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Evaluation of the Microbiological Quality of Palm Fruits in the Various Stages of Palm Oil Production

OMOKARO OBIRE & IKENNA OGUNDU HAKAM
Rivers State University of Science and Technology, Nigeria

ABSTACT The evaluation of microbiological quality of oil palm fruits in the various stages of palm oil production in a palm oil mill was investigated. This was carried out by the use of standard microbiological techniques. The bacteria counts ranged from 2.97 x 10^6 cfu/g to 1.51 x 10^7 cfu/g in the solid samples and 1.87 x 10^6 cfu/ml in the palm oil sample while the fungal counts ranged from 1.63 x 10^6 sfu/g to 3.7 x 10^6 sfu/g in the solid samples and 1.03 x 10^6 sfu/ml in the palm oil. The microbial population varied at the different stages of production up to the finished product (palm oil) with the fermented fruits having the highest population of both bacteria and fungi whereas the freshly prepared palm oil had the lowest populations. Analysis of variance (ANOVA) of the various samples showed that there is significant difference in the total heterotrophic bacteria and fungi counts. The counts obtained from the cooked fruits were quite similar to those of the freshly prepared palm oil. Generally, the bacteria occurrence (%) in all the samples was *Bacillus cereus* (21.69%), *Bacillus subtilis* (24.69%), *Escherichia coli* (12.05%), *Klebsiella sp.* (2.41%), *Serratia marcescens* (10.84%), *Pseudomonas aeruginosa* (14.46%), *Staphylococcus aureus* (12.05%) and *Staphylococcus epidermidis* (2.41%). While the fungi occurrence (%) was *Aspergillus fumigatus* (13.3%), *Aspergillus niger* (33.3%), *Mucor sp.* (20%), *Penicillium sp.* (16.7%), and *Saccharomyces cerevisiae* (16.7%). *Bacillus cereus* and *Escherichia coli* were isolated from all the samples including the fresh palm oil. The results indicated contamination at some stages of palm oil processing and that sporulating organisms survived most of the production process. The presence of *E. coli* indicates faecal contamination possibly from personnel who defecates in the surrounding bushes without washing up properly. The presence of these potential pathogenic bacteria and fungi in palm oil poses food safety challenges and health hazards. Palm oil mill workers should avoid unhygienic practices while carrying out the milling process as well as during final packaging of the products. Regulatory agencies should help monitor the activities of Palm oil mills in Nigeria. Government and Non-governmental agencies should support oil palm/palm oil production as this will surely boost the country's economy.

Keywords: Palm fruits, palm oil, *Staphylococcus*, *Bacillus*, *E. coli*, faecal contamination

Introduction

The African oil palm (*Elaeis guineensis* Jacq) is one of the most important tropical plantation plants used commercially in the production of palm oil and supplies one quarter of the crop derived oil in the world. Palm oil is an edible vegetable oil rich in anti-oxidants such as carotenoids (precursors of vitamin A) from which it derives its deep red colour. Carotenes levels in Palm fruit is higher than those from crops like carrot, tomatoes etc. Vitamin A from carotene helps in maintaining good vision as well as a strong immune system. Tocopherols and Tocotrienols (string antioxidant Vitamin E) in palm oil are present at higher levels than in other vegetable oils. The major component of its glycerides is the saturated fatty acid, palmitic acid. Hence it is a viscous semi-solid even at tropical ambient and a solid fat in temperate climates (Obire and Putheti, 2010). It also has what is now generally considered a very valuable blend of saturated, monounsaturated and polyunsaturated fatty acids, which gives it an important dietary role known to be beneficial to health (Poku, 2002; Harold, 2004). Many nutritional health experts believe that palm oil has several important benefits, particularly in lowering the risk of heart disease. Palm oil can retain its freshness and its valuable properties over long periods of storage (Sundram *et al.*, 2003). Unlike other vegetable oils, it is a solid at room temperature and less susceptible to turning rancid. These properties make palm oil increasingly valuable edible oil for use in cooking and food processing. Until 1934, Nigeria had been the world's largest producer (Ayodele, 2010). The US Department of Agriculture (USDA, 2005) calculates that global production of palm oil has now overtaken soybean oil to be the world's leading vegetable oil yielding more than five times that of other vegetable oil crops because oil palms are harvested throughout the year unlike annual crops which are harvested once a year. Palm oil has a plethora of food and industrial uses. Both palm oil and palm kernel oil are sources of basic chemicals such as olefins (alkenes) and have uses in manufacturing lubricants and detergents and shampoos. The high yields of palm oil have attracted the interest of producers of 'biodiesel', a more environmentally sound alternative fuel based on renewable resources rather than inevitably diminishing petroleum reserves (Obire and Putheti, 2010).

