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Biodeterioration of palm oils from *dura* and *tenera* varieties of oil palm *(Elaeis guineensis)* jacquin by fungi

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ABSTRACT

Palm oils extracted from *dura* and *tenera* varieties of the oil palm *(Elaeis guineensis)* were examined for their stability to fungal deterioration. Twenty six fungal species were isolated from the oil samples. The isolates were members of the following genera: *Penicillium, Aspergillus, Cladosporium, Fusarium, Saccharomyces, Mucor, Geotrichum* and *Candida.* Amongst the fungi, *Aspergillus, Saccharomyces,* and *Candida* species predominated in the *dura* (Ojukwu) oil while *Aspergillus* and *Saccharomyces* predominated in the *tenera* (Osukwu) oil. Evaluation of the ability of the contaminants to cause deterioration carried out by screen tests showed that all the fungal isolates from *tenera* oil were capable of growing on and utilizing the oil as sole carbon and energy source while all the fungal isolates from *dura* oil with the exception of *Mucor* and *Geotrichum* species grew on and utilized the oil. Fungal genera may differ depending on the fatty acid involved.

INTRODUCTION

The oil palm, *Elaeis guineensis,* is one of the main sources of vegetable oil in Nigeria. It gives the highest yield of oil per unit of any crop (Purseglove, 1975). Two distinct oil types – the palm oil (mesocarp oil) and the kernel oil (the seed oil) are obtainable from the oil palm tree. Palm oil is obtained from the fleshy mesocarp of the fruit, which contain 45 – 55% oil. The oil melts over a temperature range of 25 – 50^0C (Purseglove, 1975).

There are three basic types (varieties) of the oil palm depending on the shell thickness of the kernel. They are *dura* (thick shell), *tenera* (thin shell) and pisifera (without shell) (Opeke, 1992, Negedu, 2002). The *dura* type is characterized by thin mesocarp, thick endocarp with generally large kernel. The *tenera* type possesses thick mesocarp, thin endocarp with reasonably sized kernel. This is a dual purpose oil palm variety for the production of mesocarp oil and kernel. Palm oil consists mostly of glycerides and its characteristics and uses are dependent upon the glyceride composition. Structurally, a triglyceride is the reaction product of one molecule of glycerol with three molecules of fatty acids to yield three molecules of water and one molecule of a triglyceride.

The fatty acid composition of palm oil is myristic acid 1-2%, palmitic acid 43-46%, stearic acid 4-6%, oleic acid 37-41% and linoleic acid 9-12% (Okiy, 1979, Jacquemard, 1998). The non-glyceride fraction is less than 1% and consists of carotenes (500-900ppm) tocopherols (500-800ppm) and alcohol (800ppm) among others (Okiy, 1979).In addition to the fatty acids traditionally associated with palm oil, trace amounts of other fatty acids were presented and these include myristoleic (C14:1) margaric (C17:0), gadoleic (C20:1) and behenic (C22:0) (Ekpa, *et al*: 2001).

Microorganisms are known to cause biochemical deterioration of vegetable oils derived from the seeds or fruit pulps of plants (Okpokwasili and Molokwu, 1996a). The fatty materials ordinarily are very low in moisture, a condition that favours moulds more than other microorganisms. Moulds cause both oxidative and hydrolytic decomposition that result in rancidity. Some yeasts especially film yeasts are lipolytic (Frazier and Westhoff, 1991).Contamination of processed oil with foreign matter and microorganisms is known to result in oil spoilage through lipolytic activities of microorganisms. This result in lower quality products and decrease in the commercial value from such deteriorated products (Kuku and Agboola, 1984).

This study is aimed at examining palm oils extracted from *dura* and tenera varieties of *Elaeis guineensis* (Ojukwu and Osukwu) by isolation of the fungal contaminants, identification of the isolates and evaluation of the ability of the contaminants to cause deterioration or utilize the palm oil types.

MATERIALS AND METHODS

Collection of oil samples

The palm fruits used in the work were obtained from *Elaeis guineensis dura* (Ojukwu) and *Elaeis guineensis tenera* (Osukwu) varieties. Each sample of *dura* and *tenera* varieties was obtained from 5 locations in Abia State namely: Nvosi, Ikputu, Ndume, Ubakala and Amawom.

