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Toxicity Levels of Nitrate And Nitrite In Selected Soft Drinks Sold In Lafia, Nigeria

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ABSTRACT

This study aimed at determination of the concentrations of nitrates and nitrites in selected soft drinks sold in Lafia, Nasarawa State, Nigeria, and to assess their compliance with international safety standards. Six brands of carbonated soft drinks: (A, B, C)-orange flavour, (D, E and F)-cola flavour were purchased from local retail outlets, 5 per each brand from different batches of production. Nitrate and nitrite concentrations were determined using UV-Visible spectrophotometry, with nitrite measured via the Griess colorimetric method and nitrate determined after cadmium column reduction. The mean nitrate concentrations in various carbonated soft drinks were as follows: A (4.31±0.02 mg/L), B (4.07±0.03), C (4.25 ±0.02 mg/L), D (3.73 ±0.02 mg/L), E (4.06 ±0.03 mg/L) and F (4.32 ±0.02), all of which were below the World Health Organization (WHO) and European Commission (EC) maximum limit of 50 mg/L. The nitrite concentrations in various soft drinks were as follows: A(2.16 ±0.02 mg/L), B(1.22 ±0.02 mg/L), C(0.95 ±0.03mg/L), D(1.78 ±0.03), E(0.14 ±0.02 mg/L) and F(1.08 ±0.02) with five out of six samples exceeding the WHO/EC permissible limit of 0.5 mg/L. Comparison with literature revealed that nitrate values were similar or slightly lower than those reported in other Nigerian cities, while nitrite levels were generally higher, indicating possible regional quality control challenges. In conclusion, nitrate levels in studied soft drinks were within safe limits while nitrite contamination is a significant public health concern. Soft drink, E, was the only brand that complied fully with both nitrate and nitrite standards, highlighting the feasibility of safer production practices. The study recommends stricter regulatory monitoring by NAFDAC, improved water purification by manufacturers, proper additive control, and public awareness campaigns to reduce the risks associated with high nitrite intake.

Keywords: Concentrations, Nitrates, Nitrites, Carbonated soft drinks, Lafia.

INTRODUCTION

Nitrates (NO_3^-) and nitrites (NO_2^-) are essential inorganic compounds that play significant roles in the nitrogen cycle, contributing both to plant nutrition and potential health risks in humans. Chemically, both ions consist of nitrogen and oxygen atoms arranged in planar structures. Nitrate contains one nitrogen atom bonded to three oxygen atoms, forming a trigonal planar configuration, while nitrite consists of one nitrogen atom bonded to two oxygen atoms, forming a bent shape[1]. These compounds are naturally occurring and are found in water, soil, air, and food. They are integral to agricultural productivity but have also become key indicators of environmental contamination due to human activity[2].

Drinking water sourced from agricultural regions is prone to nitrate contamination, especially when excessive fertilizer use leads to leaching into groundwater systems[3]. Furthermore, processed meats and beverages may contain added nitrates and nitrites as preservatives and color enhancers, thereby increasing overall exposure [3. This dual exposure; natural and anthropogenic, makes nitrate and nitrite monitoring in food and water a public health priority[3].

Once ingested, nitrate is rapidly absorbed from the small intestine into the bloodstream, where it circulates and accumulates in the salivary glands. Approximately 25 % of the absorbed nitrate is actively secreted into the saliva, where it is reduced to nitrite by oral bacteria[5]. This nitrite, upon entering the stomach, may further react with amines or amides in acidic conditions to form N-nitroso compounds many of which are known to be carcinogenic[5]. While nitrate itself is relatively inert, its conversion to nitrite and subsequently to N-nitroso compounds is the primary concern regarding its health effects[5].

Recent monitoring in low- and middle-income countries indicates detectable and sometimes concerning levels of added nitrates/nitrites in mass-market food products and potable drinks, showing that regulatory enforcement may be insufficient[6].

In Nigeria, studies have shown that the water used in soft drink production, especially from boreholes or municipal supplies may already contain significant levels of nitrates due to agricultural runoff and sewage contamination[7]. When this water is not adequately purified, it acts as the primary source of nitrate introduction into soft drinks[7]. In some cases, chlorination of water may also contribute to the formation of nitrates and nitrites as side products, especially when the chlorine reacts with nitrogen-containing organic matter[7].

