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Heavy metals and microbial contamination of palm oil produced and sold at some markets in Kogi East Area, Kogi State, Nigeria

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The physicochemical, microbial and heavy metal contamination of palm oil samples randomly selected from three different markets (Ankpa, Anyigba and Idah Markets) in Kogi State, Nigeria, was investigated. The results revealed the presence of moisture (0.5, 0.4, 0.4%); impurity (0.4, 0.4, 0.3%) and free fatty acid (13, 12.8, 12.3%) for Ankpa, Anyigba and Idah Markets respectively. The microbial contaminants isolated were *Enterobacter* sp, *Bacillus* sp, *Proteus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Aspergillus* sp, *Candida* sp, *Mucor* sp, *Rhizopus* sp and *Penicillium* sp. The presence (ppm) of heavy metals in the samples was analyzed using atomic absorption spectroscopy for chromium, cadmium, lead, arsenic, copper and iron. All the tested metals except chromium and lead were detected in samples from Anyigba Market with the mean concentration of arsenic (0.29), iron (4.66), cadmium (0.001) and copper (0.006). The metals detected in samples from Idah Market were cadmium (0.46), arsenic (0.19), iron (7.34), chromium (0.04) and copper (0.03). Only three metals, arsenic (0.04) cadmium (0.01) and iron (1.88) were detected in samples from Ankpa Market. From the findings, the microbial load of the samples fell within the acceptable limits stipulated by World Health Organization limits. However, most of the heavy metals assayed for were above acceptable limits. The present findings indicate the need for refining of locally produced palm oil to eliminate metal contaminants because of the health implication of their accumulation in the body.

Key words: Heavy metals, microbial contamination, palm oil, Kogi East, Nigeria.

INTRODUCTION

Palm oil is an edible vegetable oil obtained from the fruit of the oil palm tree (*Eleais guineensis*). Palm oil is regarded as the most widely produced and consumed vegetable oil in the world (Shahbandeh, 2021) and ranks among the most important oil producing crops in Sub-Saharan Africa (Tagoe et al., 2012). Elaeis guineensis Jacqu original habitat is in West Africa's tropical rain forests (www.fao.org). Oil palm tree is an economic plant that grows abundantly in equatorial West Africa, both in the wild and in plantations. It is cultivated extensively in Southeast Asia where Malaysia and Indonesia are ranked as the largest producers and exporters of oil palm products (Uning et al., 2020). The major countries where oil palm thrives in West and Central Africa include

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Nigeria, Benin, Togo, Ghana, Cote de'Ivoire, Liberia, Sierra Leone, Cameroun, Angola, Zaire, Congo and Angola. In Nigeria the main oil palm growing area is the South, especially the South-Eastern and Mid-Western regions (Raw Materials Research and Development Council, Nigeria, 2004).

Palm oil itself is reddish because it contains high amount of beta-carotene (Akinola et al., 2010). Crude (unrefined) palm oil is an important ingredient in the diet of many people in West Africa (Uning et al., 2020). Chemically, palm oil, like other seeds is a fatty acid ester of glycerol commonly called triglycerides (Akpanabiatu et al., 2001). Palm oil and its products are useful for cooking/frying, in local dishes, soap manufacture, as source of vitamin A, E and K (Oguntibeju et al., 2009). The value of palm oil depends largely on its quality. Okogbenin et al. (2014) isolated a number of food pathogens from freshly prepared palm oil and some of them have potential to secrete toxins in the palm oil. Palm oil becomes prone to contamination by microorganisms found in the production environment, raw materials and equipment used for the processing, as well as those used for storage and distribution. Contamination of palm oil with heavy metals could pose potential risk to humans because of their bioaccumulation in the body (Engwa et al., 2018; Tchounwou et al., 2012).

