rated acids (Tsujimoto [9]) and of the ray *Dasyatis akejei* (Chinese fan fish) (Wang and Kan [12]), with 64% of saturated acids. The former has mainly palmitic and the latter mostly stearic acid.

It is worth noting that the *Carcharius gangeticus* shark belongs to the same family of Elasmobranch fishes as the ones whose liver oils have been studied by the present authors (*Carcharhinidae* family). The similar nature of all the three liver oils strongly suggests that such peculiar compositions are characteristic of this particular family of fish. Such specific effects connected with phylogenetic relationships are now very well recognized in the vegetable fats and also in the animal fats.

Lovern has pointed out in the case of eels (7) and of tunny (8) that there is a definite tendency towards the deposition of a less unsaturated fat at higher temperatures. As the present liver oils come from the fish of warmer, almost equatorial waters, their relatively saturated nature is explainable in the light of the above observations. Lovern has also visualized (5) simultaneous hydrogenation of polyethylenic and monoethylenic derivatives in the normal preformed fats of Tsujimoto's fourth group. It is evident that any final conclusions can be drawn only when a few more such reliable and detailed data are available.

Lovern has rightly remarked (6) that "it is unfortunate that out of the great range of Elasmobranch species there are only a few for which any quantitative fat analyses are available. Many qualitative and semi-quantitative investigations, largely by Japanese workers, have served to show that several distinct types of fat may occur in this group." The present analyses are probably the first detailed ones of an oil of this group, and the third illustration of this peculiar group.

Summary

1. Two samples of the liver oil of an Indian species of shark *(Galeocerdo rayneri)*, one from the Arabian Sea, and the other from the Bay of Bengal, have been studied. Their component acids are reported.

2. Tsujimoto's lithium salt acetone method has been adopted for the separation of highly unsaturated acid fraction from the mixed acids in one case while in the other the modified technique of Lovern has been followed. The insoluble acids have been further resolved into two fractions with the help of Hilditch's modified lead-salt alcohol method. The efficient column (E.H.P.) of Longenecker has been employed for fractionation in the present work.

3. The liver oils are found to belong to the fourth group of Tsujimoto's classification of the Elasmobranch fish liver oils. Shark liver oil No. 1 contains 40.9% saturated acids (palmitic 24.9%, stearic 11.1%, also myristic 3.3%, and minor proportions of lauric, arachidic, and lignoceric) and 59.1% unsaturated acids (C_{16} is 11.2%, C_{18} 19.6%, C_{20} and above 27.1%, also some C_{12} and C_{14} monoethenoids).

Shark liver oil No. 2 has the following composition: saturated acid 39.9% (palmitic 23.6%, stearic 14.5%, and myristic 1.5%, together with a minor amount of arachidic acid) and unsaturated acids 60.1% (mainly C_{16} 10.9%, C_{18} 23.3%, and C_{20} and above 25.7%; C_{14} acids are also present).

The abnormal saturated acid content is discussed. These analyses provide the third instance of this peculiar group of Elasmobranch liver oils.

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Studies on Lipolytic Molds.[1] Comparative Study of Lipases Obtained From Molds Grown on Oil Seeds

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CAMUS (1) found that lipase is present in *Aspergillus niger* and *penicillium glaucum*. Gerard (2) observed the presence of lipase in *penicillium glaucum* isolated from the vegetable kingdom. Wehmer (3) detected the presence of lipase in *Aspergillus* species. Haehn (4) had explored the possibility of fat synthesis by fungus and yeast enzymes. David Kirsh (5) had studied the different factors influencing the activity of fungus lipase. Fodor and Chari (6) studied in detail the activity of lipases present in *Aspergillus* and *Penicillium* species.

The literature just cited gives a clue that lipase can be extracted from certain strains of molds like *Aspergillus, penicillium,* etc. Ramakrishnan and Nevgi (7) investigated the various oil seeds, and Ramakrishnan and Banerjee (8) investigated the oil seedcakes for their lipolytic activity with a view to get a cheap and active lipase for fat hydrolysis. In continuation of their search for an active lipase they have investi-

[1] Read before the XIIth International Congress of Pure and Applied Chemistry, September, 1951, New York.

