of potassium bromate content, offering valuable insights for monitoring food safety standards in the bakery industry.



Quantitative analysis of Bakery Samples

Nitric Oxide (NO) Assay

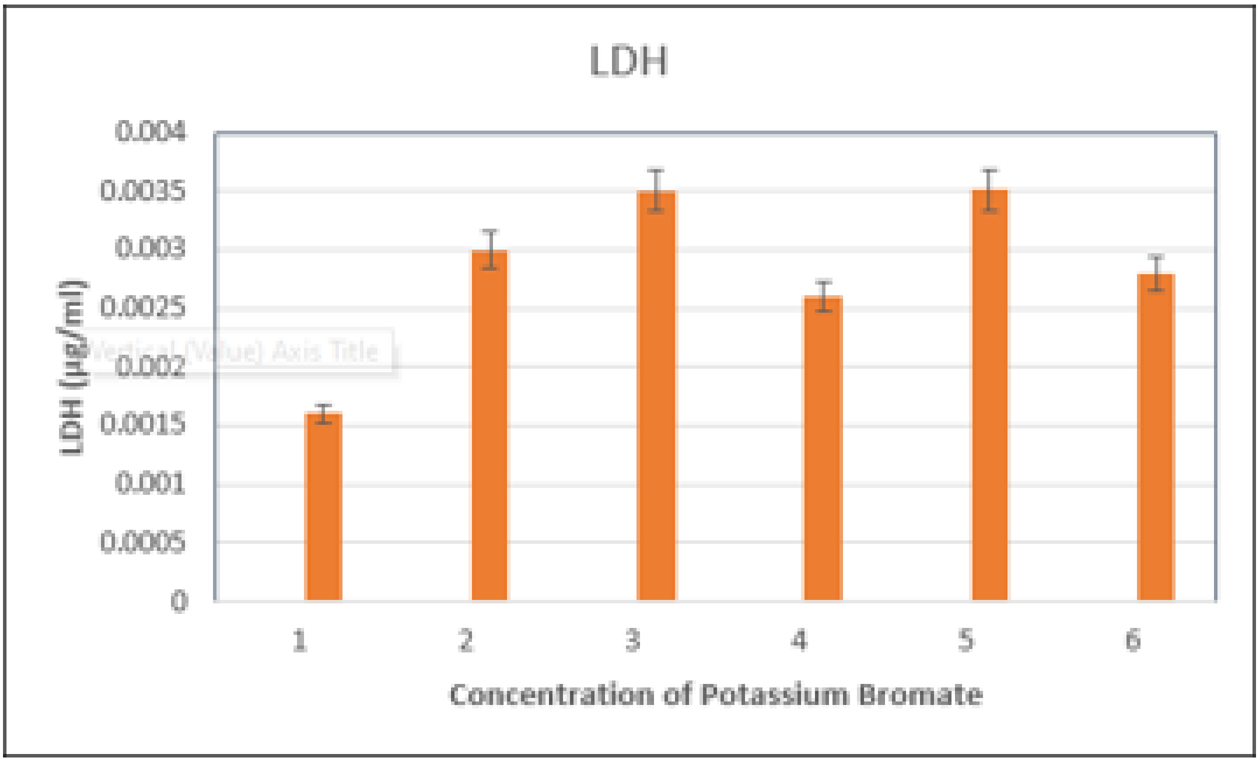
The nitric oxide (NO) assay results showed varying nitric oxide concentrations in the samples. Absorbance readings at 420 nm indicated that the 100 µg sample had the highest NO concentration, while the 1000 µg sample had the lowest. The 250 µg and 500 µg samples had moderate NO levels.



Nitric Oxide (NO) Assay of Bakery Samples

LDH (Lactate Dehydrogenase) Assay

The LDH assay revealed significant variations in enzymatic activity among the samples. The 750 µg sample showed the highest LDH activity, indicating substantial cellular damage or metabolic response. Conversely, the 100 µg sample had the lowest LDH activity, suggesting minimal damage. Samples of 500 µg and 1000 µg displayed intermediate LDH activity levels.