There are reports of heavy metals contamination of palm oil sold in markets from other areas (Olafisoye et al., 2020; Aigbemu et al., 2017; Nnorom et al., 2014; Asemave et al., 2012). Eastern part of Kogi State, Nigeria, is known for production and distribution of palm oil to other states in Nigeria, especially the Federal Capital Territory, Abuja and northern part of the country. However, earlier report in this area evaluated only the fungal contaminants in ready to use palm oil sold at Anyigba Market (Enemuor et al., 2012). There is no report on evaluation of heavy metals contamination of palm oil produced from this area. There is, therefore, the need to investigate the levels of microbial and heavy metals contamination in palm oil produced from this area and sold at the three major markets located in the area.

MATERIALS AND METHODS

Study area

The study was conducted in the Kogi East area of Kogi State, Nigeria, from three different main (local) markets located at Anyigba (located on 7.4934º N and longitude: 7.1736º E), Ankpa (located on 7.4053º N and longitude: 7.6223º E) and Idah (located on latitude 7.1138º N and longitude 6.7440º E). Kogi East area was chosen for the study because of abundance of oil palm plantations in the area and the processing of palm oil is mostly by crude traditional methods. These markets were selected because crude palm oil is distributed in commercial quantity from there to other states in Nigeria especially to the northern part of the country including Abuja, the Federal Capital Territory.

Collection of samples

From each local market ten palm oil samples were randomly selected and bought from ten different sellers (local producers) who employed traditional methods of processing making a total of thirty samples in all. The samples were collected in sterile universal bottles and care was taken not to contaminate the samples during and after collection. The samples from each market were packed in a cooler and transported to the laboratory for microbiological and heavy metals analyses.

Determination of physicochemical properties

Determination of free fatty acids

The free fatty acid content of palm oil samples was determined using the British Standard Method (1976). Five grammes of palm oil sample were measured into Erlenmeyer flask and 50 ml diethyl ether diluted with absolute ethanol in the ratio of 1:1 (v/v) was added. The mixture was heated and swirled at intervals until the oil was completely dissolved. Six drops of phenolphthalein indicator were added and the mixture was titrated against 0.1 M KOH until the end point (orange, red or pink colour which persisted for about 15 s). The titre values were recorded and the free fatty acids (FFA) content was calculated in percentage from the relation:

$$FAA = \frac{Molar\ concentration\ of\ KOH \times titre\ value \times relative\ molar\ mass\ of\ KOH}{Weight\ of\ palm\ oil\ (g)}$$

Determination of moisture content

The moisture content of the palm oil samples was determined using the British Standard Method (1976). Erlenmeyer flask was weighed (W_1) and 5 g of palm oil sample was added and reweighed (W_2). The sample was dried in the oven for 2 h at 105 ºC. It was allowed to cool in a desiccator and its final weight determined (W_3).

This process was carried out in duplicates for all the samples. The moisture content was expressed in percentage from the formula:

$$Percentage\ moisture\ content = \frac{W_2 - W_3 \times 100}{W_2 - W_1}$$

Determination of percentage impurity

The degree of impurity in the oil samples was determined by the method described by Tagoe et al. (2012). A glass funnel was lined with filter paper, washed with hexane and dried at 105 ºC for 30 min. The funnel was allowed to cool and weighed as W_1. Erlenmeyer flasks (100 ml) were weighed (W_2) and 2 g of oil added and reweighed (W_3). Hexane (20 ml) was added to the oil and the flask swirled and heated on a heating mantle to homogenize the mixtures. The mixture was then poured through the funnel and allowed to drain. The flask was rinsed with hexane to recover any remaining particles and poured through the funnel and allowed to drain completely. The funnel thereafter was dried in the oven at 105

Table 1. Some physicochemical properties of palm oil samples from the three areas.

