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Research Article

Production and characterization of alkaline lipase from *Aspergillus awamori*

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Abstract

The present study has been carried out to find out the lipase enzyme production by the fungus, *Aspergillus awamori* . In commonly available natural waste such as, Caster oil cake, Gingilly oil cake and groundnut oil cake collected from oil lotteries. The oil cake materials were diluted in the proportion of 11 grams of cakes dissolved in 100ml of water for the experimental work. Among the three natural waste employed, the groundnut oil extract showed highest lipase activity at 48hrs of incubation. Hence, groundnut oil waste was then preferred for further studies to design the production medium by supplementing different carbon, nitrogen, micro and macro nutrients, inducer compounds and also by substituting different with all these combination of gum Arabic with olive oil was also supplemented. The experiments were also carried out at different pH (2-9) and temperature (0ºC-60 ºC) levels. The interesting observation in this line revealed that, in Groundnut oil when different above-mentioned sources employed, the nitrogenous source like peptone at 2.5% level, at 7.0pH under 38 ºC and at 48 hours of incubation exhibited maximum lipase production using the fungus *Aspergillus awamori* . When the enzyme activity is analyzed or its stability at different pH (6.3-9.3) and temperature (0ºC-60 ºC), using Groundnut oil extract was done in the initial phase of study, showed maximum stability at pH 7.3 and 40ºC respectively. The above results are discussed with relevant earlier literature.

Keywords: Lipase enzyme, *Aspergillus awamori,* natural waste, Groundnut oil.

Introduction

Lipases are widely distributed among yeast, fungi and bacteria. Short chain triglycerides are also very good substrates for lipases. Enzymes such as proteases and amylases have dominated the world market owing to their hydrolytic reactions for proteins and carbohydrates. However with the realization of the biocatalytic potential of microbial lipases in both aqueous and non-aqueous media in the last one and a half decades, industrial fronts have shifted towards utilizing these enzymes for a variety for reactions of immense importance. It is in the last decade that lipases have gained importance to a certain extent over proteases and amylases, especially in the area of organic synthesis. The regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesize of cocoa butter constituents, biofuels and for synthesize of personal care products and flavor enhancers. Thus lipases are today the enzyme choice for organic chemist, pharmacists, biophysicists, biochemical and process engineers, biotechnologist, microbiologist and biochemist.

Fungal lipases

Fungal lipases have been studied since 1950s. Lawrence (1967) Brockerhoff and Jenson (1974) have presented its comprehensive reviews. These lipases are being exploited due to their low cost of extractions, thermal and pH stability, substrate specificity, and activity in organic solvents. The chief producers of commercial lipases are *Candida cylindracea, Humicola lanuginose, R.delemar, R.japonicus, R.niveus and R.ryzae* (Godfredson, 1990).

Lipases have wide applications as a good substitute for classical organic techniques in the selective transformation of complex molecules. The employment of lipases in the above reactions can reduce side reactions and easy the separation of molecules (Ashokpandey *et al*., 1999). In the present day industry, lipases have made their potential realized owing to their involvement in various industrial reactions either in aqueous or organic systems, depending on their specificity (John and Abraham, 1990; Kotting and Kibl, 1994).

Lipases have applications as industrial catalysts for the resolution of racemic alcohols in the preparation of some prostaglandin's, steroids and carboxylic nucleoside analogues. Lipases from *Aspergillus terreus* show chemo and regiospecificity in the hydrolysis of per acetates of pharmaceutically important polyphenolic compounds (Parmar *et al.,* 1998).

Lipases are widely distributed among bacteria, yeast and fungi. Short chain triglycerides are also very good substrates for lipases. The lipases of the following microorganisms have been recently isolated in an apparently homogenous form and some of them have been crystallized. They include *Aspergillus niger, Candida cylindracea, Mucor javaniscus , Pseudomonas fragi* and *Rhizopus arrhizus*. Several purified microbial lipases have shown to be glycoprotein.

Keeping in mind the importance of industrial application of fungal lipases, the present study has been undertaken with the following objectives

To estimate the lipase enzyme production from the *Aspergillus awamori* Using different natural wastes by introducing changes in

A) pH
B) Temperature
C) Carbon sources
D) Organic and inorganic nitrogen sources
E) Micro and macro nutrients level
F) Inoculum level and
G) Inducers level

- To estimate the enzyme production and stability with this fungus in lab conditions and
- Estimation of enzyme according to the growth of that fungus.

Materials and Methods

Isolation of fungi from soil sample (Kamini *et al*., 1997)

In order to initiate the proposed study, in the first phase, different fungi isolates were obtained from the soil sample by administering serial dilution technique. The selective medium for fungi, Czepak Dox and Rose Bengal Agar medium was used to isolate the fungi using 10^{-2}-10^{-7} dilutions, and poured into sterile petriplates. After plating, plates were incubated for 72 hours in room temperature at 28ºC. (Plate 1 a,b & Plate 2).