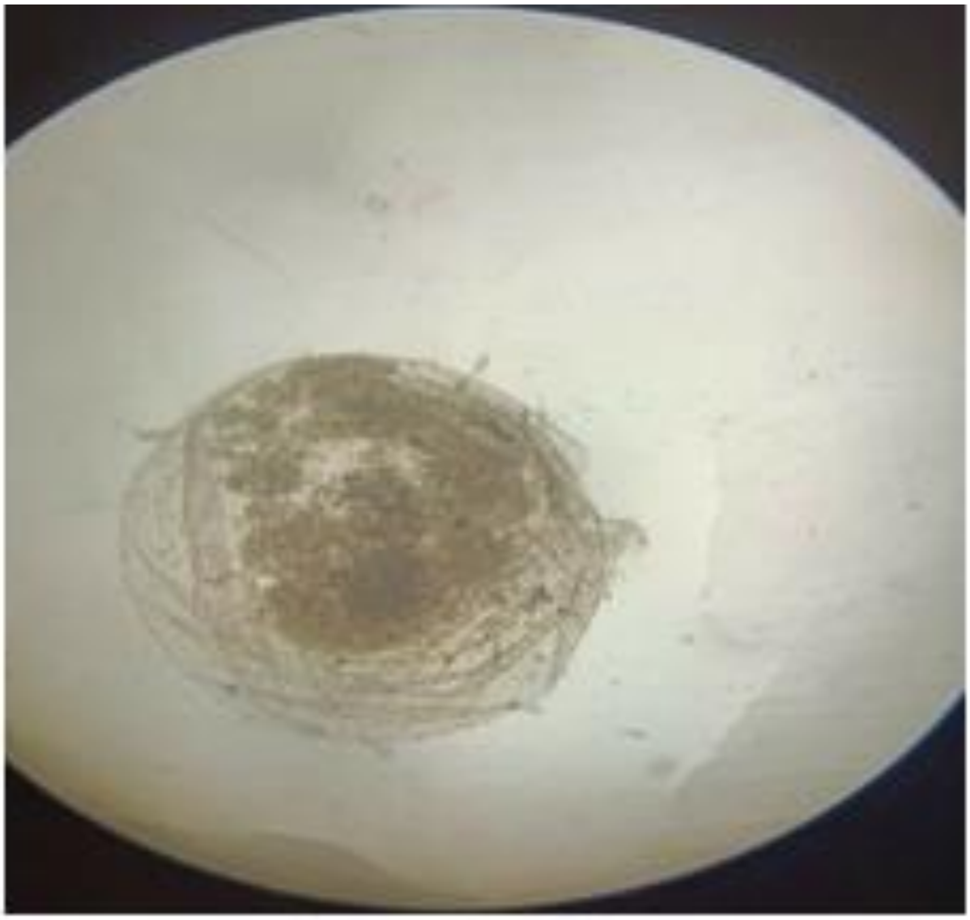


LDH (Lactate Dehydrogenase) Assay of Bakery Samples

Fish Embryo Acute Toxicity (FET) Test

The embryos are exposed to the test chemical for 72 hours, with observations recorded every 24 hours. Key indicators of lethality include: (i) egg coagulation, (ii) lack of somite formation, and (iii) failure of the tail-bud to detach from the yolk sac.