Sample	Ankpa Market			Anyigba Market			Idah Market		
	FFA content (%)	Moisture content (%)	Impurity content (%)	FFA content (%)	Moisture content (%)	Impurity content (%)	FFA content (%)	Moisture content (%)	Impurity content (%)
1	10.2 ±0.1	0.4 ± 0.1	0.0 ±0.0	10.2 ±0.2	0.4 ±0.0	0.0 ±0.0	14.2 ±0.1	0.2 ±0.0	1.0 ±0.0
2	13.0 ±0.0	0.2 ±0.0	0.5 ±0.0	13.0 ±0.1	0.2 ±0.0	0.5 ±0.0	12.5 ±0.0	0.4 ± 0.0	0.0 ±0.0
3	11.3 ±0.1	0.4 ±0.0	0.5 ±0.0	11.3 ±0.1	0.4 ±0.0	0.5 ±0.0	13.3 ±0.0	0.2 ±0.0	0.5 ±0.0
4	12.0 ±0.1	0.2 ±0.0	0.0 ±0.0	12.0 ±0.1	0.2 ±0.0	0.0 ±0.0	11.8 ±0.0	0.6 ±0.0	0.0 ±0.0
5	14.1 ± 0.0	0.6 ±0.0	0.5 ±0.0	14.1 ±0.0	0.6 ±0.0	0.5 ±0.0	10.2 ±0.0	0.4 ±0.0	0.5 ±0.0
6	12.3 ±0.1	0.6 ±0.0	0.0 ±0.0	12.3 ±0.2	0.6 ±0.0	0.0±0.0	12.5 ±0.0	0.2 ±0;0	0.0 ±0.0
7	12.8 ±0.0	0.2 ±0.1	1.0 ±0.0	12.8 ±0.0	0.2 ±0.0	1.0 ±0.0	12.3 ±0.0	0.4 ±0.1	0.0 ±0.0
8	14.4 ±0.5	0.4 ±0.0	1.0 ±0.0	14.4 ±0.1	0.4 ±0.0	1.0 ±0.0	09.8 ±0.0	0.8 ±0.0	0.0 ±0.0
9	13.6 ±0.2	0.4 ±0.1	0.0 ±0.0	13.6 ±0.0	0.4 ±0.0	0.0 ±0.0	11.5 ±0.1	0.6 ±0.0	0.5 ±0.0
10	14.1 ±0.1	0.6 ±0.0	0.0 ±0.0	14.1 ±0.0	0.6 ±0.0	0.0 ±0.0	15.5 ±0.0	0.2 ±0.0	0.0 ±0.0
Mean	12.8	0.4	0.4	12.8	0.4	0.4	12.4	0.4	0.3
SON permissible limit	3-5	0.29	0.2						

SON = Standard Organization of Nigeria.

for 30 min, cooled and reweighed (W_4). This process was carried out in duplicates for all the samples. Impurity in the oil was expressed as a percentage from the formula below:

$$Percentage\ impurity = \frac{W_4 - W_1 \ \times\ 100}{W_3 - W_2}$$

Determination of microbial contaminations

A stock solution of each of the samples was made by dissolving 1ml of each palm oil sample in 8 ml of prepared peptone water and thereafter 1 ml of sterile Tween 80 was added using sterile 2 ml syringe. Each solution was prepared in a Bijou bottle, capped and shaken vigorously to obtain a homogenized solution. Three fold serial dilutions were made from each stock solution by measuring 1ml of the stock solution into 9 ml of prepared peptone water in the test tube labelled as 10^{-1} and down to 10^{-3}). Aliquots of each dilution (10^{-1}-10^{-3}) were inoculated on three Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates using the pour plate method. The NA plates were subsequently incubated at in an incubator at 37ºC while the SDA plates were incubated at room temperature (28 ºC) for 72 to 120 h. All bacterial and fungal isolates were sub-cultured unto freshly prepared NA and SDA plates for proper identification. Standard bacteriological and mycological identification process was strictly followed.

Determination of heavy metals

The concentrations of lead (Pb), cadmium (Cd), iron (Fe), arsenic (Ar) and copper (Cu) in the palm oil samples were determined by Flame Atomic Absorption Spectrometry (AAS). Each sample (5 g) was digested with 20 ml of acid mixture (650 ml Conc. HNO_3; 80 ml perchloric acid; 20 ml Conc. H_2SO_4).