Coupled with the potential profitability and need for the diversification of crops, palm oil demand is likely to increase by more than 110% in the coming decade as reported by Akpobi and Oniah (2009). Apart from the study of oil palm in food microbiology for its nutritional value to human consumers, it is also an ideal culture media for microbial growth hence can serve as a vehicle for disease transmission as the method of processing as well as the handling sequence from producer to consumer can result in contamination by microorganisms (Okafor *et al*., 1978). Processing oil palm fruits for edible oil has been practiced in Africa for thousands of years, and the oil produced, highly coloured and flavoured, is an essential ingredient in much of the traditional West and Central African cuisine and is widely used as cooking oil as far as Europe (Obire and Putheti, 2010; Obahiagbon, 2012). Palm oil is also consumed raw as in medication and with boiled yam, sweet cassava, sweet potatoes or plantain.

Harvesting of palm fruits involves the cutting of the bunch from the tree and allowing it to fall to the ground by gravity. Fruits may be damaged in the process of pruning palm fronds to expose the bunch base to facilitate bunch cutting. As the bunch (weighing about 25 kg) falls to the ground the impact bruises the fruits and also allows contact with transient organisms from soil. The oil palm fruit bunches are transported after harvesting from the palm trees to the oil palm mill. During loading and unloading of bunches into and out of transport containers there are further opportunities for the fruit to be bruised as well as possible microbial contact. At the mill, the palm fruit bunches are split into four or six smaller pieces and left for some days to ferment so that the whole palm fruits can easily come off from the split bunches. The palm fruits are sieved from the sepals and loaded into a large cooking vessel (sterilizer) where they are cooked overnight. Cooked whole palm fruits are transferred into a digester where the fruits are digested into whole nuts, pulp, husks (fibre), and free flowing palm-oil. Hot water is added to the digester as necessary. The palm-oil is scooped off while the mixture of nuts, pulp, and husks are transferred to a mechanical "presser" where pressure is applied to extract or recover more palm-oil and liquid effluent. The palm oil is simply scooped into plastic drums or Jerry cans and stored at ambient temperature. The dried husks and kernel shells and empty bunches are used as fuel for the cooking of fermented palm fruits and for boiling the water used for processing (Obire and Putheti, 2010).

The type of microorganisms present in a finished product is influenced greatly by the environment from where the product was originally obtained, the microbial quality of the product in its raw or unprocessed state and the sanitary conditions under which the product is handled and processed (Sundram *et al*., 2003). The entire processing procedures for palm oil production, including the materials used in processing, makes the raw materials as well as the finished product prone to microbial contamination and growth.

The objectives of the study are to isolate, enumerate, characterize and identify microorganisms associated with the oil palm fruits at the various stages of palm oil production in a local mill with a view to determining the safety of the finished product; To compare the population and variety or types of microorganisms occurring at the different stages of palm oil production and to educate the palm oil mill workers on the need to carry out safe practices while processing for palm oil production.