NORMAL STAGE



ABNORMAL STAGE (After Incorporation of $KBrO_3$ to Zebrafish Embryo)

DISCUSSIONS

Potassium bromate, a once widely used dough conditioner, poses serious health risks due to its mutagenic and carcinogenic properties. Its ability to strengthen gluten and improve dough texture made it popular in baking. However, its health risks have led to its ban in several countries, including by the FSSAI (2016). Despite these bans, potassium bromate continues to be used in some regions, revealing a disconnect between regulatory measures and public health awareness. Zebrafish (Danio rerio) embryos are valuable for studying toxicity and developmental impacts due to their genetic similarity to humans, as highlighted by Scholz (2013). Research by Syed Sadman Mahmud *et al*. (2021) and Therese Ncheuveu Nkwatoh *et al*., (2023) has demonstrated effective methods for detecting potassium bromate in bakery products and its associated health risks. The study employed qualitative analysis to screen for potassium bromate presence and quantitative methods, including UV- Visible spectroscopy, to measure its concentration. Notable findings include significant potassium bromate levels in products like pizza bases, as shown by M. O. Emeje *et al*. (2009) and Rana *et al*. (2020).

The Nitric Oxide (NO) and Lactate Dehydrogenase (LDH) assays revealed disruptions in vascular function and cellular integrity, respectively. These assays, with insights from Asghar Ghasemi *et al*. (2007) and Magomya *et al*. (2020), indicate potential risks to embryonic development due to potassium bromate exposure.

While the study's methodology is comprehensive, there are limitations, such as the use of the models, which may not fully replicate human physiology, and the concentration ranges tested, which may not cover all exposure scenarios. Future research should address these limitations, explore the mechanisms of potassium bromate toxicity, and investigate a broader range of concentrations.

The study underscores the urgent need for stricter enforcement of food safety regulations and the adoption of alternative, safer dough conditioners. Continued research and regulatory vigilance are essential to safeguarding public health. By improving detection methods and enhancing consumer awareness, the food industry can ensure the safety and integrity of bakery products, ultimately protecting consumer health and promoting better food safety practices globally. Collaborative efforts between researchers, industry stakeholders, and regulatory agencies will be pivotal in advancing these objectives and addressing the challenges posed by harmful additives like potassium bromate.

LIMITATIONS

While the embryos are useful for studying developmental processes and toxicity, they do not fully replicate human physiology, which limits the direct translation of findings to human health outcomes. Additional validation using mammalian models is needed to ensure the reliability of the conclusions drawn. Budget constraints may have limited the research to specific aspects of potassium bromate toxicity, potentially overlooking other relevant factors. Additionally, the study's focus on bakery items might miss the presence of potassium bromate in other food categories, such as processed snacks or beverages. Longitudinal studies tracking potassium bromate levels over time would provide valuable insights into contamination persistence and the effectiveness of regulatory measures.

CONCLUSION

In this paper, we examine the use of potassium bromate in baking and learn its associated health risks. We conducted our study using the bakery samples such as bread, pasta, and pizza bases, employing both qualitative and quantitative analytical methods. Through the qualitative analyses, we detected the presence of potassium bromate in the sample by the change of color to a purple hue after the formation of the triiodide ions, and for quantitative analysis, we used UV-visible spectroscopy and the calibration curve of potassium bromate to find the concentration of the chemical in the given samples. For further inspection, we assessed the toxicity by conducting nitric oxide (NO) and lactate dehydrogenase (LDH) assays by means of zebrafish to evaluate the cellular damage to it.

The results of this study highlight and emphasize the urgent need to strengthen the safety legislation and to underscore the health risks associated with potassium bromate given its carcinogenic and mutagenic properties. To bring significant changes to the food supply chain and to bring about a safe food production. A multidimensional approach should be implemented where regulatory bodies should amplify the surveillance, imply strict law enforcement, and update regulations to date. In the meantime, the food industries can take measures by implementing safety protocols given by the government, conducting regular audits, and using-cutting edge technology to reduce threats. In the future researchers can focus on studying the toxic properties of potassium bromate and investigate its mechanism of action by using other animal models. They can also develop various specific and sensitive methods for the detection of Potassium bromate. Additional steps must be taken for finding alternatives. By advancing our knowledge, we can create a safer food system for the consumer.

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