Screening of lipase producing fungi (Kamini *et al*., 1997)

To screen the lipase producing from that of the isolates obtained in Czepadox agar plates, the colonies from these agar plates were then transferred to Tribytyrin agar, of which notably species *Aspergillus awamori* and another fungus with remarkable morphological feature showed clear zone formation around the colonies to ensure with their ability

Preparation of inoculation medium (Prabhakar *et al*., 2002)

In order to prepare the inoculam, the four days incubated fungal strain was taken. To these, 5ml sterile water was added; developed growth was scrapped with needle and transferred into 95 ml inoculation medium (simple nutrient broth) taken in 500ml Erlenmeyer flasks. These flasks were then incubated at 28ºC on rotary shaker at150rpm for 72 hrs. At this stage, 10% of inoculam was transferred to the production medium incubated at 28ºC on rotary shaker at 150rpm for 72hrs.

Preparation of production medium (Benjamin and Pandey, 1996)

Cheaply available natural waste such as Groundnut, Gingilly, Caster wastes, were procured from a local oil mill, soaked in 50ml (5.5g waste) distilled water and autoclaved at 121ºC for 1 hr (Plate 5). After cooling, the liquid content of the cake was squeezed out using a muslin cloth by a hand mill. This extract was used as the raw substrate and was designated as medium M1.

Different supplementations were made in the Groundnut oil to design the production media as given below. In all cases, the initial pH of the medium was maintained at 7.0.

Table: 1 Enzyme production media

Medium	Composition
M1	Ground nut waste extract
M2	M1+different carbon sources (1%)(CMC, Starch, Sucrose, Fructose, Glucose, Mannitol, Lactose, Glycerol) pH7
M3	M1+different organic/ inorganic nitrogen sources (1%)(Urea, Beef extract, Malt extract, Peptone, Yeast extract, Sodium nitrate, Ammonium nitrate, Ammonium chloride, Tryptone).
M4	M1+Various salts (1%)pH 7.0 (KCl, $MgSO_4$, $CaCl_2$, $ZnSO_4$, $MnSO_4$, $FeSO_4$, $MgCl_2$, $CuSO_4$
M5	M1+different inducers (1ml) (Coconut oil, Neem oil, Caster oil, Palm oil, sunflower oil, Groundnut oil, Gingillic oil, Olive oil)
M6	M1+ Inoculum concentrations (1%, 1.5%, 2%, 2.5% and 3.0%)
M7	M1+Olive oil (10%)+Gum Arabic (0.2-1%)
M8	M1+Different temperature (0ºC, 28ºC, 38ºC, 48ºC, 58ºC,68ºC)
M9	M1+ Different pH (2, 3, 4, 5, 6, 7, 8, 9)

Fermentation process (Shake – flask culture method):

After preparation of the production media 10% of inoculum was transferred to the production medium and incubated at 28ºC on a rotary shaker at 150rpm for 72hours.the flasks were removed at 24hr intervals, the mycelium was separated by filtration and filtrate was used for the enzyme assay.

Enzyme assay

Spectroscopy assay method (Plate .6)
Lipase activity was assayed by the modified method Safarik (1999).

Determination of lipase activity:

For this, an aliquot of olive oil (20mg) was transferred into a test tube containing 2ml of phosphate buffer (pH 6.3) and 1ml of the sample was added to it. The mixture was vortexes for 15sec and incubated at 37ºC in water bath under static conditions for 30min. after stopping the reaction by adding 1ml of concentrated HCl and vortexing for 10sec, 3ml of benzene was added and after further vortexing for 90sec, the aqueous and organic phases were allowed to separate. From this 2ml benzene layer was withdrawn and transferred to a test tube containing 1ml aqueous solution of cupric acetate (5% concentration, pH adjusted to 6.2 using pyridine) and the mixture after vortexing for 90sec, was centrifuged at 5000rpm for 10 minutes at 5C to obtain a clear organic phase. The organic (Benzene) layer was removed and used to estimate the liberated free fatty acids by measuring the optical density (OD) against distilled water at 715nm using a spectrophotometer. One unit of lipase activity is equivalent to one µM free fatty acid liberated min^{-1} ml^{-1}, under the assay conditions and expressed μg^{-1} dry substrate (ds).

