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Evaluation of Heavy Metals and Aflatoxin Contents of Selected Biscuits Sold in Makurdi, Benue State-Nigeria

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Abstract: *Biscuits are baked, edible and common flour based food products. Metallic elements with a density that is greater than 5 g/cm^3 (Heavy metals) are not easily degradable or metabolized and are usually persistent and may be biologically accumulated in food items, trapped on the outer surface or may be added due to manufacturing or processing of food for consumption. Heavy metals can develop gradually in the body tissue and overtime could exceed tolerable limits which can cause intense toxicity leading to human disease, disorders, defects, illness, malfunctioning and malformation of organs and ultimately death. Aflatoxins are poisonous, carcinogenic by-products of the growth of the molds Aspergillus flavus and Aspergillus parasiticus, and are the most studied and widely known mycotoxins. In this research, five biscuits samples were purchased at Wurukum and Modern Market, Makurdi. The samples were digested and Heavy metal (Zn, Pb, Cu, Cd, Cr, Ni, Fe, Co and Hg) concentrations and aflatoxin content evaluated using Atomic Absorption Spectrophotometer (AAS) and Enzyme-linked immunosorbent Assay (ELISA) respectively. All the metals analysed were in their permissible limits except for Cr which was 0.117±0.0001 mg/Kg in sample B, 0.122±0.0001 mg/Kg in sample C, 0.209±0.0001 in sample D and 0.166±0.0001 mg/kg in sample E against its WHO permissible limit of ≤ 0.1 mg/kg. Hg was only detected in sample D and E but were within the WHO permissible limit of ≤1.0 mg/kg. The aflatoxin in all the samples was undetected indicating that the samples were free from aflatoxin. The entire result showed that the samples were free from heavy metals and aflatoxin contaminations. Efforts should however be made to control Cr concentration as the consumption of these products with time is liable to pose health problems arising from Cr accumulation.*

Keywords: *Biscuit; Heavy metals; Aflatoxin; Toxicity; AAS; ELISA*

Introduction

Biscuits are a form of relatively small, packaged, ready-to-eat fast foods, usually taken not as a regular meal, but as a stopgap. Snacks may be eaten to temporarily hold hunger or to satisfy a craving. Biscuits are often a form of ready-to-eat food, designed to be portable, quick, and satisfying (World Health Organization, 2015). In spite of the fear expressed over foods originating from the informal food production and marketing system, many people in the urban centres cannot help eating packaged, home-made snacks due to their busy schedule (Oyelola *et al*., 2013). Contamination can occur in food due to pick up of metals from equipment, processing or packaging materials. These contaminated food products with heavy metals contribute to human dietary intakes and the levels of these metals need to be regularly observed and controlled. While the health effects of microbial contamination may be noticed within days or weeks, the effects of chemical contamination may take a longer time to manifest. Examples of chemical contaminants are metals like Pb, Cd, Zn, Hg, Mg, Mn, Cu, Co. Though some of these metals (e.g. Zn, Cu, Co, Mn) are classified as essential elements, when present in the body above certain concentrations, they can become harmful causing various health conditions. Metals like Pb and Cd have no known biological functions and may exhibit toxicological problems even at low or trace concentrations (Marsh & Bugusu, 2007; Iwegbue, 2012; Elham-Elshewey *et al.,* 2015). Depending on the type of metal, the health effects of metal poisoning include gastrointestinal disorders, tremor, diarrhoea, paralysis, vomiting, convulsion, diabetes, cancer, anaemia among others (Adefris, 2011; Duruibe *et al.,* 2007). Heavy metals disrupt the physiological functions of the body by accumulating in vital organs and glands such as the heart, brain, kidney, bone, and liver (Ray & Ray, 2009). In order to check exposure to food contaminant toxicity, the Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly released a list of maximum levels for contaminants and toxins in foods (Codex Alimentarius Commission, 2011). Frequent assessment studies are therefore necessary to determine the safety of foods that are consumed regularly. Many studies have found metal contaminants above recommended safe levels in many foods that are regularly consumed in Nigeria and other places (Lanre-Iyanda & Adekunle, 2012; Elham-Elshewey *et al.,* 2015).

Materials and Methods

Sample Collection

Three packs with different batch numbers of the same brand of biscuits each for Sample A, B, C, D and E were bought from Wurukum and Modern markets, Makurdi, Benue State.

Sample Preparation

The biscuit packs were opened and the pieces removed. The pieces were then dried in an oven at 60 ℃ for duration of 30 min for complete crispiness. Each sample was ground to fine powder

using a pre-cleaned agate mortar and pestle. The three batches were mixed and homogenized to constitute a composite sample.

Sample Digestion for AAS Analysis

2 g each of the ground samples was weighed in crucible and then 2 mL of concentrated HCl and 6 mL of concentrated HNO_3 were added (ratio of 1:3) and heated at 140 ℃ and then cooled at room temperature. After cooling, 2 mL of concentrated H_2SO_4 was added and heated at 140 ℃ and then cooled at room temperature. Later, 10 mL of HNO_3 was added and then heated again until the solution became clear. The resulting solution was cooled at room temperature and was then diluted with 25 mL of deionized water and filtered through Whatman No. 42 filter paper and <0.45 1m Millipore filter paper. It was then transferred quantitatively into well labeled sample bottles for AAS analysis.