RESULTS AND DISCUSSION

The result of some physical characteristics of the palm oil samples from the different markets are shown in Table 1. The mean moisture contents of samples from the three Markets in this study were slightly above the permissible levels for palm oil by Standard Organization of Nigeria (SON) (SON, 2000). This slight increase could be attributable to the fact that traditional methods employed in processing palm oil in these localities do not subject it to boiling at elevated temperature which could have reduced the moisture content. Orji and Mbata (2008) reported that the traditional methods employed in palm oil processing in Nigeria produced low quality product in terms of free fatty acids, moisture and impurity contents. Other workers reported on the need for improved processing technique to ensure better quality of palm oil sold in the Nigerian markets (Adebayo-Oyetero et al., 2019). However, Japir et al. (2017) reported that the physicochemical properties of high free fatty acid of crude palm oil and low

Table 2. Aerobic viable plate count of palm oil samples from the three areas.

Sample	Ankpa		Anyigba		Idah	
	Bacterial count (cfu/ml)	Fungal count (cfu/ml)	Bacterial count (cfu/ml)	Fungal count (cfu/ml)	Bacterial count (cfu/ml)	Fungal count (cfu/ml)
1	$1.05\times10^2 \pm 1.70$	$1.85\times10^2 \pm 1.50$	$1.00\times10^2 \pm 0.80$	$1.92\times10^2 \pm 1.50$	$1.20\times10^1 \pm 0.00$	$2.00\times10^2 \pm 1.00$
2	$1.31\times10^2 \pm 1.40$	$1.60\times10^2 \pm 1.20$	$2.20\times10^2 \pm 1.10$	$1.68\times10^2 \pm 1.00$	$1.70\times10^1 \pm 0.50$	$1.40\times10^2 \pm 0.90$
3	$1.80\times10^1 \pm 1.50$	$1.33\times10^2 \pm 2.20$	$2.80\times10^1 \pm 0.70$	$1.05\times10^2 \pm 0.70$	$1.50\times10^1 \pm 0.60$	$5.00\times10^1 \pm 0.30$
4	$2.30\times10^1 \pm 0.00$	$1.54\times10^2 \pm 0.80$	$1.20\times10^2 \pm 1.80$	$1.92\times10^2 \pm 1.60$	$2.00\times10^2 \pm 1.10$	$1.30\times10^1 \pm 1.50$
5	$1.73\times10^2 \pm 1.10$	$2.70\times10^1 \pm 0.40$	$2.00\times10^2 \pm 0.10$	$1.38\times10^2 \pm 0.50$	$1.30\times10^1 \pm 1.00$	$1.80\times10^1 \pm 1.20$
6	$1.54\times10^2 \pm 1.00$	$3.00\times10^1 \pm 2.80$	$1.30\times10^2 \pm 1.20$	$1.06\times10^2 \pm 1.60$	$1.50\times10^1 \pm 2.20$	$1.90\times10^2 \pm 1.00$
7	$1.30\times10^2 \pm 1.00$	$2.60\times10^1 \pm 2.40$	$3.00\times10^1 \pm 1.50$	$2.00\times10^2 \pm 1.00$	$1.90\times10^1 \pm 0.20$	$1.20\times10^1 \pm 0.80$
8	$2.07\times10^2 \pm 1.30$	$1.54\times10^2 \pm 1.70$	$1.89\times10^1 \pm 2.00$	$3.00\times10^2 \pm 1.00$	$2.80\times10^1 \pm 1.90$	$1.37\times10^2 \pm 2.40$
9	$2.00\times10^2 \pm 1.20$	$1.60\times10^2 \pm 0.00$	$1.20\times10^2 \pm 1.40$	$2.80\times10^1 \pm 00.0$	$2.00\times10^1 \pm 0.70$	$2.70\times10^1 \pm 1.00$
10	$1.20\times10^2 \pm 2.10$	$1.38\times10^2 \pm 0.00$	$1.00\times10^1 \pm 1.70$	$1.05\times10^2 \pm 2.50$	$1.06\times10^2 \pm 1.60$	$1.30\times10^1 \pm 0.20$
Mean	1.14×10^2	1.17×10^2	1.15×10^2	1.26×10^2	4.45×10^1	8.00×10^1
SON Permissible limit	2×10^4					

Legend: SON = Standard Organization of Nigeria.

free fatty acids of crude palm oil were consistent with the Malaysian standard for crude palm oil with the exception of free fatty acid percentage, hydroxyl value and moisture content.

