Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2023, Volume 14, Issue 6, Page 711-719

http://www.jmaterenvironsci.com

Investigation into some Physicochemical Parameters and Heavy Metal Status in Bread from Bakeries in Zaria, Nigeria

Okon, I. E.[1, 2], Anweting, I. B.[3], Udo, I. E.[3], Danzarami, D. A.[4]

[1]Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna, Nigeria
[2]Transport Technology Centre, Nigerian Institute of Transport Technology, Zaria, Nigeria
[3]Department of Chemistry, University of Uyo, Akwa Ibom State, Nigeria
[4]Department of Science Laboratory technology, *Nigerian Institute of Leather Science Technology,* Zaria. Kaduna State, Nigeria.
[*] *Corresponding author email: idokon22@yahoo.com*

Received *15 April 2023*
Revised *27 June 2023*
Accepted *30 June 2023*

Keywords:
- ✓ *Heavy metals;*
- ✓ *Bread;*
- ✓ *Bakeries;*
- ✓ *pH*
- ✓ *Moisture content*

Citation*: Okon I. E., Anweting I. B., Udo I. E., Danzarami D. A. (2023) Investigation into some Physicochemical Parameters and Heavy Metal Status in Bread from Bakeries in Zaria, Nigeria, J. Mater. Environ. Sci., 14(6), 711 – 719*

Abstract: This study determined the concentration of some heavy metals including lead (Pb), zinc (Zn), cadmium (Cd), iron (Fe) and nickel (Ni) and physicochemical parameters (pH and moisture content) in bread from ten different bakeries in Zaria, Nigeria. The different bread samples were prepared for analysis by wet digestion. Concentrations of the heavy metals were determined using Microwave Plasma Atomic Emission Spectrophotometer (MPAES) model 4200. The concentrations (mg/kg) were in the range 0.03 – 0.240 (Cd), 64.68 – 76.00 (Zn), 0.12 – 2.74 (Pb) and 21.04 – 44.96 (Fe). Ni was not detected in any of the bread samples. The average heavy metals levels (mg/kg) followed the order: Zn (67.38) > Fe (32.90) > Pb (1.53) > Cd (0.14). Concentrations (mg/kg) obtained for each of the metals in bread samples were higher than the 1.000, 0.003, 0.025 and 5.000 WHO permissible limits for Zn, Cd, Pb and Fe respectively. The pH content of the ten bread samples were between the range of 5.14 - 5.74. Moisture content of the bread samples ranged from 22.47 - 41.81%. This study revealed that some bread in Zaria contained heavy metals at levels that could threaten the health of consumers over prolonged regular consumption as the hazard index (HI) was far greater than 1.

1. Introduction

Heavy metals are toxic and are potential environmental contaminants, having the capability of entering into the food we eat thereby causing health problems to human, the major way in which humans are exposed to heavy metals is through soil-crop-food pathway (Adeosun and Makanjuola, 2020). Heavy metals and other chemical compounds are considered as toxic contaminants when they occur in certain concentration in food (Rather *et al.,* 2017). Bread as a food is made from flour, water and other ingredients like the leavening agent, kneaded and shaped into loaves as bread. Bread is a significant and essential food in many countries of the globe particularly the African countries and South East parts of Asia (Adeosun and Makanjuola, 2020). It is consumed extensively in homes,

restaurants and hotels in Nigeria and is one of the most consumed food with predominant consumption among poor people (Maziya-Dixon *et al.,* 2004; Olabimtan *et al*., 2014).

Some bread loaves contain additives and contaminants that are harmful to human health, some of such contaminants are heavy metals (Oyekunle *et al*., 2014; Akpambang and Onifade, 2020). Heavy metals are generally metals with relatively high density, they are not bread additive, but because they are potential environmental contaminants, they find their way into bread. Heavy metals could be introduced into bread during production from a number of sources, which includes, water used in the bakery, metal pan used for baking and bioaccumulation in flour use in the bakery (Adebiyi *et al*., 2021). The bioaccumulations in flour is linked to the possibility of heavy metal absorption from soil during growth of plants (Okon *et al*., 2023), as these plant are used for flour making. Apart from heavy metals that find their way into bread, other parameters like pH and moisture content are also quality indicators of bread. The exccedance of pH level in bread from the acceptable limit can have harmful effect on health (Hashemi *et al*., 2016). Moisture content of bread is an important factor in bread quality assessment as it indicates the tendency of bread to form mold (Olabimtan *et al*., 2014).