TABLE I

Enzymic Hydrolysis of Oil

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

pH	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank for lipase						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
3.6	7.2	8.1	5.8	3.7	4.5	3.2	2.1
4.8	8.9	9.5	6.1	4.1	5.0	4.0	2.8
5.2	12.1	11.2	7.2	4.9	5.9	4.5	3.2
6.2	15.3	13.8	9.6	5.8	6.2	5.1	3.8
6.8	11.8	10.7	10.9	7.5	5.5	4.6	4.3
7.2	9.5	9.2	9.8	6.2	5.0	4.1	3.7
7.4	8.3	8.5	8.5	5.8	4.5	3.6	3.2
7.6	7.2	6.9	7.2	4.9	4.1	3.1	2.8

gated a number of molds grown on oil seeds for their lipolytic activity, and the results are recorded.

Experimental

Isolation of molds grown on oil seeds. The molds were allowed to grow on the following oil seeds by keeping the oil seeds in different desiccators, adjusting the moisture content, and regulating the flow of air by the method of Ramakrishnan (15): castor seed *(Ricinus communis)*, peanut seed *(Arachis hypogea)*, seasame seed *(seasamum indicum)*, safflower seed *(carthamus tinctorum)*, Mysore oil seed *(guizotia abyssinica)*, cotton seed *(gossypium herbaceum)*, mustard seed *(Brassica nigra)*.

The molds grown on the oil seeds were sub-cultured in petri dishes in Czapek agar medium, and pure strains were prepared. Staining methods of Milward Bayliss, David Glick, and Robert Siem (9), as well as the biological method of Grabill and Reed (10), were used to detect the lipolytic molds from the cultures obtained. The lipase was prepared from the following best lipolytic strains isolated from the molds grown on different oil seeds: *Aspergillus niger* species strain A_1 from castor seed, *Aspergillus flavus* species strain A_2 from peanut source, *penicillium chrysogenum* species strain P_1 from seasame source, *Aspergillus oryzae* species strain A_3 from mustard source, yellow pigmented strain (unidentified) from safflower source, *Rhizopus* species strains R_1 and R_2 from Mysore seed and cotton seed source, respectively.

Preparation of the enzyme. The lipolytic strains obtained were grown in one-litre capacity, conical flasks containing 200 cc. sterilized liquid Czapek medium at 37°C., and after four days the mats were removed, washed well with sterile water, and then treated with low boiling petroleum ether to kill the cells. 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.

Effect of nature of the buffer, the substrate, and the enzyme on the activity of the lipase. First of all, the hydrolysis of freshly prepared peanut oil (Sap. value = 188.6; f.f.a. = .01%) was carried out, using these mold lipases and disodium phosphate-citric acid buffer (16) of varying pH.

Each set of the experiments consisted of 1 cc. of oil, 5 cc. of water, 2 cc. of phosphate buffer of varying pH, 0.1 gm. of mold lipase, and a few drops of toluene in a conical flask incubated for 24 hours at 37°C. A continuous shaking arrangement was provided throughout the experiment. Always blanks accompanied samples. Blanks consisted of all except enzymes. After the period of incubation the contents were taken out and titrated against N/10 NaOH after the addition of 25 cc. of neutral alcohol and warming for some time. Phenolphthalene was used as an indicator. Necessary precautions were taken to carry out the experiments under sterile conditions. The difference between the sample and the blank will give the activity of the lipase in terms of ccs. of N/10 NaOH. The results are given in Table I.

The activity of the lipases extracted from *A. niger* A_1 from castor source and *A. flavus* A_2 from peanut source are very active at optimum pH of 6.2. The optimum pH for the hydrolysis of peanut oil changes with the nature of the lipase.

The activities of the lipases extracted from *A. niger* A_1 and *A. flavus* A_2 were compared with that of the lipases extracted from castor and peanut seeds and their cakes by studying the hydrolysis of peanut oil, using these lipases and disodium phosphate-citric acid buffer. The results are given in Table II.

