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Assessment of Microbiological Quality and Heavy Metal Content in Palm Oil Sold at Selected Markets in Bayelsa State

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Abstract
This study assessed the heavy metal burden and microbial load of palm and the microbiological safety of palm oil sold in Zarama, Elebele and Tombia markets in Bayelsa State Nigeria. Heavy metal was analysed using an atomic absorption spectrophotometer. Bacteria and fungi were isolated and identified based on their cultural, morphological and biochemical characteristics. The Total viable count (cfu ml^{-1}) of the samples ranged from 24 x 10^6cfu/ml to 55 x 10^6cfu/ml with samples obtained from the Elebele market having the highest, while samples from the Tombia market had the least. The microbial contaminants isolated were *Escherichia coli, Pseudomonas sp Klebsiella sp, Bacillus* sp, *Proteus* sp, *Staphylococcus aureus, Aspergililussp, Mucor* sp *Rhizopus sp, Penicillium* sp. *Bacillus sp* had the highest frequency of occurrence of 28.6% followed by *Aspergillus sp* and, *Mucor* sp (22.2%), while *Escherichia coli, and Klebsiella sp* had the lowest (7.1%). All the tested metals were detected in the samples from Tombia and Elebele Markets. Zn concentrations were highest in Zarama with 0.25±0.01 while it was low in the Elebele market respectively. Cu was found to be highest in Zarama, having 2.47±0.05 as its concentration but very low in Elebele with a concentration of 0.01 respectively. Tombia recorded the highest concentration of Cd with 0.34±0.02 and Pb with 0.4±0.01 while Pb was not detected in Zarama. The majority of the microorganisms found in the oil are pathogenic to humans. However, most of the heavy metals assayed for were above acceptable limits.

Keywords: Heavy Metals, Microbial Quality, Palm Oil, Safety

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). Palm oil is reddish because it contains a 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 is produced by smallholder processers with poor hygienic status. As such the essential and health benefits of palm oil could be altered by environmental contaminants such as microorganisms. Microorganisms in palm oil could lead to rancidity, acidity, bitterness, soapiness and other flavours (Okechalu et al., 2011). Microorganisms are known to cause deterioration of food products which may lead to pathological effects when the pathogenic ones are ingested. Various microorganisms have been implicated in the deterioration of palm oil and other vegetable oils e.g. *Aspergillus flavus, A. niger, A fumigatus, Penicillum frequentans, Rhizopus stolonifer* etc. These moulds have lipolytic activities and their growth results in lipolysis (spoilage) of the palm oil. In general, the rate of lipolysis is proportional to the amount of free fatty acid already present in the oil (Okechalu et al., 2011).

Contamination of palm oil with heavy metals could pose a potential risk to humans because of their bioaccumulation in the body (Engwa et al., 2018; Tchounwou et al., 2012). There are reports of heavy metal contamination of palm oil sold in markets from other areas (Olafisoye et al., 2020; Aigbemu et al., 2017). There is no report on the evaluation of heavy metals contamination of palm oil produced from this area. There is, therefore, the need to investigate the levels of bacterial and heavy metals contamination in palm oil sold in Zarama, Elebele and Tombia markets within Bayelsa State.

Materials and Methods

Red oil was randomly purchased from three different sellers in three different markets (Zarama, Elebele and Tombia markets) making a total of nine samples in all. The samples were collected aseptically in sterile universal bottles with tight lids and labelled appropriately. 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 analysis.

A serial dilution was performed by adding 1 ml of the oil sample in 9 ml of sterile distilled water already emulsified with 10% v/v of tween 80 solutions which served as an emulsifying agent. Aliquots of the suspensions were transferred carefully into sterile Petri dishes with the aid of a sterile pipette. Already prepared 10ml of nutrient agar for isolation of bacteria was then poured over plates (the pour plate method) containing the samples and swirled gently to allow for proper mixing of the sample and the medium. The plates were transferred to an incubator with temperature set at 30±2°C for a 24 hour period.The various isolates were subjected toGram's staining procedure and standard biochemical test as described by Cheesbrough (2005).

