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QUANTITATIVE DETERMINATION OF SELECTED HEAVY METALS AND MICRONUTRIENTS IN BRANDED DAIRY PRODUCTS SOLD IN NIGERIA USING ATOMIC ABSORPTION SPECTROSCOPY (AAS)

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ABSTRACT

Quantitative analysis of heavy metals and nutrients in food helps indicate the safety and quality of food for final consumers. The present study was conducted to assess the presence of heavy metals (arsenic, copper, mercury, chromium, and lead) and the nutritional value of calcium in branded milk and yogurt to evaluate health risks for consumers. Ten (10) samples of branded milk and dairy products manufactured in Nigeria were purchased. The metal contents of the samples were determined using atomic absorption spectroscopy. The concentrations of calcium in the milk samples were between 9.33 ± 0.0023 and 18 ± 0.0071 ppm and were detected in all samples. Arsenic concentrations ranged from 0.45 ± 0.00042 to 2.48 ± 0.00064 ppm in eight branded samples but were undetected in two samples. Chromium levels were undetected in most samples, except for two with concentrations of 0.12±0.00049 ppm and 0.23±0.00021 ppm, respectively. Copper ranged from 0.032±0.00021 ppm to 0.129±0.00021 ppm in six samples. Mercury levels were detected in six samples at a concentration of 1.0±1.0 ppm. Lead concentrations ranged from 0.15±0.00064 to 0.29±0.00028 ppm in three samples. The study found heavy metals above the ideal concentration in branded milk and dairy products in Nigeria, highlighting the need for quality control measures during production to prevent contamination.

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INTRODUCTION

Heavy metals' toxic impact on human and animal health stems from prolonged exposure to low-level contamination in various environmental components such as the atmospheric air, water sources, and food supply [1]. Heavy metals are characterized by their density exceeding 5 g/cm3 [2]. Notably, several among them, including Co, Fe, Mn, Mo, Ni, Zn, and Cu, are vital micronutrients indispensable for regular growth and crucial metabolic function [1–3]. Elevated levels of certain metallic

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elements in the diet have been linked to several disorders, particularly those affecting the cardiovascular, renal, neurological, and skeletal systems [4]. Milk is a bioactive substance known for fostering the growth and development of infants. It is recognized as a near-complete dietary source owing to its rich reservoir of proteins, fats, sugars, vitamins, and minerals.

Consequently, milk and dairy products constitute pivotal constituents of human diets, extensively embraced by individuals across the globe, spanning both the pediatric and adult populations [5]. Various heavy metals have been identified within milk samples, notably Fe, Mn, Zn, and Cu, which benefit human health and play crucial roles in physiological processes. In contrast, Cr, Cd, Pb, and Ni, present in the same samples, exert adverse effects on human well-being, leading to detrimental health consequences [6].

Dairy products are associated with various effects on human health that warrant scrutiny. "These effects encompass aspects such as cholesterol metabolism, regulation of the immune system, management of diarrhea, elimination of Helicobacter pylori, as well as the display of antimicrobial, anti-mutagenic, anti-cancer, and antioxidant properties."[6] The concentration of toxic metals is critical in ensuring the safety and quality of milk and dairy products. Canned dairy products are regarded as a potential source of heavy metals due to their transmission from equipment to the product during processing and storage, particularly in cases where equipment has been compromised or damaged [7]. Based on the information, the contamination of milk, milk powder, and related products with toxic elements can stem from ingesting polluted water and feed by lactating animals or their exposure to environmental pollutants. Consequently, the production of tainted milk is a direct outcome of these contaminant sources [8]. Moreover, incorporating contaminated milk and water in baby food preparation introduces these elements into consumers' bodies, triggering their noxious effects. This situation is particularly perilous during infancy, as infants represent the demographic most susceptible to the harmful impacts of heavy metals, primarily attributable to heightened metal absorption via the gastrointestinal tract, accelerated metabolic activities, an incompletely matured detoxification system, and a relatively higher intake of food in proportion to body weight [8], [9].

The evaluation of metal concentrations serves a dual purpose, contributing to identifying potential risks to human health and aiding in the appraisal of environmental integrity. Given the absence of an extensive investigation concerning the quantification of trace elements in baby diary products within Nigeria and considering the significant consumption of these products in children's diets, undertaking the present study is imperative. Hence, the primary objective of this study is to assess trace elements, encompassing both essential and toxic elements, through the utilization of Atomic Absorption Spectrometry (AAS).

