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LIPASE ACTIVITY OF FOURTEEN FUNGI ON *CUCUMEROPSIS MANNII* SEEDS

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ABSTRACT

Lipase activity of fourteen pathogenic fungi on *cucumeropsis mannii* ('Egusi' melon) seeds were examined. Their effect on melon oil in ten weeks storage was also determined. All the fungi except *Mucor racemosus* increased the free fatty acid (FFA) value of melon seed oil in storage by above 5%. *Aspergillus flavus* caused the highest increase (9.09%) in the FFA value of the melon seed oil among the fungi tested. There was a slight increase in the FFA value of healthy melon oil (control) from 3.36 to 3.98%. Infected melon oil with FFA content above 6.00% were rancid. The healthy ungerminated melon seed had no lipase activity. All the fungi had varying lipase activity on infected seeds. *Aspergillus flavus* had the highest (9.03 + 1.08 sigma-titez units/ml) while *Talaromyces* sp. had the least (1.03 + 0.14 sigma titez units/ml). Germinating melon seedlings (5 days old) had lipase activity of 1.35 + 0.04 sigma-titez units/ml. The implications of these are discussed.

INTRODUCTION

Oil seeds are rich sources of triacylglycerides (fats and oils). *Cucumeropsis mannii* Naud-Holl ('Egusi' melon) seed oil has the potentials for use as a base for soap and body cream production (Akalonu and Ogbonna, 1990).

Lipid degradation occurs when seed or its oils are damaged by improper storage conditions or are exposed to certain microorganisms. Fungi are capable of producing lipases which can hydrolyse fats to fatty acid thereby increasing the free fatty acid (FFA) of the produce (Godin and Spensley, 1971). This is a deteriorating effect. The FFA produced from lipid degradation by lipase as explained by Bewley and Black (1978) is taken through oxidation pathway where they are activated and used for carbohydrate synthesis. The carbohydrate so produced might then be used by the fungi for their metabolic activities leading to growth.

Lipase (EC 3.1.1.3; Dixon & Webb, 1964) also known as Glycerol ester hydrolase is a principal enzyme involved in the hydrolysis of seed lipids (Triacylglycerol) to free fatty acids and glycerol. It is a milk clotting enzyme used industrially in the production of cheese. The presence of lipase activity has advantages in the acceleration of flavour development in some types of cheese (Smokuti and Babel, 1968).

Some workers have reported the presence and activity of fungal lipases on the deterioration of seeds. For example Dirks *et al* (1955) observed that fungal lipases of *Aspergillus* and

Penicillium species were responsible for the lipid hydrolysis of wheat grain. In 1978, Delucca *et al* reported the isolation of lipolytic microorganisms found on rough rice. Microbial lipases act on seeds to produce off flavours or cause rancidity of oils during storage. Hence, the necessity to study fungal lipase activity on seeds. Lipases produced by pathogenic fungi on *cucumeropsis mannii* seeds have not been reported.

This work reports the lipase activity of fourteen pathogenic fungi on *cucumeropsis mannii* seeds and the effect of the fungi on infected melon oil during storage.

MATERIALS AND METHODS

Fourteen out of nineteen pathogenic fungi isolates (Adekunle, 1997) collected during a 15 month, montly sampling of diseased melon seeds from Nigerian State Capitals were used. The fungi were isolated according to the methods of Booths (1971) and identified using conventional methods, thereafter their identity was confirmed by IMI mycological institute, Kew, London. Fungal pathogenicity was carried out using the methods of Booths (1971). Stock cultures were maintained on cornmeal agar slants incubated at room tenperature (28-31°C). Isolates were aseptically taken from stock cultures when needed.

Effect of fungi on the free fatty acid content of inoculated melon seed oil during storage.