Aliquots of each dilution (10^{-1}-10^{-3}) were inoculated on Sabouraud Dextrose Agar (SDA) plates using the pour plate method. The plates were incubated at room temperature (28 ºC) for 72 to 120 h. All fungal isolates were sub-cultured onto freshly prepared SDA plates for proper identification. The standard mycological identification process was strictly followed (Pál et al., 2015).

The concentrations of Zn (Zinc), Cu (Copper), Cd (Cadium), Pb (Lead), Mn (Manganese) and As (Asernic)) 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. H2SO4 (Izah & Ohimain, 2015). The data were expressed as mean ± standard deviation. Data were analysed using one-way analysis of variance (ANOVA) at a 5% level of significance (p = 0.05).

Results

The Total viable count (cfu ml^{-1}) of the samples ranged from 24 x 10^6cfu/ml to 55 x 10^6cfu/ml with samples obtained from the Elebele market having the highest bacterial load while samples from
Tombia market had the least contamination.

Table1: Total viable count (cfu ml^{-1}) of bacteria in the Various Markets

Market	Total viable count (cfu ml^{-1})
Zarama	30 x10^6
Elebele	55x10^6
Tombia	24x10^6

Table 2, 3 and 4 showed the microorganisms isolated from the oil samples obtained from the three (3)markets and their frequency of occurrence. The organisms isolated include *Escherichia coli, Pseudomonas sp Klebsiella sp, Bacillus* sp, *Proteus* sp*, and Staphylococcus aureus* (Table 2). Fungi isolated include *Aspergililus sp, Mucor* sp *Rhizopus sp* and *Penicillium* sp. *Bacillus* sp had the highest frequency of occurrence while *Escherichia coli and Klebsiella sp* had the lowest frequency of occurrence. The heavy metal contamination of Palm oil sold in the three (3) markets are as shown in Table 5.

Table 2. Bacteria isolated and identified in Palm oil sold in selected markets in Bayelsa State

Sample code	Cat	Glu	H_2S	Ind	Lac	Mal	Man	Mot	M/R	Oxi	Suc	Ure	VP	G/R	Probable Organism
ZARMa	+	+	-	-	+	+	+	+	-	-	+	-	+	+	*Bacillus species*
ZARMb	+	+	-	-	+	+	+	-	-	-	+	-	+	+	*Staphylococcus aureus*
ZARMc	+	+	+	-	-	-	+	+	-	+	-	+	-	-	*Pseudomonas aeruginosa*
ZARMd	+	+	-	-	+	+	+	+	-	-	+	-	+	+	*Bacillus species*
TOMMa	+	+	-	-	+	+	+	-	-	-	+	-	+	+	*Staphylococcus aureus*
TOMMb	+	+	-	+	+	+	+	+	+	-	-	-	-	-	*Escherichia coli*
TOMMc	+	+	-	-	+	+	+	-	-	-	+	-	+	+	*Staphylococcus aureus*
TOMMd	+	+	+	-	-	-	+	+	-	+	-	+	-	-	*Pseudomonas aeruginosa*
TOMMe	+	+	-	-	+	+	+	+	-	-	+	-	+	+	*Bacillus species*
ELEMa	+	+	+	-	-	+	-	+	+	-	-	+	-	-	*Proteus mirabilis*
ELEMb	+	+	-	-	+	+	+	+	-	-	+	-	+	+	*Bacillus species*
ELEMc	+	+	+	-	-	+	-	+	+	-	-	+	-	-	*Proteus mirabilis*
ELEMd	+	+	+	-	-	-	+	+	-	+	-	+	-	-	*Pseudomonas aeruginosa*
ELEMe	+	+	-	-	+	-	+	-	-	-	+	+	+	-	*Klebsiella pneumonia*

Note; ZARM; Zarama market, ELEM; Elebele market, TOMM; Tombia market.