Another mechanism is the thermal degradation of certain additives and preservatives during heat treatment or long-term storage[8]. For example, ascorbic acid and sodium benzoate, commonly found in soft drinks, may react under acidic and warm conditions to form nitrosamines, especially in the presence of nitrite precursors[7]. This is particularly concerning in tropical countries like Nigeria where storage conditions are not always optimal, and temperature control is rarely enforced during distribution[8].

Furthermore, interactions between container materials and drink ingredients also play a role in the formation of nitrates and nitrites[9]. Plastic bottles, especially those made from polyethylene terephthalate (PET), may release nitrogenous degradation products into the drink, especially when exposed to heat or sunlight[9]. The overall formation of these compounds in soft drinks is thus a multifactorial process that requires stringent monitoring across the supply chain[8].

A study conducted in Lagos found that 45% of sampled carbonated beverages exceeded the WHO permissible limits for nitrites, highlighting a significant regulatory gap in the industry[10]. Similarly, in a study on bottled and sachet water sold in Ibadan contained nitrate concentrations ranging from 35 to 92 mg/L, often surpassing WHO safety limits[11]. These findings raise serious public health concerns, especially in urban areas where packaged drinks are consumed daily[11].

One of the most commonly used techniques for detection and quantification of nitrates and nitrites is UV-Visible Spectrophotometry[12]. This method is widely used in Nigerian laboratories due to its simplicity, cost-effectiveness, and minimal instrumentation requirements[12]. However, its accuracy can be compromised in samples with interfering substances or when used for complex matrices like soft drinks[12].

Ion Chromatography (IC) is another advanced method that allows simultaneous separation and quantification of anions including nitrates and nitrites with high precision[13]. Though more accurate than spectrophotometry, IC is limited by its high operational costs and the need for skilled personnel, making it less accessible in underfunded laboratories in Nigeria[12]. Nonetheless, it remains the gold standard for regulatory and academic investigations in well-equipped institutions[13].

High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are also utilized, particularly for detecting nitrite-derived nitrosamines and other secondary products[13]. These techniques are more advanced and offer greater sensitivity, especially when coupled with mass spectrometry detectors (GC-MS or LC-MS), but their deployment in Nigeria is limited to a few specialized research centers due to financial and technical constraints[13].

Other methods such as capillary electrophoresis, ion-selective electrodes, and enzyme-linked assays have also been explored, though these are still largely experimental and have not been widely adopted in Nigerian nitrate/ nitrite analysis[13]. In recent years, portable nitrate sensors and test kits have been introduced for field-level analysis, but questions remain about their reproducibility and accuracy under Nigerian environmental conditions[13].

Nutritional diseases, particularly those linked to food additives and contaminants, have become a growing public health challenge in developing nations[14]. Among these concerns, excessive intake of nitrates and nitrites from processed foods and beverages has been associated with various health complications including methemoglobinemia (blue baby syndrome), gastric cancer, and neural tube defects[14]. These compounds interfere with normal physiological processes by converting hemoglobin to methemoglobin, reducing oxygen transport capacity, and forming carcinogenic nitrosamines in the acidic environment of the stomach[14]. Children and pregnant women are particularly vulnerable to these effects, making the monitoring of dietary sources crucial for public health protection[15].

Soft drinks represent a significant source of dietary nitrates and nitrites, particularly in populations where beverages are consumed regularly[16]. The popularity of carbonated drinks in Nigeria, especially among youths and low-income households, makes them an important vector for potential nitrate/nitrite exposure[17]. Studies in various Nigerian cities have detected concerning levels of these compounds in soft drinks, with some samples exceeding international safety standards[18]. The situation in Lafia requires particular attention given the city's growing population, changing consumption patterns, and potential environmental contamination from agricultural and industrial activities. Furthermore, the hot climate in Lafia may increase soft drink consumption, potentially elevating exposure risks if products contain excessive nitrate/nitrite levels. Concerns over food safety regulations have been triggered by increasing awareness of chemical additives and their potential health risks. These beverages contain nitrates and nitrites, which are less well-known but important pollutants. Although they are naturally occurring, these substances can be extremely harmful to health if ingested in excess[19].