Since the accumulation of heavy metals in human affects health, it is essential to monitor content of heavy metals in foodstuff in order to protect human health and create awareness towards the enormous consumption of heavy metal contaminated bread. The work therefore aims at preliminary investigation of selected heavy metals concentration and physicochemical parameters (pH and moisture content) in bread samples from bakeries in Zaria.

2. Methodology

2.1 Materials and reagents

Polyethylene sample containers, digital analytical balance, hot plate, Microwave Plasma Atomic Emission Spectrophotometer (MPAES), hydrochloric acid (HCl) and nitric acid (HNO_3), bread loaves obtained from Bakeries in Zaria. Distilled water was used throughout the analysis.

2.2 Collection and preparation of bread samples

Ten (10) loaves of bread each from ten different bakeries in Zaria were collected as samples for the study. Sample containers made of polyethylene were thoroughly washed with tap water using detergent, rinsed with distilled water for several times and allowed to air dry before collection of the sample. Each bread loaf was oven-dried to constant weight, ground into fine powder using an agate pestle and mortar and sieved through a 2.0 mm sieve to obtain a dried composite powdered sample that was used for all the analyses.

2.3 Procedure for digestion of bread sample

Bread samples were oven dried at a temperature of 40-45 ^{0}C and then ground to a fine powder with a Thomas-Wiley laboratory mill (Model 4) and stored in a clean polythene container for further use (Ekwumemgbo *et al*., 2023). Digestion was carried out in line with standard method as described by American Public Health Association (APHA, 1999). A 10 g of each of the bread samples was weighed using a digital analytical weighing balance with $\pm$ 0.0001 g precision and then added to a beaker containing 5 ml concentrated HNO_3 and 15 ml concentrated HCl. The mixture was then heated on a

hot plate to decrease the volume to 3 – 5 ml through evaporation. Afterwards, 10 ml of distilled water was added to the solution and then passed through No.1 Whatman filter paper. Finally, the solution was diluted to 20 ml with distilled water. The digest was then used for the studied heavy metal analysis.

2.4 Heavy metals analyses

Concentrations of five metallic elements in each of the pretreated samples were analyzed in triplicates using Microwave Plasma Atomic Emission Spectrophotometer (MPAES, model 4200, USA) in the Multi-User Science Research Laboratory of Chemistry Department, Ahmadu Bello University, Zaria. The metals determined include cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni) and iron (Fe). For quality control, calibration curves were prepared separately for each of the metals by running different concentrations of standard solutions, the instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination and were subjected to statistical analysis.

2.5 Evaluation of health risk

To evaluate the health risk associated with consumption of bread from the selected bread samples, the estimated daily intake (EDI), hazard quotient (HQ) and hazard index (HI) was calculated (Ekeanyanwu *et al*., 2015; Akpambang and Onifade, 2020)**.** The calculations are as presented by Equations 1 – 3:

$$\mathbf{EDI} = \frac{\mathbf{C_{Bread} \times D_{Bread}}}{\mathbf{BW}} \ldots\ldots \mathbf{1}$$

Where:

EDI = Estimated Daily Intake

C_{bread} = average trace metal concentration in bread (mg/kg dry weight)

D_{bread} = average daily bread consumption (kg/day) in Nigeria which is 0.170 kg/person (Akpambang and Onifade, 2020)

BW = average body weight (kg). US-EPA risk analysis, considering an adult average body weight of 70 kg (Gaofeng *et al*., 2010).

$$\mathbf{HQ} = \frac{\mathbf{EDI}}{\mathbf{RfD}} \ldots\ldots\ldots\ldots \mathbf{2}$$

Where:

HQ=Hazard quotient; EDI=Estimated daily intake & RfD=Oral reference dose for each heavy metal

$$HI = \sum_{i=1}^{n} (\mathrm{HQ})i \ldots\ldots\ldots 3$$

Where, HI is the hazard index for the overall toxic risk and n is the total number of metals under consideration. (Onyele and Anyanwu, 2018)

Where,

HI = Hazard Index

HQ = Hazard Quotient

When HI ≤ 1 and HQ ≤ 1, there would be no obvious health risk (Ekeanyanwu *et al*., 2015).