TABLE II

Enzymic Hydrolysis of Oil

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

pH	Hydrolysis in terms of difference in ccs. of N/10 NaOH between the sample and the blank for					
	Castor lipase	Castor cake lipase	Lipase from *A. niger* from castor source	Peanut seed lipase	Peanut cake lipase	Lipase from *A. flavus* from peanut source
3.2	5.8	3.2	6.2	4.3	2.9	6.0
3.6	6.3	3.9	7.2	5.3	4.0	8.1
4.8	12.2	3.2	8.9	7.2	4.9	9.5
5.2	7.8	3.0	12.1	11.8	4.5	11.2
6.2	4.2	2.5	15.3	3.9	3.8	13.8
6.8	3.9	2.3	11.8	3.4	3.6	10.7
7.2	3.3	2.1	9.5	2.1	2.5	9.2
7.4	3.0	1.7	8.3	1.9	3.0	8.5
7.6	2.7	0.8	7.2	1.7	0.5	6.9

From Table II it can be seen that the activities of the lipases extracted from *A. niger* A_1 and *A. flavus* A_2 are more than that obtained from the oil seeds and oil seed cakes. Hence the different factors which control the activities of these mold lipases were studied in detail.

The hydrolysis of different, freshly prepared vegetable oils were carried out, using these mold lipases and disodium phosphate-citric acid buffer. The re-

TABLE III

Enzymic Hydrolysis of Oil

Buffer: Disodium phosphate-citric acid.

Lipase: Lipase from *A. niger* isolated from castor source

pH	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank for						
	Castor oil f.f.a.= .03%	Peanut oil f.f.a.= .01%	Seasame oil f.f.a.= .02%	Safflower oil f.f.a.= .02%	Mysore seed oil f.f.a.= .02%	Mustard oil f.f.a.= .02%	Cotton-seed oil f.f.a.= .04%
3.6	7.5	7.2	6.8	5.0	3.2	3.0	2.5
4.8	9.1	8.9	8.0	5.7	3.7	3.2	2.9
5.2	12.8	12.1	9.5	6.1	4.1	3.8	3.2
6.2	15.9	15.3	10.1	7.8	4.8	4.2	3.8
6.8	12.0	11.8	12.1	6.6	5.2	4.9	3.4
7.2	10.8	9.5	10.0	5.8	4.7	4.2	3.0
7.4	9.1	8.3	8.5	5.1	4.2	3.6	2.7
7.6	6.5	7.2	6.2	4.2	3.6	3.1	2.2

sults are given in Table III. The hydrolysis of freshly prepared peanut oil was carried out, using these mold lipases and different buffer mixtures like disodium phosphate-citric acid, sodium phosphate-potassium phosphate and sodium hydroxide, and potassium phosphate buffer mixtures. The results are given in Tables I, IV, and V.

TABLE IV

Enzymic Hydrolysis of Oil

Buffer: Sodium phosphate-potassium phosphate. Oil: Peanut oil (f.f.a.=.01%)

pH	Hydrolysis after 24 hours in terms of differences in ccs. of N/10 NaOH between the sample and the blank for lipase from						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
5.28	4.6	3.2	2.7	2.2	1.9	2.1	1.6
5.90	5.8	4.0	3.0	2.5	2.7	2.6	2.0
6.46	7.2	5.1	3.3	2.8	3.0	2.9	2.3
6.97	6.1	4.6	3.8	3.1	2.5	2.5	2.7
7.16	5.7	3.8	3.4	2.7	2.1	2.0	2.2
7.38	5.0	3.1	2.9	2.3	1.7	1.6	1.8
7.73	4.1	2.7	2.6	1.9	1.4	1.4	1.3
8.04	3.8	2.2	2.0	1.5	1.1	1.0	0.8

TABLE V

Enzymic Hydrolysis of Oil

Buffer: Sodium hydroxide-potassium phosphate. Oil: Peanut oil (f.f.a.=.01%)

pH	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
6.0	5.8	4.2	2.6	2.1	1.9	1.6	1.3
6.2	6.4	5.9	3.0	2.5	2.3	1.9	1.7
6.4	8.5	6.2	3.2	2.8	2.6	2.2	2.1
6.6	6.1	5.4	3.8	3.2	3.0	2.8	2.5
6.8	5.7	5.0	4.2	3.6	3.6	3.2	2.8
7.0	4.8	4.2	5.1	4.2	3.2	2.9	3.0
7.2	4.1	3.8	3.5	3.2	2.8	2.5	2.6
7.4	2.9	3.1	2.9	2.5	2.2	2.0	1.8

