# COMPARATIVE STUDY OF LIPASES OBTAINED FROM MOULDS GROWN ON GROUNDNUT

by

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## INTRODUCTION

CAMUS[1], FODOR AND CHARI[2] have observed the presence of lipase in *Aspergillus niger* and *Penicillium roqueforti*. RAMAKRISHNAN AND NEVGI[3] investigated the different oil seeds and oil seed cakes for their lipolytic activity with a view to obtain a cheap and active lipase. In continuation of the search for a cheap and active lipase, RAMAKRISHNAN AND BANERJEE[4] investigated the different moulds grown on cocoanut and found that Aspergillus flavus isolated from the moulds grown on cocoanut shows appreciable lipolytic activity. A search was undertaken to analyse the molds grown on other oil seeds to see whether a lipase with better activity could be obtained. The moulds grown on the groundnut seed were analysed for their lipolytic activity and the results are recorded in this paper.

## EXPERIMENTAL PART

The moulds were grown on groundnut (*Arachis hypogea*) by keeping the groundnut seeds in a desiccator, adjusting the moisture content and regulating the flow of air. The moulds grown on the oil seeds were sub-cultured in petri dishes in Czapek agar medium and pure strains were prepared. Staining methods of BAYLISS, GLICK AND SIENT[5] as well as the biological method of GRABILL AND REED[6] were used to detect the lipolytic strains in the cultures obtained. The lipase was prepared from the following strains isolated from the moulds grown on the seed:

*Aspergillus flavus* $G_1$, *Aspergillus flavus* $G_2$, *A. oryzae* $G_3$, *A. oryzae* $G_4$, *A. fumigatus* $G_5$, *A. niger* $G_6$, *Penicillium sp.* $G_7$.

*Preparation of the enzyme*

The lipolytic strains obtained were grown in conical flasks of one litre capacity containing 200 ml sterilized liquid Czapek medium at 37° C and after four days the mats were removed, washed well with sterilized water and treated with low-boiling petroleum ether to kill the cells and remove any fatty materials present. The mats were dried at room temperature to remove the solvent completely, powdered well and sieved through a 60 mesh sieve. The powder obtained was used for the experiments.

The activity of these mould lipases was studied by investigating the hydrolysis of freshly prepared groundnut oil, using these lipases and disodium phosphate-citric acid buffer of varying $p_H$.

Each reaction mixture consisted of 1 ml of oil, 5 ml of water, 0.1 g of mould lipase, 2 ml of buffer mixture of varying $p_H$ and a few drops of toluene in a flask incubated at 37° C for 24 hours after a thorough shaking. A blank always accompanied each sample. After incubation the content was removed and titrated against $N/10$ NaOH after adding 25 ml neutral alcohol and warming for some time. Necessary precautions were observed to take readings under sterile conditions. The difference between the sample and the blank in terms of ml of $N/10$ sodium hydroxide will give the activity of the lipase.

From Table I, it can be seen that the optimum $p_H$ for the lipolytic moulds varies from 6.2 to 6.8, depending upon the nature of the mould. In particular *A. flavus* $G_1$ shows appreciable lipolytic activity.

*References p. 218.*

TABLE I

LIPOLYTIC ACTIVITY OF MOULDS LIPASES AT VARIOUS pH

Buffer: Disodium phosphate — citric acid Oil: Fresh groundnut oil (f.f.a — 0.01%)

| Name of the mould | Activity of the mould lipase in terms of difference in ml of N/10 NaOH between the sample and the blank when the pH of the mixture is | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3.1 | 3.6 | 4.2 | 4.8 | 5.4 | 6.2 | 6.8 | 7.2 |
| *Aspergillus flavus* $G_1$ | 6.2 | 8.1 | 8.8 | 9.5 | 11.2 | 13.8 | 10.7 | 9.2 |
| *Aspergillus flavus* $G_2$ | 1.0 | 2.3 | 2.8 | 3.2 | 4.1 | 4.8 | 5.2 | 4.4 |
| *A. oryzae* $G_3$ | 3.1 | 4.2 | 5.1 | 6.0 | 6.8 | 8.2 | 7.8 | 6.5 |
| *A. oryzae* $G_4$ | 0.8 | 1.2 | 2.0 | 2.7 | 3.0 | 3.6 | 4.5 | 3.1 |
| *A. fumigatus* $G_5$ | 2.5 | 3.7 | 4.6 | 4.9 | 5.4 | 7.4 | 6.1 | 5.8 |
| *A. niger* $G_6$ | 1.8 | 2.9 | 3.1 | 4.3 | 4.8 | 5.9 | 4.2 | 3.2 |
| *Penicillum sp.* $G_7$ | 4.4 | 5.2 | 6.3 | 7.2 | 8.1 | 9.8 | 7.5 | 6.1 |

These strains *A. flavus* $G_1$, *A. oryzae* $G_3$, *A. fumigatus* $G_5$, *A. niger* $G_6$ and *Penicillum* sp. $G_7$ were now grown in groundnut cake medium.