2.6 Determination of moisture contents (mc)

A container was dried and cool in a desiccator and weighed, 5 g of the bread sample was placed in the container and the weight of both was taken. The sample was then dried in an air circulation oven at 105℃ to a constant weight. The sample and the container were placed in a desiccator to cool and then weighed. The difference in weight after drying was then used to calculate the moisture content (MC) as presented in Equation 5

$$\mathbf{MC}\ (\%) = \frac{\textbf{Loss in weight on drying (g)}}{\textbf{Initial sample weight (g)}} \times \mathbf{100} \ldots \mathbf{5}$$

2.7 Procedure for determination of pH

A 10 g of the homogenized bread sample was placed in 50 ml beaker. Distilled water was then added to just sufficient depth to allow immersion of the pH meter probe. Thereafter, the suspension was shaken for 15 min and then allowed to stand for 10 minutes, after which the pH was measured using digital pH meter (Mettler MP 220, Switzerland) by immersion of the probe into the sample solution and then reading was recorded similar to Ebong *et al*. (2022)

3. Results and Discussion

From Microwave Plasma Atomic Emission Spectrophotometer analysis, the concentrations of heavy metals in the bread samples were given in ppm, the values were then converted to mg/kg as shown in Figures 1 – 4 for Cd, Zn, Pb and Fe. Moisture content and pH of bread the samples were determined and the results obtained are presented in Table 1.

3.1 Heavy metal content in bread samples

The concentrations (mg/kg) of Cd as represented in Figure 1 are 0.06, 0.03, 0.14, 0.22, 0.16, 0.20, 0.04, 0.06, 0.24 and 0.24 for bread samples from bakeries A, B, C, D, E, F, G, H, I and J respectively. Hence, Highest Cd content (0.24 mg/kg) was found in bread from bakeries J and I. Magomya *et al.* (2013) found that the concentration (mg/kg) range of Cd in eight (8) bread samples was 0.02 – 0.08 when potassium bromate ($KBrO_3$) and heavy metal content of selected bread samples produced in Zaria was studied. Therefore, in comparison, the range observed in this study (0.03 – 0.24 mk/kg) for Cd is higher than that of Magomya *et al* (2013). The result in Figure 1 shows that the concentrations of Cd in all bread samples exceeded the World Health Organization WHO (2011) permissible limit of 0.003mg/kg for Cd in food (Adebiyi *et al*., 2021; Okon *et al*, 2022). Cadmium is a non-essential element with no known beneficial effects in the human body as it is a toxic metal even in minute concentrations, it is associated with ill health in terms of osteoporosis, cardiovascular disease and cancer (Luevano and Damodaran, 2014). Zn content in the bread samples as presented in Figure 2 were high, the concentrations (mg/kg) obtained were 68.50, 67.88, 69.90, 65.66, 76.00, 59.48, 67.28, 69.18, 65.26 and 64.68 for samples A, B. C, D, E, F, G, H, I and J, respectively. The values obtained are higher than the concentration range (0.24 - 2.11 mg/kg) of Zn obtained by Magomya *et al.* (2013). This could emanate from high heavy metal contamination of bread condiments currently used by bakers in Zaria. The range of concentration for Zn (64.68 – 76.00 mg/kg) obtained is higher than the WHO (2011) standard limit of 1 mg/kg for Zn in food (Adebiyi *et al*., 2021).

