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ASSESSMENT OF HEAVY METALS IN LOCAL CHEESE (WARA) SOLD AT REMO NORTH LOCAL GOVERNMENT AREA OF OGUN STATE

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Abstract

Milk and dairy products are contaminated by different contaminants such as foodborne biological pathogens, chemical toxicants and other hazards. Heavy metal contamination is one of the most significant types of chemical contamination in milk and dairy products as it poses an important and challenging role in food safety. It can be the reason to the prevalence of diverse illnesses and lesions in the human body. The aim of this study was to determine the levels of Copper (Cu), Lead (Pb), Zinc (Zn), Chromium (Cr) and Cadmium (Cd) contents in local cheese (wara) sold at Remo-North local government area of Ogun State. Fifteen samples of local cheese were bought from Fulani women in three communities of the local government area (Ode, Isara, and Ipara). Quantitative analysis for heavy metals was performed using atomic absorption spectrometer (AAS) (Hitachi Z-8100, Japan). The highest mean content of Cu (0.18 mg kg^{-1}), Pb (0.02 mg kg^{-1}) and Cd (0.88 µg kg^{-1}) were found in samples from Ipara while the highest mean content of Zn (3.03mg kg^{-1}) was found in samples from Isara. The least content of Cu (0.14mg kg^{-1}), Pb (0.01mg kg^{-1}), Zn (2.10mg kg^{-1}) and Cd (0.63 µg kg^{-1}) were found in samples from Ode. Cr was however not found in any of the samples collected. Contents of Cu, Pb, Zn, and Cr in the local cheese samples were below the international recognized permissible limits except for Cd whose concentration was above the permissible and recommended values. Therefore, it is imperative to address the health risk associated with wara consumption in Remo-North local government area of Ogun state, by implementing effective control measures and regular testing to ensure the safety and quality of this staple food.

Keywords: Heavy metals, wara, safety, milk

Introduction

Cheese, found in over 2000 varieties worldwide, is the most common dairy product globally (O'Connor 1993; Belewu *et al.*, 2006). Approximately one-third of the milk produced in African nations is utilised in cheese production. (FAO, 2002). In addition to providing a great source of nutrients like protein, carbohydrate, lipid, minerals and vitamin, cheese also serve as a form of conserving vital mineral nutrients (Hildreth, 1977). The majority of Nigeria's traditional cheese producers are the wives of Fulani herders who market the cheese as a means of livelihood (Fabusoro and Oyegbami, 2009). Warankashi (wara for short) is the popular Nigerian local cheese produced by boiling fresh cow milk slowly at 50^{o} C in a pot with extract of leaves and stem of Sodom apple (*Calotropis procera*) a madar plant known to contain callotropin enzyme needed to cuddle casein in milk. (ILCA, 1998). The white and soft cheese produced from this process is eaten raw, as a flavoured snack, fried cake or added to soup as a meat supplement (Omotosho *et al*., 2011)

Metals whose densities are five times greater than that of water are regarded as heavy metals (Holleman and Wiberg, 1985). They are also called trace elements because they are usually present in the environment in traces (mg kg^{-1}) or ultra-traces (μg kg^{-1}) (Gagoasa *et al*., 2008). Some essential heavy metals called micronutrients (e.g. Copper, Zinc, Cobalt, Chromium, Manganese and Iron) are needed in the diet of humans and animals for overall health, normal growth, disease resistance, vitality and reproduction. Their enormous ingestion beyond tolerable limits can be toxic and harmful to living organisms (Blaylock and Huang, 2000). The non-essential metals like Barium, Aluminum, Lithium and Zirconium are not required by living organisms for optimal well-being (Mahmood *et al*; 2014)

Toxic heavy metals are metals that are harmful to humans and animals and are not tolerable even in minute quantities e.g. Cadmium and Mercury. (Mukesh *et al*., 2008). Certain consequences of excessive heavy metal buildup within the human body encompass kidney failure, genetic mutation, and disorders of the nervous system. Additionally, it may lead to cardiovascular issues, various forms of cancer, respiratory ailments, compromised immunity and infertility. (Urannet *et al*., 2019)