From Table III it is found that the optimum pH of the mold lipase varies to a certian extent with the nature of the substrate used. From Tables I, IV, and V it is found that the optimum pH of the mold lipase varies to a certain extent with the nature of the buffer, and the maximum activity decreases in the order: disodium phosphate-citric acid, sodium phosphate-potassium phosphate, and sodium hydroxide-potassium phosphate. Lipases extracted from *A. niger* A_1 and *A. flavus* A_2 show maximum activities at an optimum pH of 6.2 in dosodium phosphate-citric acid buffer for the hydrolysis of freshly prepared peanut oil.

Effect of buffer concentration on the hydrolysis of peanut oil by mold lipases. The hydrolysis of peanut oil was carried out by changing the concentration of the buffer mixture and keeping the other factors constant. Disodium phosphate-citric acid buffer mixture of 6.2 pH was used. The amount of buffer added varied from 1 to 8 cc. The results are given in Table VI.

From Table VI it can be seen that the optimum buffer concentration for the hydrolysis of peanut oil by mold lipases is 2 cc.

Effect of substrate concentration on the hydrolysis of peanut oil by mold lipases. The hydrolysis of peanut oil was carried out, using the mold lipases by changing the concentration of the oil and keeping other factors constant. Experiments were carried out using varying quantities of the substrates. Disodium phosphate-citric acid buffer mixture of pH 6.2 was used. The results are given in Table VII.

TABLE VI

Effect of Buffer Concentration on the Hydrolysis of Peanut Oil by Different Mold Lipases

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

Buffer added in ccs.	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
1.0	9.8	10.5	7.5	4.9	4.5	3.8	2.2
2.0	15.3	13.8	9.6	5.8	6.2	5.1	3.8
3.0	12.3	10.9	8.3	4.8	5.1	3.9	2.5
4.0	10.8	9.2	7.8	4.2	4.2	3.2	2.2
5.0	9.5	8.3	7.2	3.7	3.6	2.9	2.0
6.0	8.2	7.1	6.1	3.3	3.2	2.6	1.7
7.0	5.6	6.5	5.8	2.8	2.9	2.2	1.4
8.0	4.3	3.9	3.2	2.1	2.0	1.9	1.2

From Table VII it can be seen that the maximum hydrolysis of the oil is obtained when the concentration of the substance is 1-2 cc., depending upon the nature of the lipase.

Effect of enzyme concentration on the hydrolysis of peanut oil by mold lipases. The next factor is the effect of enzyme concentration on the hydrolysis of peanut oil by mold lipases. Experiments were carried out, using varying quantities of lipase and keeping all other factors constant. The results are given in Table VIII.

TABLE VII

Effect of Substrate Concentration on the Hydrolysis of Oil by Different Mold Lipases

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from						
A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
15.3	13.8	8.6	5.8	6.2	5.1	3.8
16.8	15.2	8.5	6.1	5.4	4.8	4.2
15.2	13.8	7.2	5.9	5.0	3.7	3.8
12.8	10.9	6.8	5.2	4.7	3.2	3.0
10.1	8.6	6.2	4.8	4.1	2.9	2.5
9.5	7.2	5.9	4.3	3.8	2.5	2.1
8.2	6.1	5.2	3.9	3.2	2.1	1.8
6.1	5.4	4.8	3.1	2.8	1.8	1.3

From Table VIII it is seen that the percentage hydrolysis of peanut oil goes on slowly increasing as the concentration of the enzyme increases.