Effect of fourteen pathogenic fungi on the FFA content of melon oil during storage was investigated using Kuku's (1979) methods. Five hundred millilitres of melon oil sample was poured into a 1000ml conical flask and sterilised in an autoclave at 121°C and 1.04kg/cm^3 for 15 minutes. An equal volume of previously prepared sterile semi-solid agar medium (15g of plain agar in 1 litre of distilled water) was poured into the 100ml flask containing the sterile oil. They were mixed together in a mechnical shakerfor one hour. The free fatty acid content of one gram (about 2ml) of the melon oil/agar was determined (Kuku, 1979). The oil/liquid agar mixture was distributed into fifteen sets of four 100ml conical flasks (20ml per flask). Fourteen of these sets were inoculated with seperate fungi, while the last set was used as ccontrol. The flasks were inoculated with the fungus by aseptically introducing 5mm^2 of fungal disc cut with a 5mm^2 corkborer from 14 day - old pure culture of the fungus. All the flasks were then incubated at laboratory temperature of 28-31°C for ten weeks. The free fatty acid content of 2ml of the oil/agar mixture of each sample was determined every two weeks for ten weeks using the methods of Kuku (1979).

Lipase activity of fungi cultured on melon seeds

Visually healthy shelled *cucumeropsis mannii* seeds were sterilized by washing in 0.1% mercuric chloride for 1 minute and then rinsed in 3 changes of sterilized distilled water. Ten grams of the sterilized seeds were placed on two weeks old fungal culture on potato dextrose

Table 1: *Effect of fungi on free fatty acid content of melon seed oil during ten weeks storage.*

Fungi Used	Free Fatty Acid Content (%) Period of Storage (weeks)					
	Start	2	4	6	8	10
Oil free of fungi	3.36	3.64	3.64	3.65	3.87	3.98
Multiple infection on seeds	6.42	7.91	8.02	8.90	9.73	10.80
Absidia blakelseeana	3.36	4.10	4.71	5.39	5.78	6.06
Aspergillus clavatus	3.36	4.17	5.39	6.56	7.01	8.13
Aspergillus flavus	3.36	4.60	6.51	7.67	8.75	9.09
Aspergillus niger	3.36	3.98	4.71	5.78	6.62	6.73
Aspergillus tamarii	3.36	4.10	4.77	5.39	6.06	6.34
Aspergillus wentii	3.36	5.22	5.50	5.95	6.62	6.90
Botryodiplodia theobromae	3.36	4.30	5.10	5.65	6.25	7.06
Fusarium solani	3.36	3.76	5.50	6.64	7.18	7.41
Macrophomina phaseolina	3.36	4.04	4.71	5.22	5.50	6.28
Mucor racemosus	3.36	3.65	3.93	4.10	4.38	4.60
Penicillium chrysogenum	3.36	5.44	5.50	5.55	5.67	6.12
Penicillium pinophylum	3.36	4.38	5.44	5.55	5.67	5.75
Rhizopus oryzae	3.36	4.21	5.79	6.84	7.24	7.35
Talaromyces sp	3.36	3.87	4.21	4.60	4.99	5.33

agar (PDA), of each fungal isolate used and incubated at room temperature (28 -31°C). A control was set up where 10 grams of sterilized melon seeds were placed on sterilized semi-soild agar medium without inoculating with fungus, and also incubated for 5 days. Three replicates were made for each sample. Another set of 10 gram of sterilized, visually healthy melon seeds was used for extraction of lipase enzyme at the start of the experiment.

After five days of incubating, lipase enzyme was extracted from each of the inoculated and control samples, by grinding the seeds to a paste in a sterile mortar using the method of Bergmeyer (1974). Ten mililitre of sterilized distilled water was added to the paste and filtered with sterilized muslin cloth and funnel. The filtrate was used on the crude extract of lipase enzyme assay sample. Lipase activity of the crude extract was determined by using the titrimetric method of Anon (1990). The results were statistically analysed at $P = 0.05$ using analysis of variance. The standard deviation of means was also calculated. (Parker, 1979).

RESULTS.

Effect of fungi on the free fatty acid (FFA) content of inoculated melon seed oil during storage

The result of the effect of fungi on the FFA content of inoculated melon seed oil during ten weeks storage is shown on Table 1. At the start of the experiment, oil from healthy melon seeds contained 3.36% FFA, while that extracted from multiple infected seeds contained a high FFA (6.42%). Healthy melon seeds (control) showed only slight increase in percentage FFA content. Oil artificially infected with various fungi showed significant increases in percentage FFA over the control, while that extracted from multiple infected seeds increased from 6.42% to 10.80%.