Although the mean bacterial and fungal counts (Table 2) in the oil samples were within acceptable limits the high moisture content could lend to their multiplication especially as the storage time prolongs. This could lead to ease of spoilage and rancidity and shorten the shelf-life. Other workers have isolated several microbes in ready-to-use palm oil sold in different markets in Nigeria (Okechalu et al., 2011, Enemuor et al., 2012, Okogbenin et al., 2014, Seiyaboh et al., 2018, Odoh et al., 2016). Bacillus species which was isolated can cause food poisoning, bacteremia and endocarditis. It has been reported that Bacillus spores are dormant and highly resistant to lethal effects of heat and ultraviolet radiation. Aspergillus and Mucor species also produce heat resistant spores and have been noted for their ability to survive in oil by producing lipase enzyme which can lead to rancidity of the oil. The frequency of the bacterial and fungal isolates is shown in Figures 1 and 2 respectively.

Figure 1, shows the frequency of isolation of bacterial species from palm oil samples from the three markets located at Ankpa, Anyigba and Idah. The bacterial isolates included: Bacillus, Staphylococcus, Enterobacter, Proteus, Micrococcus and Pseudomonas spp. The frequency of isolation of Bacillus sp. is highest in samples from Ankpa Market while Entrobacter sp. was highest in samples from Anyigba Market. Although Staphylococcus sp. had the highest frequency in samples from Idah Market, it should be noted that samples from Idah Market had the lowest bacterial load in comparison with Ankpa and Anyigba Markets. Bacillus, Enterobacter and Proteus spp. were all isolated from samples from the three markets.

Figure 2, shows the frequency of isolation of fungal species in the three markets located at Ankpa, Anyigba and Idah. The fungal isolates included: Candida, Aspergillus, Rhizopus, Mucor and Penicillum spp. All the fungal species were isolated from samples from the three markets. Candida sp. had the highest frequency from the three markets followed by Aspergillus sp. Samples from Anyigba Market had the highest fungal load than that from Ankpa and Idah Markets followed by samples from Ankpa Market. Although the frequency of Candida sp. in samples from Idah Market was high however, they (samples) had the least fungal load comparatively. All the microorganisms isolated in this study have been implicated in similar studies by other workers (Okechalu et al., 2011; Enemuor et al., 2012; Okogbenin et al., 2014; Ohimain and Izah, 2015; Odoh et al., 2016; Seiyaboh et al., 2018;