In different culture flasks 200 ml of groundnut cake solution containing 15% of oil-free cake were added, together with different amounts of fresh groundnut oil. They were sterilized for 10 minutes under 15 lbs. pressure and inoculated with the strains and incubated at 37° C. After four days, the mats were removed and the lipase powder prepared. The activity of these mould lipases was determined by studying the hydrolysis of freshly prepared groundnut oil using these lipases and disodium phosphate-citric acid buffer of pH 6.2.

TABLE II

LIPOLYTIC ACTIVITY OF LIPASES OF MOULDS GROWN IN MEDIA CONTAINING DIFFERENT AMOUNTS OF GROUNDNUT OIL

| Medium | Growth | Lipase activity in terms of difference in ml of N/10 NaOH between the sample and the blank for | | | | |
|---|---|---|---|---|---|---|
| | | *A. flavus* $G_1$ | *A. oryzae* $G_3$ | *A. fumigatus* $G_5$ | *A. niger* $G_6$ | *Penicillum sp.* $G_7$ |
| Czapek | Appreciable growth | 13.8 | 8.2 | 7.4 | 5.9 | 9.8 |
| Groundnut cake alone | Not satisfactory | 10.2 | 6.5 | 5.2 | 4.8 | 8.2 |
| Groundnut cake + 5% G.oil | Slight growth | 11.8 | 7.1 | 6.7 | 7.2 | 10.1 |
| Groundnut cake + 10% oil | Good growth | 15.6 | 12.3 | 10.1 | 8.5 | 13.2 |
| Groundnut cake + 15% G.oil | Slight growth | 9.5 | 6.1 | 5.0 | 3.1 | 7.8 |
| Groundnut cake + 20% G.oil | Slight growth | 6.8 | 3.8 | 2.5 | 2.0 | 6.1 |

From Table II, it can be seen that the strains grown in groundnut cake medium containing 10% oil show the highest lipolytic activity. On further investigation the cake medium may prove to be a good source for growing lipolytic moulds on a large scale.

*Synthetic activity of the lipolytic moulds*

The acetone dried lipase was prepared from the moulds according to RAMAKRISHNAN AND NEVGI's method. The synthesis of butyl oleate was carried out with the aid of these lipases.

In different conical flasks, equimolecular quantities (0.054 g mol of butyl alcohol $(CH_3(CH_2)_2 CH_2OH$, b.p. — 117° C; d = 0.809 g/ml, mol. wt. 74.1) and oleic acid $[C_8H_{17}CH=CH(CH_2)_7COOH$,

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b.p. — 286° C, d = 0.895 g/ml; mol. wt. 282.4] were added. The lipase (1 g) and ether solvent (10 ml) were added to each flask, which were well shaken and kept in the incubator at 37° C after corking well. At different intervals of time 1 ml was taken from each flask, 25 ml of neutral alcohol were added, the mixture was warmed for some time and titrated against $N/10$ NaOH. A blank always accompanied each sample. The difference between the blank and the sample was calculated in terms of ml of $N/10$ sodium hydroxide. From this the percentage synthesis was calculated.

The results are given in Table III.

TABLE III

SYNTHESIS OF BUTYL OLEATE BY MOULD LIPASES

Solvent: Ethyl ether

| Set No. | Lipase from | Percentage synthesis on | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | 5th day | 6th day | 7th day |
| 1 | *A. flavus* $G_1$ | 15.4 | 30.7 | 45.8 | 52.3 | 60.8 | 63.4 | 60.9 |
| 2 | *A. oryzae* $G_3$ | 11.3 | 14.1 | 20.9 | 28.6 | 35.9 | 40.2 | 38.7 |
| 3 | *A. fumigatus* $G_5$ | 12.1 | 15.6 | 21.8 | 30.5 | 38.6 | 40.1 | 35.2 |
| 4 | *A. niger* $G_6$ | 7.2 | 11.1 | 15.8 | 21.3 | 29.6 | 32.5 | 31.9 |
| 5 | *Penicillum sp.* $G_7$ | 13.2 | 22.8 | 31.2 | 38.6 | 45.9 | 48.3 | 40.7 |

From Table III it can be seen that these mould lipases can also synthesize esters which gives an indication that they might be tried for the study of the synthesis of fats.

The above mentioned observations prove that the mould lipases can be used for fat hydrolysis. The strains isolated from the moulds grown on other oil seeds will also be investigated for their lipolytic activity and if no other strain with better activity is found, these strains will be investigated further with the aim to grow them on a large scale and extract lipase from them in a pure form.

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SUMMARY

*A. flavus, A. oryzae, A. fumigatus, A. niger* and *Penicillium sp.* strains isolated from moulds grown on groundnut were investigated for their lipolytic activity.

RÉSUMÉ

Nous avons étudié des souches d'*A. flavus, A. oryzae, A. fumigatus, A. niger* et de *Penicillium sp.* isolées à partir de moisissures cultivées sur un milieu d'arachide en vue de leur activité lipolytique.

ZUSAMMENFASSUNG

Stämme von *A. flavus, A. oryzae, A. fumigatus, A. niger* und *Penicillium sp.*, welche aus auf Erdnuss gezüchteten Schimmeln isoliert worden waren, wurden auf ihre lipolytische Wirkung hin untersucht.

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