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Extraction of palm oil from the mesocarp of oil palm varieties

A modification of Anochili (1986) was used. The freshly harvested ripe oil palm fruits were separated from the bunches and boiled in a large container for about 4 hours. The mass of pulp was produced by pounding the boiled fruits in a special pit. The initial stages were carried out in the pit, the sides of which are coated with cement. The whole mass was immersed in water, stirred and the crude oil which rose to the surface was skimmed off into another pot. The fibre was then sifted out of the water manually and finally the nuts were collected and separated from the remaining fibre. The crude oil thus obtained was boiled in smaller vessels where any fibre still present sank to the bottom. The purer oil was again skimmed off and was then "fried" in a shallow pot to remove any last traces of water (Anochili, 1986). The oil was stored in a refrigerator (4^0C) and later used for isolation.

Isolation of fungi

Sabouraud dextrose agar (temperature 45-50^0C) in 9ml amounts was used as a blank for diluting the oil samples. Sterile streptomycin 50µg/ml was added to the Sabouraud dextrose agar to suppress bacterial growth. The agar medium was autoclaved at 121^oC for 15 minutes and 1.05Kg/cm^3.Oil samples were serially diluted in the molten agar in test tubes before pouring into sterile Petri plates. One mililitre of 10^{-3}, 10^{-4} and 10^{-5} dilutions were used and three replicate plates made for each dilution. The agar medium was allowed to cool and solidify before the plates were incubated at room temperature (30± 2^0C) for 72 hours – 120 hours. Fungal counts were taken from the oil samples after incubation to represent the fungal load of the sample. A portion of each fungal colony which developed was picked using a sterile inoculating needle and aseptically sub-cultured on the fresh Sabouraud dextrose agar plates. The plates were kept as stock cultures for identification tests.

Fungal characterization

The fungal isolates were examined visually (macroscopically) and microscopically using mounted needle and cotton blue lactophenol stain. Identification followed the scheme of Hunter and Benneth (1973) and Larone (1976).

Fungal utilization

Fungal isolates from the oil sample were tested for utilization of test oil in a mineral salts medium containing the oil, as sole source of carbon and energy. The modified mineral medium of Mills *et al*. (1978) with pH adjusted to 4.5 was used. Filter sterilized antibiotics (50µg/ml each of tetracycline and streptomycin) were incorporated into the medium to inhibit bacterial growth (Walker and Colwell, 1978). The medium was dispensed in 99ml amounts into Erlenmeyer flasks. Into each of these flasks 1ml of fresh oil sample was added. The flasks were sterilized by autoclaving at 121^0C for 15 minutes and 1.05kg/cm^3. On cooling, the flasks were divided into two sets. The flasks in one set were inoculated with an isolate from that oil while those in the other sets remained uninoculated and served as control. The flasks were incubated at 25^0C for 3-5 days and the contents were shaken daily at the end of which those showing turbidity were recorded as positive and others as negative. The turbidity (optical density) was measured by using a Pye Unicam Spectrophotometer.

RESULTS AND DISCUSSION

From the oil samples, eight species of fungi were isolated and identified namely: *Penicillium digitatum, Aspergillus niger, Cladosporium resinae, Fusarium oxysporum* and *Saccharomyces cerevisiae* were isolated from *tenera* oil samples while *Mucor rouxii, Aspergillus niger, Penicillium digitatum, Geotrichum candidum, Saccharomyces cerevisiae* and *Candida* glabrata were isolated from the *dura* oil samples. The results presented in Table 1 shows that yeasts were present in both *tenera* and *dura* oil samples. Kuku and Agboola (1984) reported that fifteen mould species were isolated from some vegetable oils and the greatest number of species were isolated from some vegetable oils and the greatest number of species (thirteen) occurred in palm oil and groundnut oil, while only eight mould species were isolated from palm kernel oil. Okpokwasili and Molokwu (1996) also reported that sixteen yeasts and thirty five mould species were isolated from six vegetable oil samples.

The distribution and frequency of fungal genera isolated in the various oil samples are presented in Table 2. Amongst the fungi encountered, *Aspergillus, Saccharomyces* and *Candida* occurred most frequently in the contaminated *dura* variety. Similarly, *Aspergillus* and *Saccharomyces* were most frequent in the *tenera* oil. *Aspergillus* occurred in five *dura* oil samples with higher frequencies and in four *tenera* with high frequencies. *Saccharomyces* occurred in four *tenera* oil samples with higher frequencies, and in three *dura* oil samples with high frequencies. *Candida* did not occur at all in *tenera* oil samples.