Table 3: Frequency of Occurrence of bacterial Isolates in Palm Oil samples from the different Markets

Organisms	**Markets**			**No of times isolated**	**% frequency of occurrence**
	Zarama	Tombia	Elebele		
Pseudomonas sp	1	1	1	3	21.4
Bacillus sp	2	1	1	4	28.6
Staphylococcus aureus	1	2	-	3	21.4
Proteus sp	-	-	2	2	14.3
Klebsiella sp	-	-	1	1	7.1
Escherichia coli	-	1	-	1	7.1

Table 4:Frequency of Occurrence of fungal Isolates in Palm Oil samples from different Markets

Organisms	**Markets**			**No of time isolated**	**% frequency of occurrence**
	Zarama	Tombia	Elebele		
Mucor sp	2	1	1	4	22.2
Aspergillus sp	2	1	1	4	22.2
Rhizopus sp	1	2	-	3	16.7
Penicillium sp	1	-	2	3	16.7

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Table 5: Heavy metal content of palm oil samples collected from three LGA in Bayelsa state

Sample	Zn	Cu	Cd	Pb	Mn	As
ZARMa	0.07±0.00bc	2.47±0.05f	0.02±0.00c	0.0±0.00a	0.04±0.00h	0.05±0.00c
ZARMb	0.26±0.01j	1.00±0.28g	0.01±0.00ab	0.0±0.00a	0.01±0.00c	0.05±0.00c
ZARMc	0.15±0.00f	0.11±0.00a	0.01±0.00abc	0.0±0.00a	0.00±0.00a	0.11±0.00e
ZARMd	0.06±0.01a	2.17±0.04e	0.02±0.00bc	0.0±0.00a	0.00±0.00a	0.01±0.00a
TOMMa	0.17±0.00g	0.02±0.00a	0.34±0.02d	0.4±0.01e	0.02±0.00f	0.10±0.00d
TOMMb	0.10±0.00d	0.03±0.01a	0.01±0.00abc	0.3±0.00d	0.01±0.00e	0.03±0.00b
TOMMc	0.20±0.00h	0.03±0.00a	0.01±0.00abc	0.1±0.00b	0.01±0.00c	0.02±0.00ab
TOMMd	0.06±0.00ab	0.23±0.02b	0.01±0.00abc	0.0±0.00a	0.00±0.00a	0.02±0.00a
TOMMe	0.25±0.01j	0.58±0.04c	0.02±0.00c	0.0±0.00a	0.00±0.00a	0.02±0.00ab
ELEMa	0.12±0.00e	0.01±0.00a	0.02±0.00bc	0.2±0.00c	0.03±0.00g	0.18±0.01f
ELEMb	0.23±0.00i	1.70±0.01d	0.00±0.00a	0.0±0.00a	0.01±0.00d	0.02±0.00ab
ELEMc	0.08±0.00c	0.01±0.00a	0.02±0.00bc	0.0±0.00a	0.00±0.00b	0.27±0.01g

Values are means of triplicate determinations. This means that the same column with different sets of superscripts is statistically different (Duncan Multiple2. Table 5, above illustrates the various levels of heavy metals present in the study areas. Heavy metals such as Zn (Zinc), Cu (Copper), and Cd (Cadium), Pb (Lead), Mn (Manganese), As (Asernic) were shown to be present. Each heavy metal was represented as a means of triplicate determination with the corresponding level of deviation as shown in each column.