Estimation of protein (Lawry's method, 1951)

To 1ml of inoculated sample was taken and centrifuged at 10,000rpm for 20 minutes and supernatant was collected. To 0.1 ml of sample, 1.4 ml of 0.1N sodium hydroxide, 4.5ml of alkaline copper reagent were added and was allowed to stand for ten minutes. After incubation, 0.5ml of diluted folin's reagent was added to tubes with continuous shaking and allowed to stand for 30 minutes. The colour developed was read at 660nm. The amount of protein was expressed as mg/g. (Plate -7)

Estimation of glucosamine (Sakurai *et al*., 1977)

The sample of varying concentration was taken for analysis. The solution were treated with 1ml of freshly prepared 2% acetyl acetone in 0.5M sodium carbonate in capped tubes and kept in boiling water bath for 15 minutes. After cooling in tap water, 5ml of 95% ethanol and 1ml of Ehrlich's reagent were added and mixed thoroughly. The purple red colour developed was read after 30 minutes at 530nm (Plate .8).

Estimation of total soluble sugar (Dubois, 1952)

2ml of the inoculation medium was taken and centrifuged. 1ml of the supernatant sample was taken in different test tubes. To each tube 4ml of the anthrone reagent was added along the side of the test tubes. A glass marble was placed on top of each tube

to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 minutes. The tubes were then removed and cooled to room temperature in a water bath. A reagent blank was treated similarly. And the absorbency of the blue green solution was red at 625nm. The amount of sugars present in sample was calculated using standard curve prepared from glucose (Plate .9).

pH stability of the enzyme

The optimum pH of the enzyme was determined was using different pH buffer (i. e) using acetete (pH 4.6-5.5), Tris acetate (pH 6.0-7.0), Tris HCl (pH7.5-9.0) and Borate buffer (pH 9.0-15). The purified buffers was incubated in these 30 minutes, 60 minutes, 90 minutes and 120 minutes at 37ºC and then assayed for the residual activity for determining its pH stability.

Inoculated medium as centrifuged at 5000rpm and supernatant enzyme fluid were only taken. To the enzyme 1ml of 250mg Olive oil was to that different pH buffers were added in a series of test tubes and kept at 37ºC for 30 minutes the lipase producing strain were tested.

Thermal stability of the enzyme:

To determine the thermal stability of the lipase enzyme. The medium was centrifuged at 5000rpm and supernatant enzyme fluid was only taken. To the enzyme 1ml of 250mg olive oil was added and 2ml of Tris acetate buffer was added to make the pH 7. These preparation were taken in a series of test tubes and incubated at different temperature, from 30ºC to 90ºC and the incubation temperature various from 30minutes, 60minutes, 90 minutes and 120minutes etc. This test was done in lipase producing strain and the thermal stability of the enzyme was noted.

Growth curve

Groundnut oil waste extract was prepared in the medium. The fungal organism *Mucor* sp. was inoculated and kept in shaker at 28C. After 12 hours intervals O.D was measured at 600nm using spectrophotometer. The O.D value was measured up to 600nm using spectrophotometer. The O.D value was measured up to 72hours. Lipase activity was also analyzed in this sample.

Results and Discussion

Plate assay

The fungal strain isolated from soil samples in our laboratory was identified as *Aspergillus awamori* (Plate .4). Tributyrin was used as substrate for examining lipase production on Czapak dox agar plates. A halo zone of 5mm diameter around colony in the Tributyrin agar plates clearly indicated the production of lipase

In this study, the lipase production by the fungus *Aspergillus awamori* was studied using cheap common natural waste (Oil cake) like Gingilly, Caster and Groundnut oil. The lipase activity was determined at 24h, 48h, and 72h intervals, the lipase activity increased up to 48h, after which it decreased (Table-2 & Fig-2)

Table-2 shows the fermentation behavior of *Aspergillus awamori* in Groundnut extract by shake flask culture. The lipase activity at 24h, 48h, and 72h were10.43U/ml^{-1}, 12.23U/ml^{-1}, 09.0U/ml^{-1} respectively. Among the three durations, at 48h optimum lipase production was observed. The respective soluble sugar consumption at this stage was 8.05mg/ml, 09.53mg/ml, and 10.40mg/ml, as against the soluble protein content 20.70 mg/ml, 23.00 mg/ml, and 18.60 mg/ml, for 24 h, 48h and 72h durations.

There was very slight variation in the pH of media, which were 7.2, 7.3, and 7.4, at 24h, 48h, and 72h duration respectively. In view of high product titers in groundnut oil extract, further experiments were carried out with groundnut oil extract.

In order to determine the suitable carbon, nitrogen, inducers, salts etc, for optimum lipase production by *Aspergillus awamori* Various nutrient sources like carbon, nitrogen, inducers, salts, phosphate and Gum Arabic were added individually to groundnut oil extract. More over the lipase activity has been checked at various temperature and pH level.