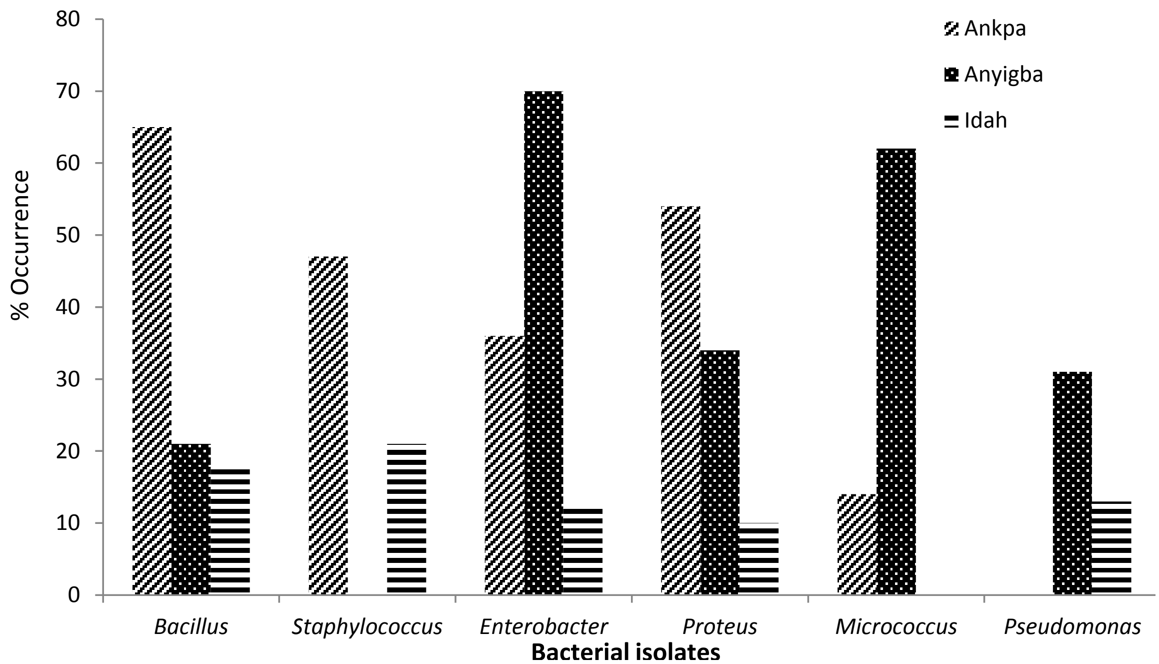

Figure 1. Percentage frequency of occurrence of bacterial isolates in palm oil samples.

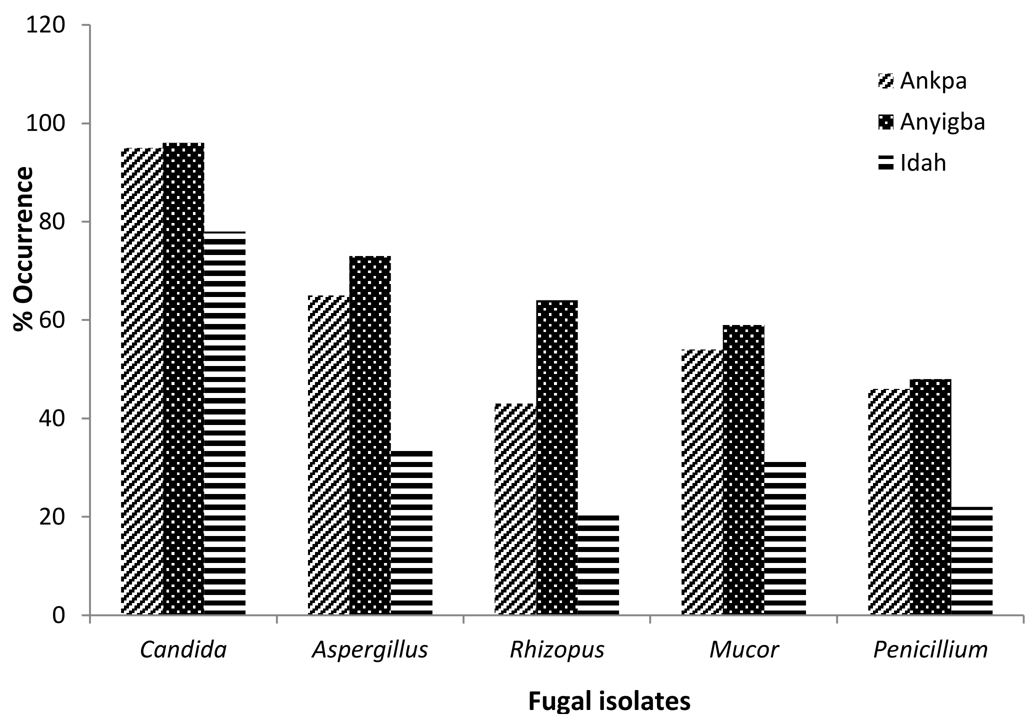

Figure 2. Percentage frequency of occurrence fungal isolates in palm oil samples.

Ngangjoh et al., 2020). It was observed that palm oil samples from Idah area had lowest microbial load and frequency of the isolated microorganisms (Table 2, Figures 1 and 2). The fact that the mean concentrations of Cd and Fe in palm oil samples from Idah (Table 3) were higher than in samples from Ankpa and Anyigba (and above WHO acceptable limit) could offer some explanations for the low microbial load. Several reports

Table 3. Comparison of detectable metals in palm oil samples from Ankpa, Anyigba and Idah areas.