The European Union maintain strict limits of 50 mg/L for nitrates and 0.5 mg/L for nitrites in drinking water[20]. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) adopts similar thresholds but faces challenges in consistent enforcement across all regions[21]. The agency's guidelines reference WHO standards but require more robust monitoring systems, particularly for small-scale producers and regional markets[15]. Current regulatory limits for soft drinks in Nigeria specify maximum levels of 500 mg/kg for nitrates and 200 mg/kg for nitrites, though compliance data remains limited (Standards Organisation of Nigeria[21]. This study aims to provide empirical evidence to support regulatory decisions and consumer protection measures in Lafia City, where such data is currently scarce. By evaluating nitrate and nitrite levels in popular carbonated soft drink brands available in Lafia markets, this research will contribute to understanding compliance with safety standards and potential health risks associated with regular consumption. The findings may inform policy recommendations for NAFDAC and other stakeholders to strengthen food safety monitoring and protect public health in Nasarawa State.

MATERIALS AND METHODS

Chemicals and reagents: All reagents used were of analytical grade and included:sodium nitrite ($NaNO_2$), sulfanilamide, N-(1-naphthyl) ethylenediaminedihydrochloride (NED), hydrochloric acid (HCl, concentrated), zinc or copperized cadmium granules (for cadmium column)andde-ionized water (used throughout the procedures)[22].

Apparatus and equipment: Apparatus and equipment used include; UV-Visible Spectrophotometer, Glass cuvettes (1 cm path length), Volumetric flasks (100 mL, 250 mL, and 1000 mL), Beakers, conical flasks, and measuring cylinders, Analytical balance (accuracy±0.001 g), Pipettes and micropipettes, Cadmium reduction column, Filter paper and funnel[23].

Methods

Sample collection and preparation: A total of 30 samples were collected. Six brands of carbonated soft drinks: (A, B, C)-orange flavour, (D, E and F)-cola flavour were purchased from local retail outlets, 5 per each brand from different batches of production in Lafia City, Nigeria. Each bottle was inspected for expiry date, brand labeling, and physical integrity. The samples were transported to the laboratory at room temperature and collected as filtrate for analysis.

The filtered samples were stored at 4°C and analyze within 24 h to prevent alteration of nitrite/nitrate levels[23,24].

A 10 mL of each of the soft drink was measured into a clean 50 mL beaker, stirred for 10 min to remove dissolved CO_2, and then allowed to sit for 5 min in order to allow the foam and gas to dissipate. Each degassed sample was filtered through a 0.45 µm membrane filter into a labeled 50 mL beaker stored in a clean, dry environment prior to analysis[24,25].

Preparation of Standard Solutions: A stock solution of 1000 mg/L sodium nitrite was prepared by dissolving 1.5 g of sodium nitrite ($NaNO_2$) in distilled water and diluting to 1000 mL in a volumetric flask. From this stock, working standard solutions of 2, 4, 6, 8, and 10 mg/L nitrite were prepared by serial dilution. These were used to construct a calibration curve[26,27].

Determination of Nitrite (NO_2^-): Determination of nitrite in carbonated soft drink samples was carried out using the Griess colorimetric method, a procedure that relies on diazotization and azo dye formation for colorimetric quantification[26,27]. In this study, 10 mL of each sample or standard nitrite solution was transferred into a clean test tube. Subsequently, 1 mL of sulfanilamide reagent was prepared by dissolving 10 g of sulfanilamide in 100 mL of 1 M HCl was added to the solution. After a 2-min standing period, 1 mL of NED (N-(1-naphthyl) ethylenediaminedihydrochloride) reagent at 0.1% concentration was introduced[26,27].

The mixture was gently mixed and allowed to stand for 10 min to ensure full colour development. A pink colouration, characteristic of an azo dye complex, was observed, which is formed between the diazonium salt and the NED reagent. This colouration was measured at 540nm using a UV-Visible spectrophotometer, as this wavelength corresponds to the maximum absorbance of the Griess complex[26,27]. Standard nitrite solutions ranging from 0.1 to 1.0 mg/L were prepared and analyzed similarly to generate a calibration curve. The concentrations of nitrite in unknown soft drink samples were then estimated by comparing their absorbance values to the standard calibration curve[28,29].