Materials and Methods

Location, Sample Description and Sample Collection

The palm oil mill from which study samples were collected was located at Egbu community in Etche Local Government area of Rivers State in Nigeria. Oil palm fruits at different stages of palm oil processing in the oil mill include the freshly harvested fruits which had a characteristic red/yellow colour, (sample 1), fermented/ready to process fruits was dark red to dark brown in colour (sample 2), and boiled fruits from cooking vessel was dark brown in colour (sample 3). The finished product is the freshly produced palm oil which had a characteristic deep red colour (sample 4). Samples were aseptically collected and placed in a sterile bag, appropriately labelled and put into cool box containing ice packs. The samples were immediately transported to the laboratory where sample preparation was carried out for analysis.

Sample Preparation

Fibre from the oil fruits were extracted with a very sharp sterile kitchen knife. Extracts were then mashed into a mixture of fine particles using a sterile mortar and pestle and were homogenized. After homogenizing, 1.0g of each sample was placed into a test tube containing 9.0mls of sterile normal saline already labelled 10^{-1}. One millilitre (1.0ml) of the palm oil sample was also dispensed into a test tube labelled 10^{-1}. One millilitre (1.0ml) of each sample was transferred into a separate test tube and diluted serially in one tenth stepwise for all four samples to 10^{-5} dilution (Paul and Clark, 1988).

Cultivation of Bacteria and Fungi

An aliquot (0.1ml) from the dilution 10^{-2} and 10^{-4} of each sample was transferred aseptically onto freshly prepared nutrient agar plates. Spreading was done using bent glass rod (Paul and Clark, 1988; Harrigan and McCance 1990). Inoculated plates were inverted and incubated at $37^{o}C$ for 20-24 hours after which the plates were examined for growth. The discrete colonies which developed on the plates were counted and mean of duplicate plates were recorded as total viable heterotrophic bacteria. An aliquot (0.1ml) from dilutions 10^{-2} and 10^{-4} was transferred aseptically onto freshly prepared Sabouraud dextrose agar plates to which 0.2ml of 0.5% Ampicillin and tetracycline has been added to inhibit the growth of bacteria and allowing the growth of fungi (Harrigan and McCance, 1990). The inoculums was spread using a sterile bent glass rod. The inoculated plates were incubated at 37^oC for 5 to 7 days after which the plates were observed for growth. The colonies which developed were counted and mean of duplicate plates were recorded as total fungi. Pure cultures of fungi were obtained by subculturing discrete colonies onto freshly prepared sabouraud dextrose agar plates and incubated at 37^oC for 5 to 7 days.

Isolation and Preparation of Stock cultures and Identification of Bacteria

Pure culture of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared nutrient agar plates and incubated at 37^oC for 20 to 24 hours which then served as the subculture. Subsequent streak from the pure culture onto an agar slant (McCartney bottle containing nutrient agar) which was incubated for 20 – 24 hours at 37^oC and these served as pure stock cultures for subsequent characterization tests. Bacteria isolates were identified on the basis of colonial and morphological characteristics, reaction to biochemical tests and sugar fermentation tests.

Microscopic Examination of Fungi Using Wet Mount Method

The wet mount method as described by (Cheesebrough, 1991; Olds, 1983) was used. A small portion of the isolate was picked with a sterile wire loop and placed on a clean grease free microscope slide. A drop of lactophenol was added and smeared; it was covered with cover slip and was examined under microscope under ×10 objective lens was used to check the hyphae and ×40 objective lenses to see the fruiting body.

Results

The result of the total aerobic heterotrophic bacteria count of the various samples showed that counts ranged from 3.9×10^6 to 9.9×10^6cfu/g with a mean of 6.9×10^6cfu/g for freshly harvested fruits; from 1.11×10^7 to 1.92×10^7cfu/g with a mean of 1.51×10^7cfu/g for fermented fruits; 2.3×10^6 to 3.3×10^6cfu/g with a mean of 2.97×10^6cfu/g for cooked fruits and from 1.7 ×

10^6 to 2.1×10^6cfu/ml with a mean of 1.87×10^6cfu/ml for freshly prepared palm oil.