Heavy metals commonly infiltrate the ecosystem via natural mechanisms, like volcanic activity, erosion, wind-blown dust, precipitation, and human induced activities such as mining, metallurgy, industrial processes, fuel combustion, increased vehicular traffic, and agricultural practices such as the utilization of synthetic fertilizers, bio-solids, agrochemicals, animal manure distribution and waste disposal ((Herewatic *et al*.,2000). The food chain serves as a primary conduit through which enduring, toxic heavy metals gain access to the bodies of higher organisms. Typically, organisms occupying the upper tiers of the food chain can amass substantial quantities of these metals in their tissues, with accumulation influenced by factors such as age, size, and dietary preferences (Mahmood *et al*., 2014) The well-documented toxicity resulting from elevated levels of certain elements, including Chromium (Cr), Cadmium (Cd), Lead (Pb) and Mercury (Hg), is widely recognized. (Llobet *et al*., 2003)

In Nigeria, milk serves as a key source of animal protein and is commonly consumed in various forms including local yoghurt, sour milk, boiled or fried cheese, as well as powdered, condensed, or evaporated milk (Ganguly *et al*., 2019). Although many Nigerians consume milk which is industrially processed in different forms, the consumption of locally produced milk by settled Fulani Pastoralists (SFPs) is of economic significance. The SFPs are local dairy producers that migrate in search of an ideal condition for raising their herds and means of survival. Several studies have proven that local production of milk by SFPs is carried out under unhygienic conditions that influence contaminations (Omotayo *et al*., 2013). Despite several laws guiding the production of food, local producers such as the SFPs and consumers may not be aware of the health implications of unhygienic food and the conditions of preparation of the food derivatives and other products: milk and cheese which this study considered.

Different contaminants, such as heavy metals, mycotoxins, and residues of veterinary drug can affect the quality of milk and dairy products. Among these, heavy metals like Lead and Cadmium pose significant challenges due to their potential public health risks including various illnesses and lesions (Rokni, 2007; Mahmoudi *et al*., 2013; Fallah *et al*., 2015; Mahmoudi *et al*., 2015). These metals which are not metabolized in the body, increase susceptibility to bacterial, viral, and fungal diseases. High concentration of heavy metals over a short period have similar effect to lower concentration over a longer period. Environmental organizations identify Lead and Cadmium as particularly harmful (Eskandari & Pakfetrat., 2014; Hamidpour et al., 2011; Mahmoudi et al., 2015; Rokni, 2007). This study aims to evaluate the concentration of heavy metals in wara (soft cheese) sold in Remo-North local government, Ogun state; to ascertain the quality and safety for human consumption.

Materials and Methods

Study Area

Remo North local government was carved out of Ikenne local government on October 1996. The area is located at latitude 7°00'N and longitude 3°41'E. The local government comprises of three major communities; Ode-Remo, Isara and Ipara with the headquarters situated in Isara. A notable area in Remo North local government is Saapade, a place where it is assumed that the three major communities of the local government intersect therefore the name saa-pade – "we meet". Bounded by Ijebu North, Ikenne and Obafemi Owode (to the East, South West and North respectively), Remo-North local government has a population of 59,911 (as at 2006 Census figure) and occupies 199 square km. It is less than forty minutes-drive to Lagos and Ibadan (the two most populous cities in Nigeria). Agriculture is the main source for livelihood. However, some industries already exist there while dominant commerce is gradually setting in.

Sample Collection

Fifteen samples of local cheese were bought from Fulani women in three different communities, (Ode, Isara, and Ipara) of the Remo North local government area. Three (3) samples each were bought randomly from five Fulani women in the selected locations. Each sample of the local cheese bought was packed individually in sterile Ziploc plastic bags and taken to the laboratory within 24 hours for proper analysis.

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Laboratory Analysis

The method for analysis of heavy metals in wara involved a two-stage procedure;

A. Nitric–perchloric Acid Digestion

Ten grams of sample was placed in a 250 ml digestion tube followed by the addition of 4ml of concentrated H_2SO_4, 20ml HNO_3, and 5ml of perchloric acid ($HClO_4$). The mixture was left to stand at room temperature for 30 min before being heated at 250 °C for 30 min. Afterwards, the digestion tube was removed from the heating mantle and allowed to cool. Once the solution became clear after cooling, it was filtered through Whatman No. 42 filter paper and <0.45 Millipore filter paper. The filtrate was then transferred quantitatively to a 25 ml volumetric flask by adding distilled water.

B. The use of Atomic Absorption spectrometer (AAS)

The concentrations of each of the heavy metals - Cd, Cr, Cu, Pb, and Zn in the final solution of 25 ml volumetric flask were determined by an atomic absorption spectrometer (AAS) (Hitachi Z-8100, Japan). AOAC (1990).