The next most frequently isolated in all the samples was *Penicillium, Mucor* and *Geotrichum* which also occurred in only one *tenera* oil sample and at very low frequencies too.

The predominance of *Aspergillus* in the oils could be attributed to the fact that the species of this genus are found almost everywhere, on every conceivable type of substratum. *Aspergillus* sp. have also been reported to occur frequently in vegetable oils (Kuku and Agboola, 1984). The presence may be due to contamination during

handling and storage of the product. However, the survival of these fungi may be due to their lipolytic capability (Frazier and Westhoff, 1991), and their ability to produce heat resistant strains.

The results of the screen test for the utilization of the samples by the fungal isolates presented in Table 3 shows that all the fungi isolates from *tenera* oil were capable of growing and utilizing the oil as sole carbon and energy sources. All the fungi isolated from *dura* oil were capable of growing and utilizing the oil as sole carbon and energy sources except two fungi. This suggested that the two fungi might merely be contaminants and might not have any oil biodeteriogenic activity.

The fungal count in the oil samples are shown in Table 4. The *dura* oil samples 30J, 70J and 100J have the highest bioburden while 10S,40J,50S,60J and 90S have the lowest. The results indicate the level of contamination of the oils during processing and handling.

In conclusion, the fungal contaminants wore isolated, identified and the ability of the contaminants to cause deterioration was evaluated. *Aspergillus* was observed to be one of the fungi which occurred with the highest frequency in both the *tenera* and *dura* oils. It is therefore, important to always keep the oils at a safe moisture level, since fungi are generally moisture lovers.

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Table 1. Characterization of the yeast isolates

Isolates code numbers	Colonial appearance	Gram reaction	Growth at 37^0C	Citrate reaction	Growth in 10% Nacl	Sugar fermentation					
						Glucose	Lactose	Sucrose	Mannitol	Maltose	
J7	Oval	+	+	+	+	+	-	+	-	+	*Saccharomyces cerevisiae*
S7	Oval	+	+	+	+	+	-	+	V	+	*Saccharomyces cerevisiae*
J8	Cylindrical	+	-	+	+	V	-	V	+	+	*Candida glabrata*

\+ = positive

\- = negative

v = variable

Table 2. Distribution of fungal isolates from the palm oil types

Genus	*Dura*	Frequency occurrence (%)[a]	*Tenera*	Frequency of occurrence (%)a
(a) *Moulds*				
Penicillium	1	3.85	*1*	3.85
Mucor	1	3.85	-	0.00
Aspergillus	*5*	19.23	*4*	15.38
Cladosporium	-	0.00	*1*	3.85
Geotrichum	1	3.85	-	0.00
Fusarium	-	0.00	*1*	3.85
(b)yeast				
Saccharomyces	3	11.54	*4*	15.38
Candida	4	15.38	-	0.00

a Number of times occurring in all fungal colonies examined x100

n = 26

Table 3. Screen test for the utilization of the palm oil samples by the contaminating fungi isolated from the oils

Tenera Isolates	*Tenera* oil	*Dura* isolates	*Dura* oil
Penicillium digitatum S1	+	*Penicillium digitatum* J1	+
Aspergillus niger S2	+	*Aspergillus niger* J2	+
Cladosporium resinae S5	+	*Mucor rouxill* J3	-
Fusarium oxysporum S6	+	*Geotricham candidum* J4	-
Saccharomyces cerevisiae S7	+	*Saccharomyces cerevisiae* J7	+
		Candida glabrata J8	+

Table 4. Fungal counts in the oil samples

	Oil samples	Counts (cfu/ml)
1	OS	40 ± 0.00
2	OS	50 ± 2.49
3	OJ	60 ± 2.49
4	OJ	40 ± 4.30
5	OS	40 ± 0.00
6	OJ	40 ± 2.49
7	OJ	60 ± 2.49
8	OS	30 ± 0.00
9	OS	40 ± 2.49
10	OJ	60 ± 2.49

OS = Osukwu; OJ = Ojukwu

The counts are means of three replicates ± standard deviation.

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