LIPOLYTIC ACTIVITIES OF ISOLATED MOULDS ON PALM OIL DURING STORAGE

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Abstract
Palm oil samples from a mechanized oil processing factory (NIFOR) and a locally manufacturing depot at Aisegba Ekiti, Ekiti State were investigated and the lipolytic activities of the moulds isolated were studied. Aspergillus flavus ,A. fumigatus, A. niger and Rhizopus stolonifer were isolated from both locally and mechanically produced palm oil. A. flavus is more lipolytic than any other fungi with respect to the fatty acid content, peroxide value and moisture content. The ffa for locally produced oil ranged from 11.50 to 21.00 from initial value of 4.00%.The mechanically produced oil increased from1.0% to between 1.7 to 4.00 after 14 days of storage .The peroxide values for both the locally and mechanically produced oil increased from initial values of 5.0 MEqKg to between 12.96 and 19.20 fort locally produced oil and between 4.00 and 6.30 for mechanically produced oil after 56 days in storage.

Keywords: *Palm oil, rancidity, peroxide value, fungi, inoculation, biodeterioration*

Introduction

Palm oil is used worldwide as cooking oil, medical and cosmetic products. It is reddish in colour, the colour being due to its high of carotene. Carotene is the precursor of vitamin A.

Composition of palm, oil fatty acid are myristic (0.5 2.5%), palmitic (36 42%) stearic (1 6%), oleic (30 50%), linolenic (2 14%) and linolenic (0.1 0.4%). Edible oil from plant sources are of important interest in various food and application industries.

(Odoemelam, 2005) and can also serve as oleo chemicals (Movrison, et al., 1995). Oleo chemical are completely biodegradable. Vegetable oil had made an important contribution to the diet in many countries, serving as a good source of protein, lipid and fatty acid for human nutrition including the repair of worn out tissues, new cells formation as well as a useful source of energy (Grosso et al., 1999). Oil quanlity and its stability are therefore very important for the consumers and application industries (Jambunathan et al., 1993). The traditional method of oil extraction in part of West Africa is to cook the flesh pulp with a large volume of water, the oil floats on the surface and is skimmed off.

However technology of palm oil production has improved and new technology and innovations are being introduced yearly. The quality of mechanically processed palm oil was compared with traditionally processed ones by Denenu and Eze (1985). They reported that mechanically processed oil had relatively low free fatty acid content of 3 5%, moisture content 0.09 1.70% and impurities 0.46 2.26%. The traditionally processed oil samples were characterized by high free fatty acid greater than 18%, impurities above 5% and moisture content above 3%. The important quality parameters of palm oil include free fatty acid, moisture content, impurities, peroxide value, rancidity and bleach ability (Denenu and Eze 1983).

Oils in general are known to be susceptible to microbial attack. The

composition of the various oils determines the extent and types of organisms likely to thrive in them (Okpokwasili and Williams, 1991). Palm oil is known to support the growth of fungi and bacteria especially when it contains moisture. Their lipolytic enzymes are so active that even under unfavorable conditions palm oil is seldom produced with free fatty acid content (FFA) of less than 2% and under favorable conditions of processing, the FFA content of this oil reaches 20% and higher. When the fruit is bruised, lipolytic action occurs and a near maximum FFA (8-40%) is reached within 40mintes. The FFA of un-bruised fruits may increase only 0.2% or less in the course of 4 days. The smell, taste, colour, texture, and chemical composition of the food may be sufficiently altered by the micro organisms growing on it which could make the food inedible (Williams and Shaw, 1992). The moisture content in food, its location, and availability is one of the most important factors influencing microbial growth. One of the major changes taking place in lipids is generally referred to as rancidity.

Moulds are known to cause biochemical changes in oils. These changes can take the form of decrease in bleach ability or increase in free fatty acid. The moulds which are capable of increasing the free fatty acid contents of oils are referred to as lipolytic moulds and they contain enzymes known as lipases (Price and Steven, 1990). Moulds constitute the largest groups of spoilage micro organisms in all varieties of food and materials (Abba-Kareem et al, 1990). This study is aimed at examining the biodeteriogenic effects of isolated moulds from locally and mechanically produced palm oil.

Materials and methods

Freshly milled samples of palm oil were collected from Nigerian Institute for Oil palm Research (NIFOR) Benin, Edo State and a local manufacturing depot at Aisegba Ekiti, Ekiti State. The two samples were collected into plastic containers and closed instantly to prevent contamination. The samples were stored at 28±2oC and relative humidity of 76 ± 5%.