On the other hand, the total fungi count ranged from 1.2×10^6 to 2.4×10^6sfu/g with a mean of 1.8×10^6sfu/g for freshly harvested fruits; from 2.9×10^7 to 4.3×10^6sfu/g with a mean of 3.7×10^7sfu/g for fermented fruits; 9.0×10^5 to 2.2×10^6sfu/g with a mean of 1.63×10^6sfu/g for cooked fruits and from 7.0×10^5 to 1.4×10^6sfu/ml with a mean of 1.03×10^6sfu/ml for freshly prepared palm oil. Generally, the total bacteria and total fungi counts were highest in the fermented fruits and lowest in the fresh palm oil.

Generally, the bacteria and their frequency of occurrence (%) in all the various palm fruits and palm oil samples were *Bacillus cereus* (21.69%), *Bacillus subtilis* (24.69%), *Escherichia coli* (12.05%), *Klebsiella sp.* (2.41%), *Serratia marcescens* (10.84%), *Pseudomonas aeruginosa* (14.46%), *Staphylococcus aureus* (12.05%) and *Staphylococcus epidermidis* (2.41%). While the fungi and their frequency of occurrence (%) in all the samples were *Aspergillus fumigatus* (13.3%), *Aspergillus niger* (33.3%), *Mucor sp.* (20%), *Penicillium sp.* (16.7%), and *Saccharomyces cerevisiae* (16.7%). The occurrence (%) of bacteria and fungi isolated from each palm fruit and palm oil sample are shown in Figure1 and Figure 2 respectively.

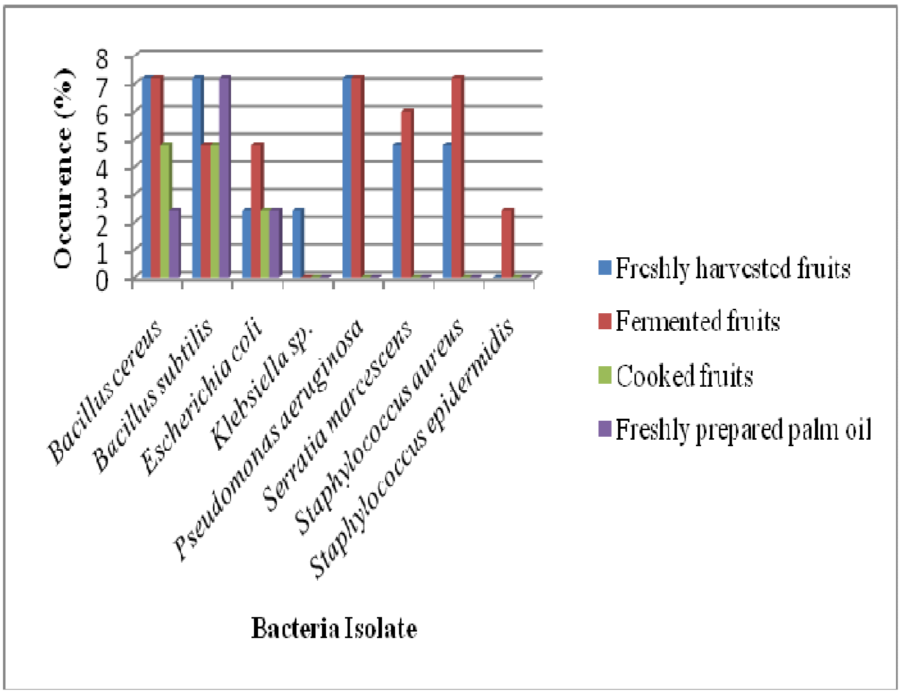

Fig. 1: Occurrence (%) of bacteria isolated from the palm fruit and palm oil samples