Discussion

The frequency of isolation of *Bacillus sp*. was highest in samples from Zarama Market while *Proteus sp* was highest in samples from Elebele Market. Although *Staphylococcus sp*. had the highest frequency in samples from Tombia Market, it should be noted that samples from Tombia Market had the lowest bacterial load in comparison with Zarama and Elebele Markets. *Bacillus sp* and *Pseudomonas sp* were all isolated from samples from the three markets. The frequency of *Mucor sp* and *Aspergillus sp* s. in samples from Zarama Market was higher than in other markets. The organism associated with the palm oil samples are in agreement with the reports of Okechalu et al. (2011) who reported the occurrence of *Bacillus sp, Proteus sp, Micrococcus sp, Staphylococcus aureus, Aspergillus niger, A. flavus, A. fumigatus and Candida* in all palm oil samples obtained from Jos plateau state Nigeria. Similarly, Izah and Ohiman (2013) have reported the occurrence of *Enterobacter sp, Bacillus sp, Pseudomonas sp, Staphylococcus sp, Aspergillus niger, A. flavus, A fumigatus, Mucor sp and Penicillum* in palm oil obtained in Delta State. Bacteria of the genera *Bacillus*, *Pseudomonas* and *Staphylococcus* are lipase producers and are associated with pathogenicity (Okogbenin et al., 2014). 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. These microbes cause deterioration of palm oil leading to changes in its physico-chemical characteristics. For instance, *Pseudomonas* species are known to cause spoilage of dairy products and food that contain high amounts of fats (Okogbenin et al., 2014). *Pseudomonas* species can adapt themselves to different kinds of environmental conditions and can survive and replicate in minimum nutritional conditions such as in drinking water containing traces of organic compounds (Okogbenin et al., 2014). *Staphylococcus aureus* is a virulent species of staphylococci frequently encountered. *Staphylococcus aureus* is found in the skin, perineal areas, mucosa colonizer, nasopharynx and normal flora of humans. Some species of *Staphylococcus* such as *S. aureus* and *Staphylococcus saprophyticus* cause genitourinary disease among sexually active persons, especially females (Ohimain & Izah, 2015). *Pseudomonas* species are pathogenic and able to cause several diseases. Some species of *Pseudomonas* such as *Pseudomonas aeruginosa* an opportunistic pathogen, that can cause issues with individuals who have their immune systems compromised (Chacko et al., 2003). *Bacillus* species alongside *Micrococcus* species and *S. aureus* are common bacteria contaminants of several substrates. Like *P. aeruginosa*, *Bacillus* species are found in several substrates. *Bacillus species* can cause several diseases such as Bacteremia and endocarditis in humans (Carmelita &Tuazon, 2014).

Also, several other microbes that are found in palm oil such as *Enterobacter*, *Aspergillus* species, etc could be attributed to contamination from the environment including water and soil. *Mucor* species are known to be thermotolerant microorganisms that hardly cause infection in humans. This could be attributed to the fact that most *Mucor* species grow at temperatures above 30ºC. However, some species of *Mucor* including *Mucor hiemalis* and *Mucor racemosus* have also been reported as infectious agents (Ellis et al., 2007). *Aspergillus* species is a cosmopolitan mould that can thrive everywhere in different substrates. *Aspergillus* species including *Aspergillus fumigatus* and *Aspergillus flavus*, produce aflatoxin (toxins and carcinogens) that cause adverse health conditions in humans. *Penicillium* species are contaminants that produce mycotoxin. The inhalation of conidia of *Penicillium* species from contaminated food or products affects health (Ellis et al., 2007). *Penicillium* species are opportunistic microorganisms that cause diseases like mycotic keratitis (Boretsky et al., 2011).

Several studies conducted in different parts of Nigeria reported detectable amounts of heavy metals including Cd, Cr, Ar and Pb in palm oil samples sold at the local markets (Aigbemu et al., 2017; Asemave et al., 2012; Tor et al., 2017). Some of the detected metal concentrations in these studies were reported to fall within permissible limits. They also speculated that the source of metals in edible oils could be from soil, environment or implements during processing. 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 the treated water supply. The concentration of Pb in the sample from this study was higher in the Tombia market with 0.4±0.01 followed by Elebele with 0.2±0.00 and was not detected in the Zarama market. Copper (Cu) was detected high in Zarama with 11.00±0.028. Cadmium (Cd) possesses risk at the slightest level of exposure to animals and plants. This is because the body does not have much capacity to degrade it to a less harmful chemical form. 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). Cadium could be found high in some areas of study due to how the extraction of the palm oil was done through traditional methods of milling palm. This metal was found higher in the Tombia market with 0.34±0.02 but least in other areas.

Conclusion

The results of this study showed that the palm oil contained some pathogenic microorganisms. Some of the microorganisms isolated can cause health problems in individuals who consume the product without heat processing. Some are food spoilage organisms and may accelerate the deterioration of palm oil. This is indicating improper processing and handling of palm oil by extractors and vendors. However, Palm oil sold at Tombia could be said to be toxic based on the presence of non-essential heavy metals (Cd and Pb) which are toxic to health.

Recommendation

The present findings indicate that Palm oil handlers should ensure a hygienic environment during oil processing and storage. Further studies should be done on the activities of the microorganisms found in the oil which is an indicator for safe oil.

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