TABLE VIII

Effect of Enzyme Concentration on the Hydrolysis of Oil by Different Mold Lipases

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

Enzyme in gms.	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
0.1	15.3	13.8	9.6	5.8	6.2	5.1	3.8
0.2	16.2	13.9	10.1	6.1	6.8	6.1	4.1
0.3	16.8	15.2	11.2	7.2	7.5	6.6	5.2
0.4	17.1	15.8	11.8	8.3	8.6	7.1	5.8
0.5	17.3	16.5	12.3	9.5	10.1	7.5	6.2
0.6	18.5	17.1	13.1	10.6	11.2	7.9	6.5
0.7	19.1	18.2	14.2	11.8	11.5	8.2	7.2

Effect of temperature on the hydrolysis of peanut oil by mold lipases. The next factor studied is the effect of temperature on the hydrolysis of peanut oil by mold lipases. Different sets of experiments were carried out by keeping the contents in the incubator at different temperatures for a fixed time and then titrating against N/10 sodium hydroxide. The hydrolysis of the peanut oil was carried out by keeping the contents in the incubator for two hours at temperatures 28°C., 30°C., 35°C., 37°C., 40°C., 75°C., and 100°C. The results are given in Table IX.

TABLE IX

Effect of Temperature on the Hydrolysis of Oil by Different Mold Lipases

Buffer: Disodium phosphate-citric acid. Oil: Peanut oil (f.f.a.=.01%)

Temperature in °C.	Hydrolysis after 2 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from						
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1	*Rhizopus* R_1	*Rhizopus* R_2
28.0	10.1	8.2	7.3	4.8	4.5	4.1	2.2
30.0	13.8	10.9	9.5	5.2	5.1	4.9	2.7
35.0	14.2	12.1	10.1	6.3	5.8	5.8	3.1
37.0	15.3	13.8	9.6	5.8	6.2	5.1	3.8
40.0	13.2	11.6	5.2	5.2	4.8	3.2	3.0
75.0	2.2	2.1	1.1	0.9	0.5	0.4	0.4
100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

From Table IX it can be seen that the optimum temperature for the hydrolysis of peanut oil by mold lipases is 35-37°C., depending upon the nature of the lipase.

Effect of some salts and other organic substances on the enzymic hydrolysis of peanut oil by mold lipases. The action of the following substances on the hydrolysis of peanut oil by mold lipases was studied: albumin, sodium taurocholate, gum arabic, ascorbic acid, citric acid, acetic acid, strychnine chloride and sulphate glycine, sodium acetate, sodium chloride, sodium nitrate, ammonium sulphate, manganese sulphate, sodium phosphate, potassium phosphate, calcium chloride, and hydrochloric acid.

Each set of the experiments consisted of 1 cc. of peanut oil, 5 cc. of water, 2 cc. of phosphate buffer of 6.2 pH, 0.1 gm. of mold lipase, and 0.1 gm. of the substance (or 1 cc. of N/10 solution in case of hydrochloric acid and acetic acid) incubated at 37°C. for 24 hours. Blanks accompanied samples. After the period of incubation the contents were titrated against N/10 NaOH after the addition of 25 cc. of neutral alcohol and warming for some time. The difference between the sample and the blank will give the activity of the lipase in terms of ccs. of N/10 NaOH. The difference shows whether the particular substance is an accelerator or retarder, depending upon whether the difference is positive (showing the accelerating effect) or negative (showing the retarding effect). The results are given in Table X.

TABLE X

Effect of Some Salts and Certain Organic Substances on the Hydrolysis of Peanut Oil by Different Lipases

Name of additive	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank in case of lipase from			
	A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3
Albumin	+0.2	+0.8	+0.2	+0.3
Sodium taurocholate	+3.2	+4.9	+1.8	+2.1
Gum arabic	+1.2	+0.8	+0.5	+0.8
Ascorbic acid	+1.5	+0.5	+0.4	+0.6
Citric acid	+2.8	+0.3	+0.4	+1.2
Acetic acid	+2.3	+1.3	+1.0	+2.3
Strychnine chloride	−2.5	−1.2	−0.8	−0.2
Strychnine sulphate	+0.3	−0.9	−0.5	+0.1
Glycine	−1.0	+1.4	+1.2	+1.3
Sodium acetate	+2.2	+2.4	+1.8	+1.6
NaCl	+1.8	+1.2	+0.7	+1.2
$NaNO_3$	−2.1	−1.3	−0.5	−0.8
$(NH_4)_2SO_4$	+1.2	+1.7	+1.2	+1.8
$MnSO_4$	+2.3	+2.5	+2.2	+1.5
Na_2HPO_4	+1.4	+1.2	+1.8	+1.2
KH_2PO_4	+2.3	+4.8	+2.5	+2.2
$CaCl_2$	−1.2	+0.7	+0.5	−0.5
HCl	+2.6	+1.6	+1.2	+1.2

From Table X it can be seen that sodium taurocholate, sodium acetate, citric acid, acetic acid, manganese sulphate, and KH_2PO_4 act as good accelerators.