Microbiological analysis

One milliliter of oil sample was aseptically withdrawn from each container and mixed with nine milliliter of sterol peptone water medium. The mixture was vigorously shaken to dislodge the microbial propagates from the palm oil samples into the peptone water medium. One milliliter of the aqueous fraction was aseptically transferred into sterile Petridis after which molten sterile Potato Dextrose Agar was aseptically poured into the plates. Sterile streptomycin 50µg/ml was added to the agar to suppress bacteria growth. The plates were gently rocked on the bench to allow proper mixing of the content. The plates were allowed to set and incubated at 28 ± 2°C for five days. The moulds were allowed to grow and were sub-cultured unto fresh sterile potato dextrose agar medium until pure cultures were obtained.

Identification of fungi

A small piece of mycelium free of medium was transferred using inoculating needle on to a glass slide containing a drop of cotton built-in-lacto phenol. The mycelium was spread properly with another sterile needle. The preparation was covered with a cover slip and observed under the microscope using (X10) and later high power (X40) objectives. Details of the hyphae, spore coloration, shape and surface marking were studied. The types of fungi present were finally identified by reference to Barnett (1960).

Inoculation of samples

Sixty milliliters of hot palm oil was measured aseptically into the sterile bottles. The samples were further sterilized in an oven at 160°C for fifteen minutes.

About 200 spores of the following

isolated and identified fungi:- Aspergillus flavus, A. niger, A. fumigatus and Rhizopus stolonifer were inoculated into 60mls sterilized palm oil and incubated at 300C. The effects of the inoculated moulds on the chemical attributes of palm oil sample were determined at fourteen, twenty eight, and fifty six day's interval respectively.

Determination of free fatty acid content

The free fatty acid content of fat/oil is the number of milligrams of KOH required to neutralize 1g of FFA present in ft/oil sample. The free fatty acid contents of the palm oil types/samples were determined according to the method described by reason 1976. The acid value is the number of mg of KOH necessary to neutralize the free acid in 1g of sample. The acid value is given by T-B X 5.61/W0.1M KOH contains 5.66mg/ml or 5.6/1 where T= Titre value of the sample; B = Titre value of a blank. The blank was provided as a control by titrating 2.5ml of the neutral alcohol without sample. The free fatty acid (FFA) is normally determined as oleic acid where by the acid value = 2x FFA.

NaOH may be used and a generalized formula may be used (for palm oil fractions): 25.6 x MNaOH X v/w WHERE v = Volume of NaOH solution used in ml; W= weight of sample.

Determination of peroxide value

The peroxide value was determined by the method described by Pearson 1976 as the mg weight of iodine, which is formed by 1kg of fat/oil sample. When potassium iodine was subjected to the oxidation effect of peroxide forming iodine at room temperature, the iodine, which was liberated, was titrated against standard sodium thiosulphate solution (0.02N Na2 S2O3). The peroxide value was reported as the volume of 0.02N Na2S2O3 used in the titration

Rancidity test on stored samples

The test for rancidity of oil was carried out according to Pearson (1981). Ten milliliters of oil sample was poured into a dry conical flask. Then ten milliliters of 0.1 percentage phloroglucinol solution in ether were added and the mixture was shaken vigorously to mix the content properly. Ten milliliters of concentrated hydrochloric acid were added and was again shaken vigorously for twenty seconds, until pink colour persistently appeared.

The samples that changed to pink at exactly twenty seconds were termed rancid.

Results and Discussion

The findings contained in this report showed that Aspergillus flavus was more lipolytic than other fungi. From figure 1 the locally produced palm oil with initial 4.00% free fatty acid content increased to 16.00, 21.00, 14.00 and 11.50 percentages with Aspergillus niger, A. flavus, A. fumigatus and Rhizopus stolonifer contaminated oil respectively after 14 days. This order of increase was maintained till the last day of storage. The mechanically produced palm oil with initial value of 1.00% FFA increased to 2.00, 3.10, 4.00 and 1.7% after inoculating with Aspergillus fumigatus, A. niger, A. flavus and Rhizopus stolonifer respectively after 14 days of storage. The locally produced palm oil showed higher initial free fatty acid percentage of 4.00 which eventually reflected in more free fatty acid formation with length of incubation. Post-processing local method of oil preparation increase the free fatty acid in most cases as a result of microbial activities that infect the bruised palm oil fruit and this occurs in the presence of enough moisture and dirt content which allowed micro organisms to multiply freely. The free fatty acid of palm oil may be formed by the action of lipolytic enzymes. The peroxide values of 5.00Meqkg in locally produced palm oil increased to 16.80,

19.20, 12.96 and 14.40 Meqkg for Aspergillus niger, A. flavus, Rhizopus stolonifer and Aspergillus fumigatus respectively for 56 days in storage as shown in figure 2. The mechanically produced palm oil had its peroxide values increased from initial value of 3.25Meqkg to 5.90, 6.30, 5.10 and 4.00 for same organisms with locally produced palm oil for 56 days in storage.