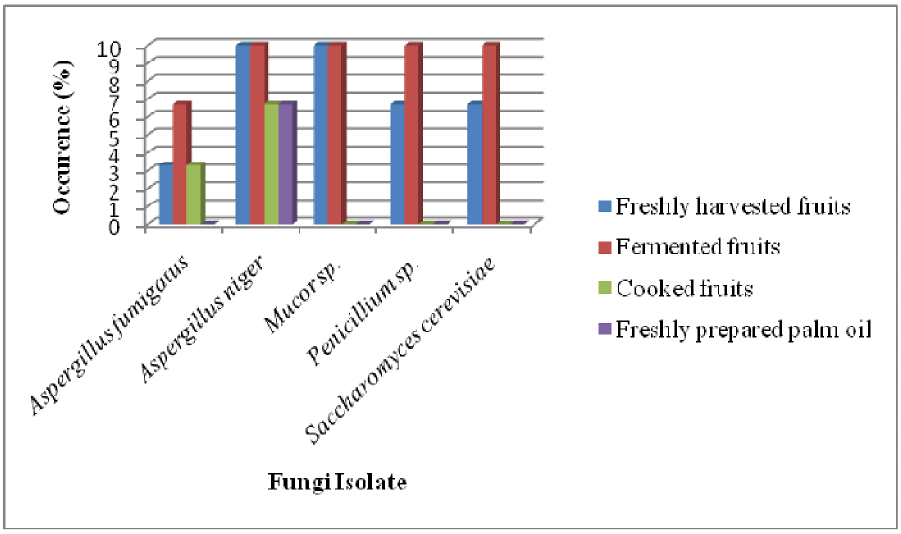

Fig. 2: Occurrence (%) of fungi isolated from the palm fruit and palm oil samples

Discussion

The present study has revealed the population and types of bacteria and fungi associated with palm fruits in the various stages of palm oil production in a palm oil mill and hence the microbiological quality in Egbu Etche in Rivers Nigeria. The results obtained showed that the population and variety of bacteria and fungi in the samples (freshly harvested fruits, fermented fruits, cooked fruits and freshly prepared palm oil) vary considerably and that a wide range of microorganisms are consistently associated with palm fruits, the material of palm oil production (Kuku and Adeniji, 1976).

The fermented fruits has been shown to contain the highest number of microorganisms (both bacteria and fungi) while the least number was recorded in the cooked fruits and freshly produced palm oil. This reduction in number and variety of microorganism is attributed to sterilization (the cooking process).

Statistical analysis using ANOVA on the population of bacteria in the samples using Analysis of variance (ANOVA) showed that there is significant difference at $p \geq 0.05$ in bacteria populations of the samples. There is also a significant difference at $p \geq 0.05$ in fungi populations of the samples. Comparison of the population of samples in pairs using the least significant difference of ANOVA showed that there is significant difference in bacteria populations in freshly harvested fruit and freshly prepared palm oil but there was no significant difference in the bacteria population of the cooked fruit and freshly prepared palm oil.

The bacteria isolates identified were *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella sp*., *Pseudomonas aeruginosa*, *Staphylococcus aereus*, *Staphylococcus epidermidis*, and *Serratia marcescens.* Fungi identified were *Mucor sp*., *Aspergillus niger*, *Penicillium Sp*., *Aspergillus fumigatus* and *Saccharomyces cerevisiae. Bacillus cereus*, *Bacillus subtilis*, and *Escherichia coli*, were isolated from all the palm fruit samples and the fresh palm oil while *Pseudomonas aeruginosa*, *Staphylococcus aereus*, and *Serratia marcescens* occurred only in the freshly harvested and fermented palm fruits. On the other hand, *Klebsiella sp*. and *Staphylococcus epidermidis* occurred only in the freshly harvested fruits and in the fermented fruits respectively. The continuous presence of *Bacillus* species is attributed to its possession of tough, protective endospores that allows it to tolerate extreme environmental conditions of temperature and desiccation (Madigan and Martinko 2005).