Comparison between different preparations of mold lipase as regards their activity. It was found that the mold lipases lose their activity after some time. Hence different preparations were made and their activities studied at different intervals in order to see whether a stable lipase can be obtained.

Three samples of lipase were prepared by draining for two hours: one with low boiling petroleum ether, the other with acetone, and the third with the mixture of acetone and low boiling petroleum ether. All the samples were completely dried, powdered, and sieved through a 60-mesh sieve before being used for the experiment. The hydrolysis of peanut oil was carried out at different intervals of time, using each sample. The results are given in Table XI.

TABLE XI

Comparison Between Different Preparations of Lipase

Buffer: Disodium phosphate-citric acid.

Oil: Peanut oil (f.f.a.=.01%)
Lipase: Lipase from *A. niger*

Time of aging	Hydrolysis after 2 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank for		
	Petroleum ether dried sample	Petroleum ether and acetone mixture dried sample	Acetone dried sample
0 minute	15.3	12.8	6.2
24 hours	16.1	12.9	6.2
2 days	16.8	13.1	6.4
4 days	17.0	13.2	6.5
8 days	16.5	12.8	6.1
12 days	16.0	10.1	6.1
15 days	14.8	8.6	5.8
23 days	12.1	8.2	5.8
45 days	8.8	8.0	5.8
55 days	8.7	7.5	5.7
65 days	7.2	6.2	5.7
75 days	7.0	5.8	5.7
85 days	6.5	5.2	5.5
95 days	6.1	4.7	5.5
105 days	5.8	4.2	5.4
115 days	5.1	3.7	5.3
125 days	4.6	3.1	5.3

From Table XI it can be seen that in all cases the activity increases up to the fourth day and then slowly decreases. In the case of the acetone-dried sample the change in activity with time is the least even though the activity is low. Hence acetone-dried sample can be used as its activity does not change much with time.

Comparison of the synthetic activity of the lipolytic molds with that of oil seed and oil seed cake lipases.

TABLE XII

Synthesis of Butyl Oleate by Mold Lipases

Solvent: Ethyl ether

Name of the Lipase	Percentage synthesis on						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Castor seed lipase	16.5	29.7	40.2	50.7	52.5	62.7	60.3
Castor cake lipase	8.5	10.2	15.8	17.5	28.2	25.7	......
Lipase from *A. niger* isolated from castor source	17.2	28.9	41.6	49.8	52.3	65.8	61.3
Peanut seed lipase	15.9	32.8	49.5	56.2	61.8	58.5	......
Peanut cake lipase	7.2	9.9	14.5	16.2	23.8	26.3	14.8
Lipase from *A. flavus* isolated from peanut source	15.4	30.7	45.8	52.3	60.8	63.4	60.9

The acetone-dried lipase was prepared according to the method of Ramakrishnan and Nevgi (11). The synthesis of butyl oleate was carried out, using these lipases.

In different conical flasks, equimolecular quantities (0.054 gm. mol.) of butyl alcohol [$CH_3(CH_2)_2CH_2OH$ — B.P. 117°C., d = 0.809 gm./cc. mol. wt. 74.1] and oleic acid [$C_8H_{17}CH = CH(CH_2)_7COOH$ B.P. 286°C., d = 0.895 gm./cc. mol. wt. 282.4] were added. The lipase (1 gm.) and ether solvent (10 cc.) were added to each flask, shaken well, and kept in the incubator at 37°C. after corking well. At different intervals of time 1 cc. from each flask was taken, 25 cc. of neutral alcohol added, warmed for some time, and titrated against N/10 sodium hydroxide. Always blanks accompanied samples. The difference between the blank and the sample was calculated in terms of ccs. of N/10 sodium hydroxide. From this the percentage synthesis was calculated. The results are given in Table XII.