Results and Discussion

The concentration of Pb, Zn, Cu, Cd and Cr in the wara samples bought from the three sampling locations in the study area are shown in Table 1

Table 1: Concentration of Heavy metals in wara samples

Location	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cr (mg kg^{-1})	Cd (µg kg^{-1})
ODE$_1$	0.14±0.00^{def}	0.01±0.00^{cde}	2.07±0.01^{i}	0.00±0.00	0.65±0.01^{d}
ODE$_2$	0.13±0.00^{f}	0.01±0.00^{de}	2.12±0.00^{h}	0.00±0.00	0.61±0.06^{d}
ODE$_3$	0.14±0.00^{ef}	0.01±0.00^{cde}	2.11±0.00^{h}	0.00±0.00	0.64±0.00^{d}
ISARA 1	0.14±0.00^{cd}	0.01±0.00^{e}	3.00±0.00^{b}	0.00±0.00	0.81±0.00^{bc}
ISARA 2	0.14±0.00^{cde}	0.01±0.00^{de}	3.12±0.00^{a}	0.00±0.00	0.78±0.00^{c}
ISARA 3	0.15±0.00^{c}	0.01±0.00^{e}	2.98±0.00^{c}	0.00±0.00	0.85±0.00^{ab}
IPARA 1	0.18±0.00^{ab}	0.01±0.00^{bc}	2.66±0.00^{d}	0.00±0.00	0.90±0.00^{a}
IPARA 2	0.18±0.00^{b}	0.02±0.00^{a}	2.48±0.00^{g}	0.00±0.00	0.87±0.00^{a}
IPARA 3	0.18±0.00^{a}	0.02±0.00^{ab}	2.56±0.00^{f}	0.00±0.00	0.88±0.00^{a}
IPARA 4	0.18±0.00^{ab}	0.01±0.00^{bc}	2.58±0.00^{e}	0.00±0.00	0.87±0.00^{a}

Means with different superscripts along same column are significantly different ($p<0.05$)

Table 2: Mean values of concentration of heavy metals in wara samples

Metals	ODE	ISARA	IPARA
Cu (mg kg^{-1})	0.14±0.00^{c}	0.14±0.00^{b}	0.18±0.00^{a}
Pb (mg kg^{-1})	0.01±0.00^{b}	0.01±0.00^{c}	0.02±0.00^{a}
Zn (mg kg^{-1})	2.10±0.01^{c}	3.03±0.02^{a}	2.57±0.02^{b}
Cr (mg kg^{-1})	0.00±0.00	0.00±0.00	0.00±0.00
Cd (µg kg^{-1})	0.63±0.02^{c}	0.82±0.01^{b}	0.88±0.00^{a}

Means with different superscripts along same row are significantly different ($p<0.05$)

Mean values of the heavy metal content in the wara samples from all the

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sampling sites followed the order Zn > Cd > Cu > Pb > Cr.

Zn concentration was the highest in the wara samples analyzed in this study (Table 2). The mean concentration values ranged from 2.17 mg kg^{-1} to 3.03 mg kg^{-1} which coincides with what was reported by Simsek *et al*., (2000), Licata *et al*., (2004), Dobrzanski *et al*., (2005), Enb *et al*., (2009) and Pilarezyk *et al*., (2013) who determined Zn concentration in milk in the ranges of 2.3-6.46, 0.0247-4.961,3.09-3.16, 3.001-3.940 and 2.026-4.8 mg kg^{-1} respectively. Malhat *et al*., (2012) in a similar study of dairy products in Egypt reported higher values between 4.770-10.75µg g^{-1}, Meshref *et al*., (2014) reported values in the range of 2.73-18.316mg kg^{-1} in milk and dairy products from Egypt. Monika *et al*., (2013) reported values between 11.21mg kg^{-1} -54.47mg kg^{-1} in cottage cheese from various regions of Poland, Mas *et al*., (2011) obtained range of 33.66-63.41µg g^{-1} in cheese from France. Fayed *et al*. (2013) in his study stated that zinc was the major contaminant in milk and milk products samples, followed by iron, but copper, lead, manganese and cadmium were found in lower concentrations.

Zn stands as a vital for growth and functional performance of the immune system, serving as a key component of enzymes and playing a significant role in cellular growth and tissue differentiation. The recommended maximum level of zinc is 0.028 mg kg^{-1}, (IDF Standard 1979). Exceeding this limit can result in various health issues. (Faa *et al*., 2008). Environmental sources of zinc pollution include fossils fuel combustion, industrial activities, and municipal sewage with zinc from mining and industrial waste potentially contaminating groundwater due to its high solubility.