The continuous presence of some microorganisms after cooking showed that complete sterilization was not attained. The presence of microorganisms after cooking was not unexpected as the cooking temperatures did not get to 100^{o}C in many cases because the cooking vessels were heavily loaded with oil palm fruits that had constantly been in contact with the soil making it extensively different from pure water which boils at 100^{o}C.

In this study, *Bacillus* species and *Pseudomonas aeruginosa*, are the dominant bacteria in the palm fruits while *Aspergillus* species are the dominant fungi. This is in support of the report of Kuku and Adeniji (1976). Isolated microorganisms from palm fruits of western Nigeria were found to be dominated by *Rhizopus* and *Mucor* species while *Aspergillus* species predominated from Eastern and Mid-western Nigeria (Kuku and Adeniji, 1976).

Pseudomonas aeruginosa, *Aspergillus niger* and *Penicillium* species have been reported as normal flora of oil palm fruits (Kuku and Adeniji, 1976). They are present at the substratum, are saprophytic in soil, air, water and sewage. They are expected to play an important role in spoilage of palm fruits through their lipolytic activities. This is as a result of their production of the enzyme lipase.

The occurrence of *Staphylococcus epidermidis* in very few occasions is in the fact that it is part of the normal flora of the skin and hence a frequent contaminant of specimens. Occurrence of *Bacillus cereus* is not unexpected since it is present in large numbers in the soil and is able to survive long periods in the environment due to production of spores. Its presence in food is of great concern since it causes food poisoning by production of an enterotoxin on ingestion of the spores (Okafor *et al*., 1976).

Serratia marcescens occurred in the freshly harvested and fermented palm fruit samples. It produces a reddish-orange tripyrrole pigment called ***prodigiosin***. Its presence can be attributed to its preference for damp conditions and its feeding off phosphorus-containing materials or fatty substances

(Hejazi *et al*., 1997). *Serratia marcescens* thrives in many diverse environments, sometimes extreme conditions due to its production of a variety of extracellular enzymes and can be implicated in the spoilage of palm oil. It is frequently found in food, water, soil, animals, and plants and in rhizosphere where it is associated with suppression of soil-borne fungal pathogens acting as a biocontrol agent.

The presence of *Klebsiella* and *Escherichia coli* which are indicators of faecal contamination is attributed to the unhygienic practices of processors or handlers, who defecates along the roads and nearby bushes as their toilets and do not really wash up properly after use.

Conclusion

Palm oil has been shown to be of enormous economic importance to Nigeria and the global community at large hence, there is need to ensure that the processes leading to its production are effectively monitored to obtain a product that will not pose a health hazard microbiologically and otherwise. The evaluation of microbiological quality of palm oil is essential because of its dietary uses. Typically, over 90% of palm oil produced in Nigeria is used for food purposes. The presence of lipolytic microorganisms play important roles in the milling process, loosening the fruits from the bunch, some of these microbes are also normal flora of the oil palm fruit but can become pathogenic if their populations are enhanced. The transportation of palm fruit bunches to the palm oil mill should be carried out with adequate care to reduce bruising of fruits and contamination. Palm oil mill personnel should ensure that the cooking temperatures (Sterilization) are monitored to ascertain that they are high enough to destroy sporulating organisms. Palm oil mill workers should avoid unhygienic practices while carrying out the milling process as well as during final packaging of the products. Individuals who consume uncooked oil palm fruits should ensure that the fruits are properly washed to reduce the microbial load. Palm oil should be heated up as to sterilize it before consumption for medication or consumption with boiled yam, sweet cassava, sweet potatoes or plantain. Regulatory agencies should help monitor the activities of Palm oil mills in Nigeria. Government and Non-governmental agencies should support oil palm/ palm oil production as this will surely boost the country's economy.

Correspondence
Omokaro Obire
Department of Applied and Environmental Biology
Rivers State University of Science and Technology
P.M.B 5080, Port Harcourt, Nigeria
E-mail: omokaro515@yahoo.com

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