From Table XII it can be seen that the mold lipases can also synthesize esters, and the synthetic activity of the mold lipase is greater than that of seed or cake lipase.

Vitamin contents of lipolytic molds. Srinivasan and Ramakrishnan (12) have found that the molds grown on oil seeds contain water-soluble vitamins like thiamine, ascorbic acid, etc. The vitamin contents of the lipolytic molds were determined just to see whether there is any relation between the vitamins present and the lipolytic activity of the molds. The thiamine content of the molds was found according to the method of Clausen and Brown (13) and the ascorbic acid content of the molds according to the method of Gawron and Berg (14). The results are given in Table XIII.

TABLE XIII

Vitamin Contents of Lipolytic Molds

Name of the lipase	Lipolytic activity in terms of difference in ccs. of N/10 NaOH between sample and the blank	Amount of thiamine present microgram /gm. of mat	Amount of ascorbic acid present mgm./gm. of mat
A. niger A_1	15.3	1.212	0.180
A. flavus A_2	13.8	5,395	0.200
Penicillin chrysogenum P_1	9.6	0.2676	0.120
A. oryzae A_3	5.8	2.752	0.183
Yellow mold Y_1	6.2	0.1345	0.085

From Table XIII it is found that, in the case of *A. flavus,* lipase activity as well as the vitamin contents are high. But, in general, there could not be found any definite relation between the vitamin contents and the lipase activity of the molds.

Effect of different media on the activity of mold lipase. It has been found that the mold lipase is more active than seed or cake lipase. An attempt was made to find a cheap medium in which to grow the lipolytic molds on a large scale.

In different conical flasks of one-litre capacity 250 cc. of peanut cake medium containing 20% oil-free cake and different amounts of fresh peanut oil were taken, autoclaved for 15 minutes under 15-lb. pressure, and inoculated with the strains. They were incubated at 37°C., and after a week the mats were removed and lipases prepared from them. The hydrolysis of peanut oil was carried out, using these lipases and disodium phosphate-citric acid buffer mixture of pH 6.2. The results are given in Table XIV.

TABLE XIV

Effect of Different Media on the Activity of Mold Lipase

Medium	Growth	Hydrolysis after 24 hours in terms of difference in ccs. of N/10 NaOH between the sample and the blank for lipase from				
		A. niger A_1	*A. flavus* A_2	*P. chrysogenum* P_1	*A. oryzae* A_3	Yellow mold Y_1
Czapek liquid medium	Moderate	15.3	13.8	9.6	5.8	6.2
Peanut cake medium	Fair	11.3	10.9	8.7	5.1	6.1
+ 5% peanut oil	Good	14.8	12.6	10.9	6.5	8.2
+10% peanut oil	Very good	22.6	20.2	16.1	12.4	13.9
+15% peanut oil	Fair	15.3	14.1	10.3	6.2	6.7

From Table XIV it can be seen that the lipolytic molds grow well in a peanut cake medium containing 10% oil, and the activity of the lipase extracted from the mat in this case is also more than that in other cases. It seems possible that the cake medium, if investigated further, may prove to be a very cheap source in which to grow the lipolytic molds on a large scale. The work is under progress to study the different factors like Hion concentration of the cake medium, moisture content, temperature, effect of different salts, etc., which control the growth of lipolytic molds in a cake medium.

Summary

A. niger and *A. flavus* isolated from oil seed source show an appreciable amount of lipolytic activity.

The optimum pH of the mold lipase varies to a certain extent with the nature of the substrate and buffer used.

In the experiments carried out the optimum buffer concentration is 2 cc., the optimum substrate concentration is 1-2 cc., and the optimum temperature is 35-37°C. for hydrolysis of peanut oil by mold lipases.

Sodium taurocholate, manganese sulphate, KH_2PO_4, sodium acetate, etc., accelerate the activity of the mold lipase.

The change in activity of acetone-dried sample of mold lipase with time is negligible.

The synthetic activity of the mold lipase is higher than that of seed and cake lipases.