MATERIALS AND METHODS

Sample Collection

Ten (10) distinct brands of liquid milk and yogurt, originating from various regions across Nigeria, were purchased from reputable malls and supermarkets before their designated expiry dates. These products were securely stored in hermetically sealed containers at room temperature until required for analysis. The samples were labeled A1, A2, C1, C2, C3, D1, W1, W2, W3, and W4. Notably, the samples produced in the Northern region were designated as C1, C2, and C3; those from the Eastern region were identified as A1 and A2; the single sample from the Southern region was marked as D1, while the Western region yielded samples labeled as W1, W2, W3, and W4.

Digestion

All samples were digested in triplicate following the procedures described in the literature [3]. One-gram powder sample was weighed into tubes and dissolved using a mixture of hydrochloric and nitric acids in a 1:3 volume ratio. The solution, consisting of 10 ml HCl and 30 ml HNO_3 (3M HNO_3), was heated in a water bath at 70ºC for one hour to ensure complete dissolution.

At intervals throughout the heating process, the tubes were gently agitated. Once the mixture cooled to room temperature (27°C), each tube was filtered through Whatman filter paper, collecting the filtrate in a volumetric flask. The filtrates were then diluted with deionized water to reach a final volume of 50 mL. The tubes were capped with polyethylene film and stored at ambient temperature until analysis for heavy metal content using a flame atomic absorption spectrometer.

Preparation of standard Concentration

Standard solutions (2 ppm, 4 ppm, and 6 ppm) were prepared from 1000 ppm stock solution of the metals using Equation 1:

$$C1 \times V1 = C2 \times V2 \text{.....Equation 1}$$

where;

C1 = Concentration of the stock solution

C2= Concentration of the standard solution

V1= Volume of the stock solution

V2 = Volume of the standard solution

To prepare a 4 ppm solution, 0.2 mL of a 1000 ppm stock solution was carefully aliquoted using a pipette into a 100 mL volumetric flask. Deionized water was then added to reach the graduation mark on the flask, diluting the stock solution 250-fold. This same procedure was repeated with a separate aliquot of the stock solution to prepare a 6 ppm solution, adjusting the final volume in the flask accordingly.

Instrumentation

The ignition chamber achieved high temperatures, creating an environment that facilitated the efficient reduction of various metals and minerals into their atomic forms. A nebulizer atomized each standard solution by converting it into a fine mist, mixing it with supporting gases, and ultimately breaking it down

into individual atoms. This process was repeated for all standards, and the instrument automatically generated calibration curves for each metal or mineral of interest based on their specific absorption wavelengths. The instrument then analyzed each sample at its corresponding wavelength and determined its concentration by referencing the previously generated calibration curve.

Calibration Curve and Measurement of Metal Concentrations

The linear type of calibration curve obtained from each metal's standard solutions was used to determine the concentration of Ca, Ar, Cr, Cu, Hg, and Pb. Standard solutions of Ca, Ar, Cr, Cu, Hg, and Pb were prepared from dilution of Ca, Ar, Cr, Cu, Hg, and Pb 1000 ppm stock solution, with a concentration range, i.e., 2, 4, 8 ppm of each metal. A blank and standard solution were run in AAS, and three points of calibration curves were established. Each standard solution was measured three times, and the mean of the value was plotted. The concentrations of each heavy metal were determined by interpolation from the calibration curves.

Statistical Analysis

Values expressed as mean ± SEM of triplicate measurement. Analysis of variance was performed to compare the differences among the groups.

RESULTS AND DISCUSSION

Concentration of Micronutrients and Heavy Metals in Dairy Products

Using atomic absorption spectrometry, this study employed wet digestion techniques to assess and compare sample preparation methodologies for analyzing macro, micro, and toxic elements in dairy products. The efficacy of these sample preparation techniques was ascertained through the determination of element concentrations in standard reference materials, which evaluated the accuracy of the procedures.

Calcium Concentration

Table 1 outlines calcium concentrations in the milk samples, ranging from 9.33 ± 0.0023 to 18 ± 0.0071 ppm. The highest calcium concentration was observed in samples C1 and D1 (18 ± 0.0071 and 18.00±0.0035 ppm, respectively), followed by W3 and W4 at 17.3333 ± 0.0023 ppm. Conversely, the lowest concentration was noted in W1 at 9.33 ± 0.0023 ppm. These values are below the maximum permissible limit (MPL), and a similar result was also observed by Bakircioglu *et al*. [14]. The recommended dietary allowance for calcium ranges from 700–1000 mg for children aged 1-8 years and 1000-1300 mg for adults, while the tolerable upper intake level is 2000-3000 mg, according to FAO/WHO guidelines. Therefore, while milk is a good calcium source, several branded dairy products may not provide the recommended amount per serving.