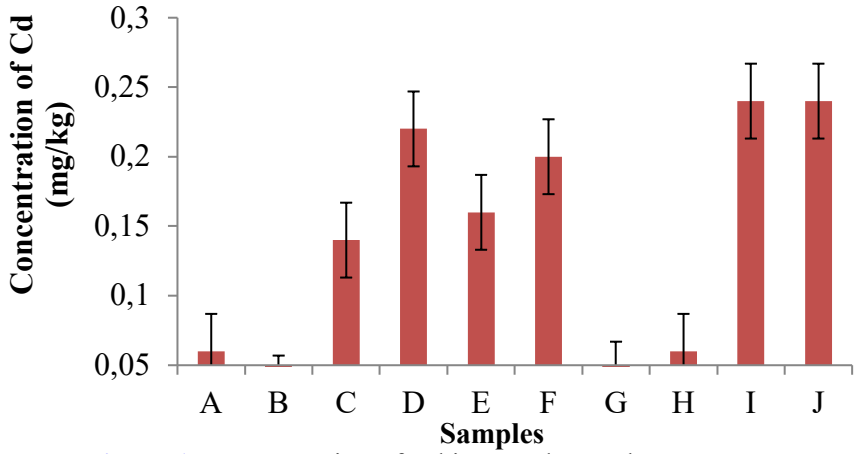

Figure 1. Concentration of Cd in Bread Samples

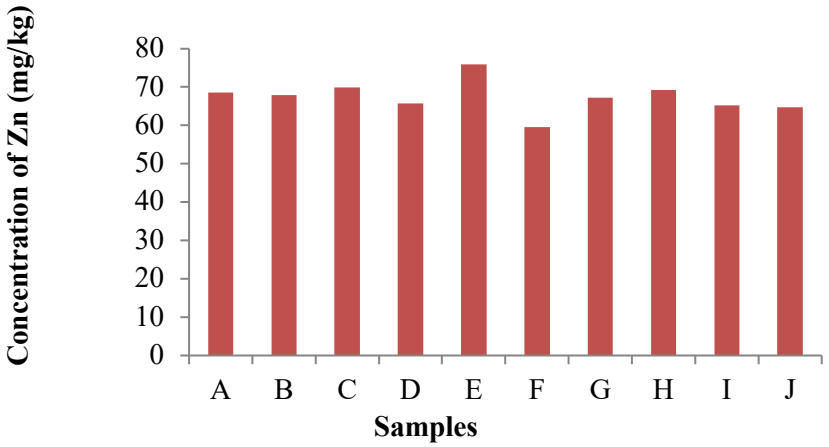

Figure 2. Concentration of Zn in Bread Samples

The concentration of Pb as shown in Figure 3 shows maximum concentration in sample A (2.74 mg/kg) and minimum concentration (0.12 mg/kg) in sample E. in sample F – J, Pb was not detected. Concentration range of Pb (0.12 – 2.74 mg/kg) in this study is higher than that of Dariush *et al.* (2014) which was 0.33 – 0.90 mg/kg. This could resulted from higher heavy metal content of bread condiments, it could also emanate from different environment of research as Dariush *et al.* (2014) carried out their study in Iran. Flour used for bread making can contain Pb resulting from the soil in which the major flour precursor (wheat) is grown; this can lead to high Pb in Bread. As shown in Figure 3, Pb levels in all the bread samples are above the WHO (2011) standard limit of 0.025 mg/kg (Adebiyi *et al*., 2021).

Fe concentrations ranged from 21.04 – 44.96 mg/kg as presented in Figure 4. Magomya *et al.* (2013) obtained 7.05 mg/kg as the maximum concentration and 0.53 mg/kg as minimum concentration for Fe in bread samples. Therefore, comparing the result with that of Magomya *et al*. (2013), Fe contamination observed in this study is higher. The range of concentration (21.04 – 44.96 mg/kg) across the ten samples is above the permissible limit range of 2.2 – 5.0 mg/kg for Fe in food (Adeosun and Makanjuola, 2020).