Determination of Nitrate (NO_3^-): Determination of nitrate involved chemical reduction, where nitrate was first converted to nitrite before colourimetric analysis. The reduction was carried out using zinc powder as described by standard protocols[28,29,30]. A 0.10 g of zinc powder was measured into10 mL of each soft drink sample, which effectively reduced nitrate ions to nitrite[28,29,30].

After reduction, 10 mL of the resulting solution was transferred into a clean test tube. According to the method adopted, 1 mL of sulfanilamide reagent was then added and the mixture was left to stand for 2 minutes. Thereafter, 1 mL of 0.1% NED reagent was added, and the mixture was allowed to stand for 10 min to allow complete development of the pink azo dye[28,29,30].

Furthermore, absorbance readings were taken at 540nm using a UV-Vis spectrophotometer. Since nitrate had been quantitatively reduced to nitrite, the total amount of nitrate+nitrite was expressed as nitrite. To determine the concentration of nitrate alone, a separate sample that was not subjected to reduction was analyzed for nitrite content, and the value obtained was subtracted from the total[28,29,30].

Statistical Analysis: In this study, statistical method was used to evaluate the results obtained from the analysis of nitrate and nitrite concentrations in bottled carbonated soft drinks. A descriptive statistics such as the mean, standard deviation, and range help summarize and understand variations in analytical data. The data was processed using software such as Microsoft Excel and SPSS.

RESULTS AND DISCUSSION

Results

Table 1: Concentration of Nitrates and Nitrites in Carbonated Soft Drinks Sold in Lafia

Samples	Nitrates (mg/L)	Nitrites (mg/L)
A (orange flavour)	4.31±0.02	2.16±0.02
B (orange flavour)	4.07±0.03	1.22±0.02
C (orange flavour)	4.25±0.02	0.95±0.03
D (cola flavour)	3.73±0.02	1.78±0.03
E (cola flavour) F	4.06±0.03	0.14±0.02
(cola flavour)	4.32±0.02	1.08±0.02

DISCUSSION

The concentrations of both nitrate and nitrite in all six carbonated soft drink samples collected from Lafia are given in Table 1. Nitrate concentrations across the samples were generally close in range, suggesting some consistency in production water or ingredient sources. The highest mean nitrate concentration was observed in sample F, which had a value of4.32 ± 0.02 mg/L, closely followed by sample A at 4.31 ± 0.02 mg/Land then sample C at 4.25 ± 0.02 mg/L. There was no statistical significant difference amongst samples A, C and F on nitrate concentration ($P< 0.05$). Samples B and E showed nearly similar nitrate contents of 4.07 ± 0.03 mg/L and 4.06 ± 0.03 mg/L, respectively. There was no statistical significant difference between samples B and E on nitrate concentration ($P< 0.05$). The lowest nitrate concentration was found in sample D, which had 3.73 ± 0.02 mg/L. There was a statistical significant difference of sample D and other studied samples ($P< 0.05$).

There was a variation in nitrite concentrations across the samples. The highest mean nitrite concentration was indicated in sample A, 2.16 ± 0.02 mg/L, followed by sample D at 1.78 ± 0.03 mg/Land then sample B at 1.22 ± 0.02 mg/L. There was a statistical significant difference amongst samples A, B and D on nitrite concentration ($P< 0.05$). Sample C and sample F showed closely similar nitrate contents of 0.95 ± 0.03 mg/Land 1.08 ± 0.02 mg/L, respectively. There was no statistical significant difference between samples C and F on nitrite concentration ($P< 0.05$). The lowest nitrite concentration was found in sample E, which had 0.14 ± 0.02 mg/L. There was a statistical significant difference of sample E and other studied samples ($P< 0.05$).

The mean nitrate values showed minor differences among brands, and all fell well below the World Health Organization (WHO)andEuropean Commission (EC)recommended maximum of 50 mg/L for nitrates in drinking water[15,20]. This suggested that nitrate contamination in the soft drinks studied is minimal and unlikely to pose a direct health risk to consumers under current consumption levels. Even the highest value, 4.32 mg/L in sample F represents less than 9% of the maximum allowable limit, indicating general compliance with nitrate safety standards.