Arsenic Concentration

The study findings demonstrated that the concentration of arsenic ranged from 0.45 ± 0.00042 to 2.48 ± 0.00064 ppm in eight brand samples (A1, C1, C3, D1, W1, W2, W3, W4), while it was not detectable in two samples (A2, C2). Notably, the arsenic levels in these samples exceeded the recommended level of 0.00001 ppm set by the United States Department of Agriculture (USDA). Arsenic can impede the activity of antioxidant enzymes, and such inhibition can give rise to heightened oxidative stress, potentially leading to membrane impairment and the reduction of membrane-bound enzymes, such as ATPases [15]. Recent research has demonstrated positive correlations between low-dose arsenic exposures and the cumulative incidence ratios of lung, bladder, and urinary-related cancers [16], [17].

Chromium Concentration

The levels of Chromium (Cr), as presented in Table 1, were not detectable in most of the samples, except for samples A2 and W1, where they were measured at 0.12±0.00049 ppm and 0.23±0.00021 ppm, respectively. This is below the MPL as given in Table 2. It was consistent with the result by Sani *et al*. [18] and the report by Chowdhury [9]. Chromium (Cr) is a trace element that has garnered attention recently due to its essential nature. It is a cofactor in regulating normal glucose metabolism [19].

Copper Concentration

The result obtained as the Copper (Cu) concentration at 324.8nm in the ten (10) samples is represented in Table 1. The concentrations of copper varied from 0.032±0.00021 ppm to 0.129±0.00021 ppm across samples A1, D1, W1, W2, W3, and W4. Among the samples, W3 exhibited the highest copper concentration (0.129±0.00021 ppm), while W4 displayed the lowest (0.032±0.00021 ppm). Additional values were recorded as 0.065±0.00014 ppm, 0.097±0.00071 ppm, 0.032±0.00021 ppm, and 0.065±0.00014 ppm for A1, D1, W1, and W2, respectively.

Copper, an essential trace element, is vital for promoting optimal growth, maintaining integrity of the cardiovascular system, ensuring lung elasticity, regulating neuroendocrine function, and facilitating iron metabolism [20]. The MPL of Cu from the milk and dairy products is 0.001 (refer to Table 2). Consequently, milk and dairy products are regarded as relatively poor sources of copper. However, occurrences of copper deficiency are not frequent, except for cases of severe malnutrition. The investigation of copper (Cu) levels in milk samples was reported by Meshref *et al*. as 0.095 mg/kg and by Bakircioglu *et al*. as 0.138 mg/kg [14], [20]. Our current study findings indicate that the Cu concentrations in our samples closely align with those from the studies.

Iron Concentration

Sample W3 exhibited the highest iron (Fe) content among all samples. Upon comparison of these outcomes with the established permissible limits, it was observed that the levels of

Table1: Trace elements concentration in branded dairy products (ppm)

Sample code	Ca	Ar	Cr	Cu	Hg	Pb
A1	12.67±0.0083	0.45± 0.00042	0.00±0.00000	0.065± 0.00014	1.00±1.00	0.00±0.00000
A2	12.00±0.0035	0.00±0.00000	0.12±0.00049	0.000±0.00000	1.00±1.00	0.00±0.00000
C1	18.00±0.0071	1.35±0.00014	0.00±0.00000	0.000±0.00000	0.00±0.00	0.00±0.00000
C2	12.00±0.0071	0.00±0.00000	0.00±0.00000	0.000±0.00000	1.00±1.00	0.00±0.00000
C3	10.67±0.0047	0.90±0.00014	0.00±0.00000	0.000±0.00000	0.00±0.00	0.00±0.00000
D1	18.00±0.0035	1.58±0.00042	0.00±0.00000	0.097±0.00071	1.00±1.00	0.00±0.00000
W1	9.33±0.0023	0.68±0.00028	0.23±0.00021	0.032±0.00021	0.00±0.00	0.29±0.00028
W2	16.67±0.0012	1.35±0.00057	0.00±0.00000	0.065±0.00014	1.00±1.00	0.15±0.00064
W3	17.33±0.0023	2.48±0.00064	0.00±0.00000	0.129±0.00021	1.00±0.00	0.29±0.00028
W4	17.33±0.0023	1.35±0.00057	0.00±0.00000	0.032±0.00021	0.00±0.00	0.00±0.00000