A definite relation between the vitamin contents and the lipolytic activity of the molds could not be found.

Peanut cake medium appears to be a good source to grow the lipolytic molds on a large scale.

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Fatty Acid Composition of Hydrogenated Linseed Oil

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THE development in recent years in the fields of spectrophotometric analysis has led to improved methods for the determination of linoleic and linolenic acids in fats. Applying the method of Mitchell, Kraybill, and Zscheile (1), a number of investigators have examined the catalytic hydrogenation of unsaturated oils.

On investigating the composition of hydrogenated linseed oil, Lemon (2) found an isomer of linoleic acid which did not undergo conjugation upon treatment with alkali. This acid was believed to be 9,15-octadecadienoic acid and assumed to be produced by the selective hydrogenation of the central double bond of linolenic acid. Subsequently Mattil (3) and Filer (4) ascertained the presence of 9,15-octadecadienoic acid in hydrogenated soybean oil. The acid is named isolinoleic acid. Fischer, O'Connor, and Dollear (5) stated that iso-linoleic acid is not produced during hydrogenation of cottonseed and peanut oils. Bailey and Fischer (6) reported the relative reactivities toward hydrogenation of oleic, iso-linoleic, linoleic, and linolenic acids. They found iso-linoleic acid to be hydrogenated only about three times as fast as oleic acid whereas linoleic and linolenic acid are hydrogenated respectively 20 and 40 times as fast as oleic acid. Later Bailey (7) made a more complete analysis of the reaction kinetics through a mathematical approach. The reactions occurring in the hydrogenation of linseed oil are shown diagrammatically in Figure 1.

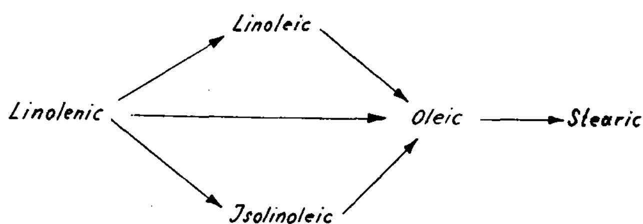

FIG. 1.

Rebello and Daubert (8) hydrogenated methyl linolenate and isolated a fraction rich in methyl isolinoleate which was found to be a mixture of at least three isomers, the 8,14-, 9,15-, and 10,14-isolinoleic acids. Thompson (9) hydrogenated a mixture of 50% tung oil and 50% linseed oil. Reactivity ratios for the various fatty acids were calculated, and it was found that during the initial stage of hydrogenation the elaostearin was about 20 times more reactive than normal linolenin.

Our present investigation will prove that during hydrogenation of linseed oil comparatively great amounts of conjugated linoleic acid are formed. The reactions pictured in Figure 1 are apparently only part of those involved, and in our opinion the hydrogenation of linolenic acid is more likely to proceed as shown in Figure 2.

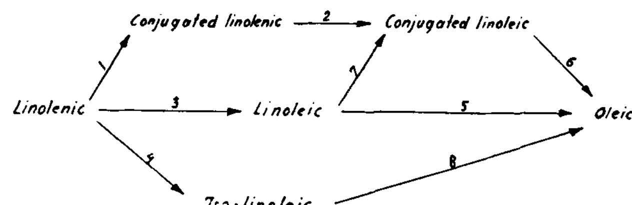

FIG. 2.

The Isomerization Effect of Ni-catalyst. Among several catalysts reported to be effective in conjugating unsaturated fatty acids, nickel catalysts are well known. Radlove, Teeter, Bond, Cowan, and Kass (10) examined the conjugation effect of nickel catalysts upon soybean oil and linseed oil and found that about 35% of the di- and tri-unsaturated acids were converted to conjugated forms. Mattil (3) observed a slight conjugation when heating soybean oil with a nickel catalyst at 200°C. Blekkingh (11) reports that a nickel hydrogenation catalyst in contact with hydrogen has the property of shifting the double bonds in unsaturated fatty acids.

In investigations regarding the kinetics of the conjugation effect of nickel catalysts the temperature is found to have a very great influence on the reaction rate of the isomerization (10). The present investigation is concerned with catalytic hydrogenations at dif-