Metal	Mean concentration (ppm)			
	Ankpa Market	Anyigba Market	Idah Market	WHO limits
Cadmium (Cd)	0.010	0.001	0.460	0.050
Chromium (Cr)	<0.001	<0.001	0.040	0.100
Lead (Pb)	<0.001	<0.001	<0.001	0.010
Arsenic (As)	0.040	0.290	0.190	0.010
Copper (Cu)	<0.001	0.006	0.030	1.300
Iron (Fe)	1.880	4.660	7.340	1.000

WHO: World Health Organization; ppm: parts per million.

have elucidated on the toxicity of heavy metals on microbial growth by interfering with the biochemical and physiological properties of microorganisms (Igiri et al., 2018; Oijagbe et al., 2018; Xie et al., 2016; Chen et al., 2014; Das et al., 2012). Table 3 shows the mean concentrations of some heavy metals in the palm oil sample from the three local markets.

The mean concentrations of As and Fe were above acceptable limits in all the samples from the three area markets.

Arsenic can cause a number of human health effects and diet has been reported as the largest source of exposure to arsenic (Tchounwou et al., 2012). Therefore, consumption of the palm oil sold in these markets exposes the consumers to these health risks. The mean concentration of Cd in the samples from Idah Market was also above WHO permissible limits. It has also been reported that human exposure to Cd is possible through eating contaminated food (Tchounwou et al., 2012). Blood vessels are considered to be the organs of Cd toxicity and Cadmium compounds are classified as human carcinogens by several regulatory agencies (Tchounwou et al., 2012).

Several studies conducted in different parts of Nigeria reported detectable amounts of heavy metals including Cd, Cr, Ar and Pb in the ready-to-consume red palm oil samples sold at the local markets (Adepoju-Bello et al., 2012; Aigbemu et al., 2017; Asemave et al., 2012; Tor et al., 2017; Nnorom et al., 2014; Ogabiela et al., 2010). Some of the detected metal concentrations in these studies were reported to fall within permissible limits. They also speculated that source of metals in edible oils could be from soil, environmental or implements during processing.

Obviously, palm oil sold in markets in urban areas and cities in Nigeria could be supplied from different geographical locations within the country that have oil palm plantations. Soils from different locations differ in metallic content. Idah area has oil palm plantations and is located at the bank of River Niger and upstream are located Ajaokuta Steel Company and iron ore mining activities at Itakpe within the same region. These industrial activities tend to confirm the possible metallic content of soil in this area. Again, when the river overflows its banks during the long raining seasons the soil could be contaminated the more with metals coming down from the wash offs from the industries located upstream. The bioavailability of metals in soils on oil palm plantations in Nigeria has been assessed (Olafisoye et al., 2016). They concluded that the concentrations of Pb and Cu were highest in the organic fraction and that metals in the organic phase are more easily released into soil solution comparatively. In Malaysia, other workers determined selected heavy metal concentrations in an oil palm plantation soil and concluded that the soil was uncontaminated but noted that the detectable amounts of metals were from application of chemical fertilizers (Ab-Manan et al., 2018). In another study on the accumulation and risk assessment of metals in palm oil from contaminated oil palm plantation soils, Olafisoye et al. (2020) reported that there is a correlation between the accumulations of metals from soil to palm oil. Apart from oil palm trees assimilating these metals from the soil more contamination of the palm oil by these metals could come from the contaminated water used by locals in the processing because they do have access to treated water supply.

The source of Fe contamination could as well be from wears of metallic implements and equipment used in local processing of palm oil in these three localities as its average concentration in the samples from these zones is above permissible limits. Iron poisoning has always been of interest mainly to pediatricians because children are highly susceptible to iron toxicity (Erber, 2012; Gupta, 2016; Kleiner, 2018).

Conclusion

From the findings, the microbial load of the samples fell within the acceptable limits stipulated by regulatory agencies. However, most of the heavy metals assayed for were above acceptable limits. The present findings indicate the need for refining of our locally produced palm oil to eliminate metal contaminants because of the health implication of their accumulation in the body.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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