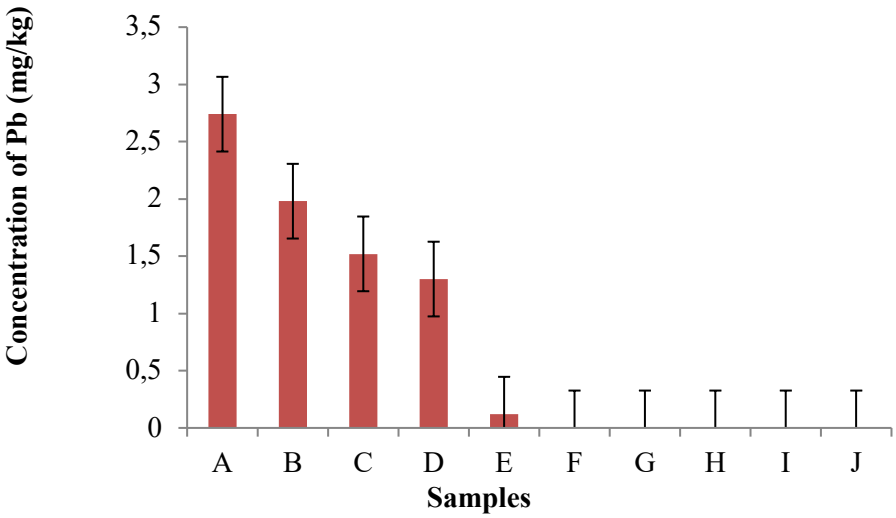

Figure 3. Concentration of Pb in Bread Samples

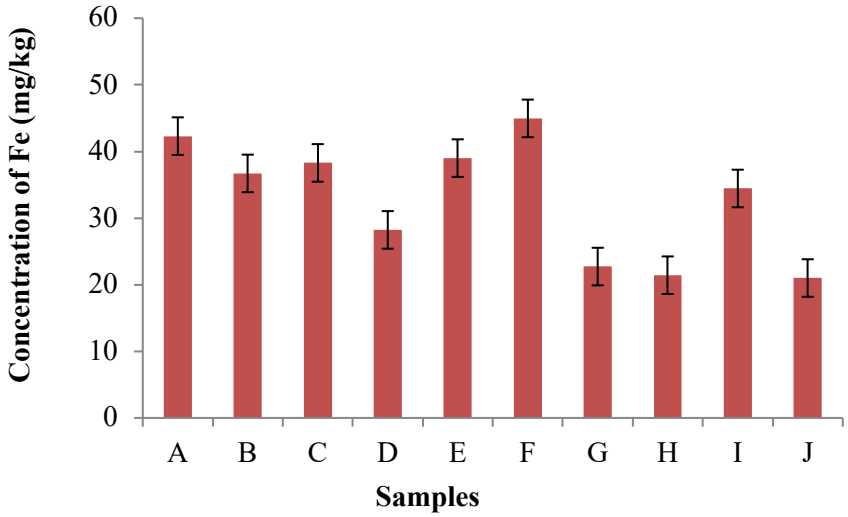

Figure 4. Concentration of Fe in Bread Samples

3.2 Health risk assessment

Health risk associated with consumption of the bread was determined considering estimated daily intake (EDI), hazard Quotient (HQ) and hazard index (HI). EDI was estimated based on concentration of each metal measured in bread, average daily bread consumption of 0.17 kg/day (Akpambang and Onifade, 2020) and average body weight of 70 Kg (Gaofeng *et al*., 2010). Hazard quotient (HQ) values were determined based on the oral reference dose (mg/kg/day) of Cd (0.001), Zn (0.3), Pb (0.004) and Fe (0.007) as given by USEPA IRIS (2011). Hazard index (HI) was calculated by the summation of hazard quotients (HQ) of all the metals studied. The EDI values (mg/kg) were 0.0003, 0.1636, 0.0037 and 0.0799 for Cd, Zn, Pb and Fe respectively. That HQ were 0.0075, 0.5455, 0.9301 and 11.4142 for Cd, Zn, Pb and Fe respectively while the HI was 13.23. This HI of 13.23, which is the sum of health

risk from the metals studied shows that bread consumption in Zaria has some heath risk despite the fact that HQ was less than 1 for Cd, Zn, Pb since HI is far greater than 1 (Ekeanyanwu *et al*., 2015).

3.3 The pH of bread samples

The pH of all ten bread samples as shown in Table 1 is between the range of 5.15 – 5.74, an indication that all the bread samples were somewhat acidic. The pH range observed was within the acceptable limit range (4.6 – 6.0) of pH for bread (Ghajarbeygi *et al*., 2018). The low pH range (5.15 – 5.74) in bread samples can constrain the growth of bacteria and mold as they do not really thrive in acidic medium.

3.4 Moisture content

High moisture content in bred can cause an increase in the rate of microbial growth; this can affect the freshness and shelf-life of the bread. The values of moisture content as shown in Table 1, reveals that bread from bakery H has the highest moisture content (41.81%) with sample C having the lowest (22.47%). Following the fact that low moisture content implies less tendency of bread to form mold while high moisture content will result in it having molds (Olabimtan *et al*., 2014), bread from bakery H may have higher tendency of mold growth and shorter shelf-life compared to others. The high moisture content in sample H could have resulted from moisture content of the bread making ingredients, production process such as low baking temperature and the amount of $KBrO_3$ additive used during baking. Low moisture content of other bread samples calls for concern as it could emanate from higher level of $KBrO_3$ in them. This is because since the ban on $KBrO_3$ by National Agency of Food and Drug Administration and Control (NAFDAC) due to its toxicity, many bakers in Nigeria have not complied with the ban (Olabimtan *et al*., 2014).

Table 1. Selected Physicochemical Parameters of Bread Samples

Bread sample	pH	Moisture Content (%)
A	5.52	29.67
B	5.65	25.98
C	5.57	22.47
D	5.73	26.00
E	5.48	32.68
F	5.61	27.22
G	5.46	28.84
H	5.74	41.81
I	5.15	33.91
J	5.69	33.00

Conclusion

The result of this analysis has shown that the level of cadmium, zinc, lead and iron in the bread samples are higher than the WHO (2011) permissible limits. The pH level of the bread samples are within the acceptable limit while water content was higher in one of the samples and this could lead to growth of

microbes. The study revealed that some bread in Zaria contained heavy metals at levels that could threaten the health of consumers over prolonged regular consumption as the hazard index (HI) was far greater than 1. Hence, there is need for regulatory bodies to enforce strict conditions regarding bread production by carrying out regular monitoring of bread production process to prevent contamination by heavy metal and to align with all standards.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

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