Aspergillus flavus caused the highest increase in FFA value (from 3.36%-9.09%) while *mucor racemosus* had the least effect (from 3.36%-4.60%). Only healthy melon oil and oil extracted from *mucor racemosus* infected seed had less than 5% FFA value; and all the five species of *Aspergillus* infected oil tested had more than 5% FFA content after 10 weeks. Storage oil with FFA above 6.0% was rancid. Rancidity was observed in oil infected with *Aspergillus* species,

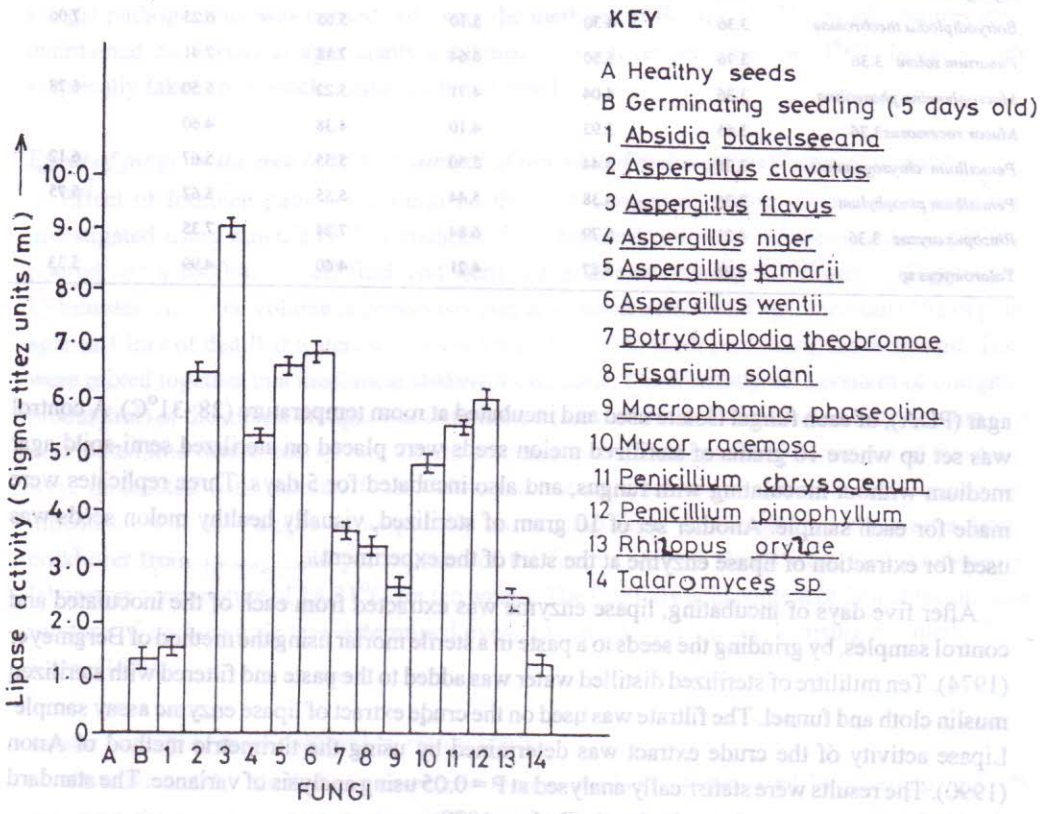

Fig. 1: Lipase activity of fungal isolates in infected melon seeds

Penicillium species were responsible for the lipid hydrolysis of wheat grain. In 1978, Delucca *et al* reported the isolation of lipolytic microorganisms found on rough rice. Microbial lipases act on seeds to produce off flavours or cause rancidity of oils during storage. Hence, the necessity to study fungal lipase activity on seeds. Lipases produced by pathogenic fungi on *cucumeropsis mannii* seeds have not been reported.

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high lipase activity in ungerminated fungal infected seeds in this study showed that these fungi were responsible for the production of more lipase enzyme. This work confirms the work of St. Angele and Robert (1983) that lipases are principal enzymes involved in degradation of lipids by microorganisms.

It is suggested from the results of this study that lipase production from the fungi used could be exploited on a large scale for industrial purpose, and that stored melon seeds should be free of fungi to prevent deterioration, especially in seeds used for oil production.

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