In contrast, nitrite concentrations showed greater variability and raised more significant safety concerns. The highest nitrite level was recorded in sample A, with 2.16 ± 0.02 mg/L, a value over four times the internationally accepted maximum limit of 0.5 mg/L for nitrites in drinking water[15,20]. Sample D also showed a dangerously high nitrite concentration of 1.78 ± 0.03 mg/L, followed by sample B at1.22 ± 0.02 mg/L, andsample F at 1.08 ± 0.02 mg/L. Even sample C, often considered a standard product, contained 0.95 ± 0.03 mg/L of nitrite, which is nearly double the permissible limit. Only sample E, with 0.14 ± 0.02 mg/L, remained within the recommended safety margin for nitrite, showing better compliance and possibly superior water treatment or ingredient control[15,20].

The nitrate concentrations in the six carbonated soft drink samples in Table 1 ranged from 3.73 ± 0.02 mg/L to 4.32 ± 0.02 mg/L. These values fall within a narrow band, suggesting relatively uniform nitrate contamination, possibly arising from water used during production or residual agricultural runoff in ingredient sources.

When compared with values reported in other Nigerian studies, these concentrations are comparable or slightly lower. For instance, a study conducted by Oluwafemi *et al.,*[29] reported nitrate concentrations in soft drinks ranging from 3.5 to 5.1 mg/L, overlapping closely with the range observed in this study. Similarly, Onyekwere and Okoro.[31] in their analysis of beverages documented nitrate levels averaging around 4.8 mg/L, which is slightly higher than most values observed in carbonated soft drinks samples in Lafia. Additionally, a study by Nduka *et al.,*[25] found nitrate levels in sachet and bottled drinks ranging between 4.0 and 6.3 mg/L, again placing the Lafia results in a relatively moderate bracket.

The nitrite levels of the analyzed samples in Table 1 varied more significantly than nitrates, ranging from 0.14 ± 0.02 mg/L to 2.16 ± 0.02 mg/L. This wide variation suggests inconsistent nitrite control during production and possibly differences in water purification, preservative breakdown, microbial activity, or packaging conditions.

Compared to existing literature, the nitrite concentration in sample A (2.16 mg/L) was higherthan many previous findings. In a study conducted by Onyekwere and Okoro[31], nitrite levels in soft drinks ranged from 0.8 to 2.0 mg/L, indicating that the value for sample A from Lafia exceeds that range. Similarly, Oluwafemi *et al.,*[29] reported nitrite levels of 1.0 to 1.9 mg/L in carbonated drinks. Nduka *et al*.,[25] documented nitrite levels between 0.4 and 1.5 mg/L, which were lower than nearly all values recorded in the current study, except for sample E. sample E, which had 0.14 mg/L, was the only sample consistent with WHO/EC safety limits (0.5 mg/L). This supports the possibility that multinational companies may employ more robust quality control systems, explaining the significantly lower values compared to locally branded alternatives like sample F.

CONCLUSION

The results indicated that nitrates contamination in carbonated soft drinks sold in Lafia is generally within safe limits and does not pose an immediate health hazard under current consumption patterns. However, nitrite contamination was a significant concern, with five out of six brands exceeding international safety limits, some by more than fourfold. This suggested that nitrite control in the production process is inadequate for most brands analyzed, potentially exposing consumers to health risks such as methemoglobinemia and the formation of carcinogenic N-nitroso compounds.

Sample E was the only sample to comply fully with both nitrate and nitrite safety standards, suggesting that stricter quality control and better water purification are possible within the Nigerian soft drink industry when enforced.

Recommendations

i. **Regulatory Oversight:** The National Agency for Food and Drug Administration and Control (NAFDAC) should enforce routine nitrate and nitrite testing for all beverage manufacturers, with penalties for non-compliance.

ii. **Water Treatment:** All soft drink producers should adopt advanced water purification techniques such as reverse osmosis or ion exchange to minimize nitrate/nitrite contamination.

iii. **Ingredient and Additive Control:** Manufacturers should ensure that additives and preservatives used in the production do not degrade into nitrite under typical storage conditions.

iv. **Storage and Distribution:** Retailers should be educated on proper storage practices, including avoiding prolonged exposure to heat and sunlight, which can promote chemical degradation.

v. **Public Awareness:** Consumer education campaigns should inform about potential chemical contaminants in beverages and encourage moderation in consumption especially among vulnerable populations such as children and pregnant women.

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Conflict of interest
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