Values are expressed as Mean±SD, n=3, SD= standard deviation , n= no. of readings taken per sample

Table 2. Maximum permissible limits (MPL) of heavy metal and micronutrient

Heavy metals	MPL (ppm)
Ca	2000[a]
Hg	0.01[b]
As	0.00001[b]
Pb	0.02[c]
Cr	0.3[b]
Cu	0.01[d]

[a]Food and Agriculture Organization [10]; [b]USDA [11]; [c]Commission Regulation (EU) [12]; [d]SAC [13]

Fe surpassed the accepted legal threshold (0.002 ppm) on average. This observation suggests the likelihood of Fe contamination originating from the metallic container of the condensed product, as previously suggested in a separate report.

Mercury Concentration

The mercury (Hg) levels, as presented in Table 1, were not detectable in samples C1, C3, W1, and W4. However, in samples A1, A2, C2, D1, W2, and W3, mercury was detected at a concentration of 1.0 ± 1.0 ppm. Mercury and methyl mercury (organic mercury) are considered neurological toxicants classified as a group C carcinogen, denoting that they are not classifiable as to their carcinogenicity to humans [17].

Lead Concentration

The lead (Pb) concentrations span from 0.15±0.00064 to 0.29±0.00028 ppm, as outlined in Table 1 and determined across three samples. The highest lead concentration was observed in samples W1 and W3 (0.29±0.00028 ppm), while the lowest was detected in sample W2 (0.15±0.00064 ppm). Lead concentrations were found to be undetectable in the remaining samples. Notably, the recorded values exceeded the maximum residue limit (0.01ppm) established by the United States Department of Agriculture (USDA). Our result was similar to other findings. Abu-Shaala *et al.* [7] reported 0.185ppm in dried dairy products, and Rahaman [6] reported 0.189 ppm in powdered milk. In contrast, [18] reported significantly lower mean values (0.0009–0.0014 ppm) for various branded and unbranded cow milk samples in selected regions of Kaduna Metropolis, Nigeria.

Lead (Pb) in milk and dairy products might originate from various environmental origins, including atmospheric deposition, waste disposal, vehicle emissions, and urban effluents [20]. Notably, it is crucial to recognize that the lead levels in milk and dairy products were notably elevated, surpassing the permissible threshold of 0.02 mg/kg, as established by the Commission Regulation for milk and related secondary dairy products. Lead (Pb) in infant milk raises significant concerns, necessitating urgent measures to reduce its levels, as it can accumulate to hazardous concentrations [21]. This concern is particularly crucial as children heavily rely on milk during this critical developmental stage. Implementing strategies to minimize lead levels in infant milk is essential to safeguard the health and well-being of infants. Such strategies include assessment of acceptable levels of heavy metal intake in food should be conducted using several methodologies recommended by WHO, USEPA, SON, and FAO/WHO, and ensuring good manufacturing practices such as evaluation of raw materials that serve as sources of contamination.

CONCLUSION

Using atomic absorption spectroscopy to quantitatively assess certain heavy metals and micronutrients in commercially available dairy products in Nigeria yielded significant results. The research revealed the heightened levels of specific heavy

metals in multiple samples, including lead, arsenic, and mercury, exceeding the acceptable thresholds established by regulatory authorities. Furthermore, it brought attention to the insufficiency of calcium levels found in certain products, which could impact their nutritional value. The findings necessitate rigorous quality control protocols within the dairy sector to guarantee customer safety and facilitate manufacturing dairy products adhering to crucial nutritional criteria. The study's significance lies in providing insights into potential health hazards linked to the consumption of specific branded dairy products, emphasizing the need for stakeholders to prioritize adopting efficient monitoring and regulation approaches to protect public health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Okafor SN, Osadebe PO, and Okafor CJ designed the experiments. Ogbonna ET and Okafor CJ conducted the experiments. Okafor SN, Osadebe PO, Okafor CJ, Ogbonna ET, and Ezenwafor C. contributed to interpreting the results. Ogbonna ET and Ezenwafor C. prepared the manuscript with contributions from all co-authors.

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