In both types of oil, it was generally noticed that the peroxide values increased with the increase periods of incubation. During storage, peroxide formation is slow at first during induction period, which may vary from weeks to several months according to the particular oil or fat and the temperature (Yeah and Chooi 1977). The increase in the peroxide value may have been due to the increase in the oxidation of unsaturated fatty acid that form peroxide and this frequently leads to oxidative rancidity. All the moulds species inoculated into mechanically produced palm oil did not make the oil rancid at the end of 56 days of incubation. The reason may be due to low peroxide value development because according to Mehlenbacher (1960), oil begins to show incipient rancidity when peroxide value ranges between 15 20 Meqkg. In locally produced palm oil, Rhizopus stolonifer and Aspergillus fumigatus did not make the oil rancid even at 56 days incubation periods while palm oil inoculated with both Aspergillus niger and A. flavus showed little rancidity and this showed that these two fungi were more biologically active in palm oil than the other inoculated mould species.

The initial moisture content of locally produced palm oil before inoculation was 4.20% and with inoculated moulds the value increased to 4.80, 5.10, 4.90 and 4.20 for A. niger, A.flavus A.fumigatus and Rhizopus stolonifer respectively for incubation period of 56 days. For mechanically produced palm oil, the initial content of 0.09 increased to 0.30, 0.50, 0.90 and 0.29% for A. fumigatus, A. niger and A flavus and Rhizopus stolonifer after inoculation and incubated at 30OC for 56 days as shown in figure 3.

Olie and Tjeng (1994) recommended 0.08 1.00% moisture content for safe storage. The initial high moisture content of locally produced palm oil was as a result of the processing methods involved which include pounding in mortal and then put in a drum where large volume of water was always used for cooking before oil content that float are skimmed off.

In conclusion, mechanically processed palm oils are more stable to oxidative rancidity that are caused by moulds because of its low moisture content and the processing is done under hygienic condition contrary to locally produced palm oil.

TABLE 1: Result of rancidity test on locally and mechanically produced palm oil after inoculation and incubation at 30^0C

Mould species		Period of incubation (days)		
		14	28	56
Control		-ve	-ve	-ve
Aspergillus niger (LPPO)		-ve	-ve	+ve
Aspergillus niger (MPPO)		-ve	-ve	-ve
Aspergillus flavus (LPPO)		-ve	-ve	+ve
Aspergillus flavus (MPPO)		-ve	-ve	-ve
Rhizopus stolonifer(LPPO)		-ve	-ve	-ve
Rhizopus stolonifer(MPPO)		- ve	-ve	-ve
Aspergillus fumigatus (LPPO)	-ve	-ve	-ve	
Aspergillus fumigatus (MPPO)	-ve	-ve	-ve	

LPPO= Locally produced palm oil
MPPO= Mechanically produced palm oil
-ve= Negative (Not rancid)
+ve= Positive (Rancid)

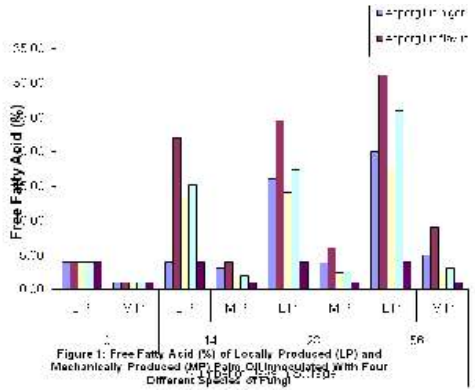


Figure 1: Free Fatty Acid (%) of Locally Produced (LP) and Mechanically Produced (MP) Palm Oil Inoculated with Four Different Species of Fungi

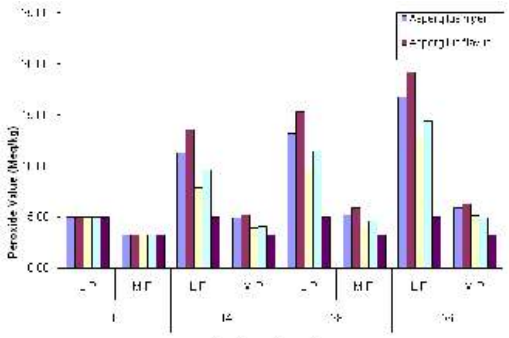
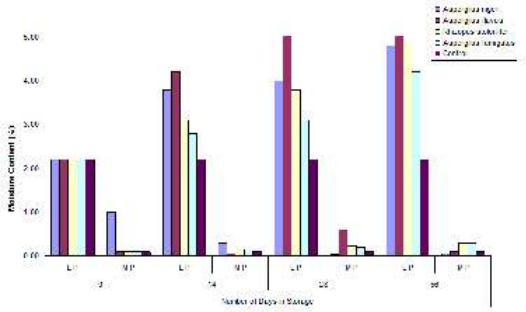


Figure 2: Graph of Peroxide Value (meq/kg) of Locally Produced (LP) and Mechanically Produced (MP) of Palm Oil Innoculated with Four Different Species of Fungi



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