Sample Digestion for Aflatoxin Evaluation

50 g of prepared sample was taken in a 500 mL conical flask and 25 mL water, 25 g diatomaceous earth and 250 mL chloroform were then added to it. The flask was securely stoppered with masking tape and shaken on a risk action shaker for 30 min to extract the toxin. The mixture was filtered through fluted filter paper and the first 50 mL was collected and kept for aflatoxin determination.

Sample Analysis for Heavy Metal Concentration

The well labeled samples were then analyzed for some heavy metal concentrations at the Federal Ministry of Agriculture and Rural Development, Zaria office, Kaduna State using the Atomic Absorption Spectrophotometer (AAS) model PG990.

Sample Analysis for Aflatoxin

The prepared and well labeled samples were then analysed for aflatoxin at National Agency for Food and Drug Administration and Control (NAFDAC) Laboratory, Kaduna, Kaduna State using ELISA.

Determination of moisture content

The air oven method was used to determine the moisture contents of the biscuits. 3 g of the biscuit samples was weighed into different petri dishes, dried separately at 105 ℃ for 4 hr and cooled in a dessiccator. The samples were finally dried to a constant weight and the percentage moisture content was calculated as follows:

$$\% \text{ moisture content} = \frac{W2-W3}{W2-W1} \times 100$$

Where W_1= Weight of crucible

W_2=Weight of crucible + sample before drying

W_3=Weight of crucible + sample after drying

Results

The results for the analysis are as shown below:

Table 1: Concentrations of Heavy Metals in the Samples

Heavy Metals	Concentration (mg/kg) A	B	C	D	E	WHO
Zn	2.315±0.0046	2.507±0.0036	1.568±0.0007	1.425±0.0021	2.663±0.0027	≤ 99.4
Pb	1.215±0.0004	0.851±0.0004	1.191±0.0008	1.178±0.0005	2.915±0.0005	≤ 3.0
Cu	0.316±0.0004	0.334±0.0003	0.324±0.0002	0.328±0.0003	0.376±0.0002	≤ 73.3
Cd	0.182±0.0014	0.167±0.0009	0.180±0.0014	0.162±0.0016	0.220±0.019	≤ 0.2
Cr	0.154±0.0004	0.117±0.0001	0.122±0.0001	0.209± 0.0001	0.166±0.0001	≤ 0.1
Ni	0.395±0.0006	0.444±0.0002	0.422±0.0007	0.483±0.0018	0.524±0.0006	≤67.9
Fe	2.392±0.0012	1.896±0.0011	1.409±0.0002	1.477±0.0008	7.439±0.0074	≤42.5
Co	0.268±0.0008	0.219±0.0009	0.221±0.0007	0.272±0.0002	0.309±0.0006	≤ 2.0
Hg	ND	ND	ND	0.125±0.0001	0.117±0.0002	≤1.0

- ND=Not Detected

Table 2: Concentrations of Aflatoxin in the Samples

Parameter (µg/kg)	Samples A	B	C	D	E	FAO
Aflatoxin	ND	ND	ND	ND	ND	<0.05

- ND: Not detected

Table 3: Moisture Content of the Samples

Parameter (%)	Samples A	B	C	D	E	WHO
Moisture content	4.95±0.05	5.21±0.04	5.15±0.45	4.98±0.02	5.00±0.04	1-6 %

Discussion

The concentrations of all the heavy metals evaluated in sample A were within WHO acceptable range in food thus indicating no harm to consumers (Table 1). For the rest of the samples, only Cr out of the nine evaluated metals was higher than the permissible limit set by WHO. The concentration of Cr was 0.117±0.0001 mg/kg, 0.122±0.0001 mg/kg, 0.209± 0.0001 mg/kg and 0.166±0.0001 mg/kg in B, C, D and E respectively against the WHO permissible limit of ≤ 0.1 mg/kg.

In general, all the heavy metals detected were within their respective WHO standards except Cr. The reasons for the high levels of the metal in the samples could arise from unsafe storage conditions or at any point in the production chain. High levels of Cr indicates that, continuous intake of sample B, C, D and E can cause serious health problems such as bloody diarrhea, muscle weakness, kidney problems among others.

The aflatoxin in all the samples was not detected indicating that the raw materials were free from this mycotoxin. This also indicates that both the processing and the storage conditions were also appropriate. The moisture content in all the samples were very low and within the WHO standards thus confirming that microorganisms including aflatoxins cannot thrive.

Conclusion

The concentrations of heavy metals in the studied samples were generally low and within the permissible limits set by WHO. Chromium was however higher than the recommended concentrations in sample B, C, D and E. The assessment provides some preliminary evidence that these products contained unacceptable levels of Cr with potential serious consequences for children and adults who consume the products. Regulation on food should be strongly enforced so as to checkmate this ugly occurrence in order to protect human health.

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