Cd stands out as the most hazardous among heavy metals, possessing significant carcinogenic and mutagenic characteristics (Piglowski, 2018; Gonzalez *et al*., 2006). In this research, the mean concentration of Cd ranged from 0.63 to 0.88 mg kg^{-1} contrasting with findings by Lante *et al*. (2006), who found no Cadmium in the milk samples he analyzed. However, these values surpass those reported by Malhat *et al*., (2012) in local cheese samples (0.288µg g^{-1}) and Sarsembayeva *et al*., 2020 (0.0025-0029mg kg^{-1}) in a similar study. Cd is not readily absorbed by the body but upon absorption, it is slowly excreted and builds up in the kidneys, leading to renal damage. The permitted levels of Cadmium in raw milk according to the Codex Alimentarius 2000 (Committee on Food Additives, Geneva Study) is 0.010 mg kg^{-1} (Bonyadian et al., 2006).

Cd is widely used in industry for electroplating, production of steel, plastics, batteries, ceramics and textiles. Industrial emission (waste incineration) is also a potential source of Cd .Cd is also the major impurity present in phosphate fertilizers. High content of Cd in milk products might be due to high exposure of soil and water to the source of Cd near hazardous waste sites. Cd exhibits a high phyto-accumulation index due to its low adsorption coefficient and high

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mobility in soil-plant systems, potentially allowing it to enter the food chain. Some studies have shown that the Cd concentration in cow's milk that are fed in industrial areas, along highways or animal feed contaminated with heavy metals are much higher than those fed in clean areas (Mahmoudi *et al*., 2017) Presence of cadmium in wara samples could also be due to fodder contamination through the soil.

Copper is essential for growth, nerve function, and energy release (IM, 2001). It is stored primarily in the liver, possesses antioxidant properties, and participates in gene expression. However, excessive intake of copper can result in Wilson's disease, characterized by nerve cell destruction, liver cirrhosis, ascites, oedema and hepatic failure (Gossel and Bricker, 2019). Level of Cu in the analysed samples varied from 0.14-0.18mg kg^{-1}. There is no notable distinction, as the mean copper concentrations measured at the three sampling sites are similar irrespective of the sampling location with a p-value at $P>0.05$. The mean Cu concentration in the three locations are lower than those reported by other authors who have worked on cow's milk and dairy products (Tripathi *et al*., 1999; Kira and Maihara, 2007; Kondyii *et al*., 2007; Semaghuil *et al*., 2008, Jigam *et al*., 2011). Previous studies showed that cow milk from rural area often contained copper concentration less than 0.39mg/l (Licata *et al*., 2004). Plausible reason for this may be due to rural agrarian setting with low industrial activities.

Pb concentration recorded in this study is between 0.02 and 0.1mg kg^{-1} which is lower than that reported by Jigam *et al*., 2011(0.16-0.63mg kg^{-1}) and Caggiano *et al*., 2005 (0.24-0.77µg kg^{-1}) The occurrence of lead in milk and dairy products may result from factors like transhumance along motorways, contamination of fodder, and climatic influences such as wind as well as the use of pesticide compounds. Lead (Pb) is recognised as one of the most significant metals capable of contaminating food items. Damage to the central nervous system is a notable and frequent indication, especially in children, due to their limited tolerance to lead (Johansen *et al*., 2017). It inhibits the biosynthesis of haem groups of blood and thereby affects the membrane permeability of liver, kidney and brain cells, reducing their functions or complete damaging these organs (Ahmed and El-Boushy, 2018). Chromium was not detected in any of the wara samples bought from the three sampling sites.

The trace levels of heavy metals observed in this research are probably linked to the contamination of animal feed and water. These contaminants can be found in milk and dairy products to varying degrees, as highlighted by Caggiano *et al.,* (2005) and can be transmitted to these products during processing procedures. With environmental pollution from heavy metals on the rise annually due to industrial expansion, there is a likelihood of a corresponding increase in the presence of these contaminants in the dairy sector over time.

Conclusion

The results showed that the content of the heavy metals studied in wara was within the permissible concentration except for Cadmium whose concentration was above the permissible and recommended values. Continuous consumption implies possible bioaccumulation of both heavy metals and phthalate esters. Low public awareness of food safety and indifferent health beliefs would worsen the adverse health effects

Recommendations

As the livelihoods of settled Fulani pastoralists women depend largely on the marketing of wara, this study recommend the need for food regulatory agents' interventions to upgrade milk and cheese processing methods for quality and safe products. It is also necessary for public health officials to be involved and actively participate in sensitization and quality control programs.

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