Effect of various carbon sources in Groundnut oil extract on lipase production by *Aspergillus awamori* **in shake flask culture**

Several workers who use different carbon sources have reported an increased yield of alkaline lipase. In

order to determine the suitable carbon source for maximum lipase production, different carbon sources were incorporated into the medium by replacing glucose. Maximum lipase production was observed with sucrose 26.06U/ml^{-1} and minimum with fructose 9.86 U/ml^{-1} (Table-3 & Fig-3). The results were in accordance with that observed for *Rhizopus oryzae* by Pal and Kundu, (1978); Salleh *et al*., (1993) and Kamini *et al*. (1997). These authors have reported that maximum lipase production in *Rhizopus* species occurred when sucrose was used as the carbon source.

Effect of various Nitrogen sources in Groundnut oil on lipase production in *Aspergillus awamori* **in shake flask culture**

To determine the suitable nitrogen source for maximum lipase production, different nitrogen sources were incorporated in to the medium, maximum lipase production (24.46U/ml^{-1}) was observed when peptone was used as the nitrogen source and minimum with ammonium nitrate (12.80 U/ml^{-1}), (Table-4 & Fig-4) Similar reports were obtained from the study of lipase production by *Candida rugosa* on copra waste carried out by Benjamin and Pandey, (1996).

Effect of various inducers in groundnut oil on lipase production by *Aspergillus awamori* **, in shake flask culture**

The lipase production by *Aspergillus awamori* was studied in Groundnut oil by adding various inducers like coconut oil, neem oil, caster oil, Gingilly oil, Groundnut oil, palm oil, sunflower oil, and obtained data's confirmed that among the various types of inducers, olive oil was found to be most effective in inducing the lipase of *Aspergillus awamori* . Maximum lipase production was observed in olive oil 24.36U/ml^{-1} and minimum lipase production was observed in coconut oil 7.10U/ml^{-1} (Table-5 & Fig-5).
It was found that maximum lipase production occurred at 2.5ml (Table-8 & Fig-8). Similar results have been reported by Akhtar *et al*., (1980) for *Mucor heimalis* and *Aspergillus niger* by Kamini *et al*., (1997).

Effect of various salts in groundnut oil on lipase production by *Aspergillus awamori* **in shake flask culture**

In order to determine the suitable salts for maximum lipase production, different salts were incorporated into the medium. Maximum lipase production was observed with Ferrous Sulphate (Table- 6& Figure-6).

Effect of various inoculum concentrations in Groundnut oil on lipase production by *Aspergillus awamori* **in shake culture**

The inoculum levels were also checked with various concenations to find out the correlation between inoculum level and lipase activity. Various inoculum levels (1% to 3%) were taken and tested. 10% inoculum promoted the higher rate of lipase yield (37.66U/ml^{-1}) (Table-7). Similar results have been reported by Prabhakar *et al*. (2002), who obtained maximum lipase production with 10% level inoculum.

Effect of Gum Arabic in Groundnut oil on lipase production by *Aspergillus awamori* **in shake flask culture**

The results of the studies on the effect of supplementation of Gum Arabic at different concentration (0.2%, 0.4%, 0.6%, 0.8%, and 1%) in the presence of groundnut oil is presented in (Table-8 & Figure-8). As evident from the table, among different concentration of Gum Arabic, 0.4% was the best for inducing enzyme production. The effect of Gum Arabic could raise lipase yield to 23.51U/ml^{-1}. Similarly Benjamin and Pandey, (1996) reported the gum Arabic with 0.4% concentration as a favourable inducer of lipase production
.

Effect of various pH in Groundnut oil on lipase production by *Aspergillus awamori* **in shake flask culture**

In order to determine the optimum pH for maximum lipase production, the organism was inoculated in media of varying pH. The enzyme functioned optimally at pH 7 after 48 h when compared with other pH values in waste extract (Table-9 & Figure-9), Kamini *et al*., (1997) studied the correlation between pH and lipase production and confirmed that pH 7 is favourable maximum lipase production. Similar reports have been reported by Omar et al., (1987) for *Mucor hiemalis.*

Effect of various temperatures in Groundnut oil on lipase production by *Aspergillus awamori* in shake flask culture

Optimum temperature for maximum lipase production was found to be 38ºC (Table-10 & Figure-10). The

Table -2 Fermentation behaviour of *Aspergillus awamori* on Groundnut oil extract

Sl.No	Time (hours)	Lipase activity (mg/ml)	Soluble sugar (mg/ml)	Soluble protein (mg/ml)	pH
1	24	10.43	08.05	20.70	7.2
2	48	12.23	09.53	23.00	7.3
3	72	09.00	10.40	18.60	7.4

Figure –2 Fermentation behaviour of *Aspergillus awamori* on Groundnut oil extract

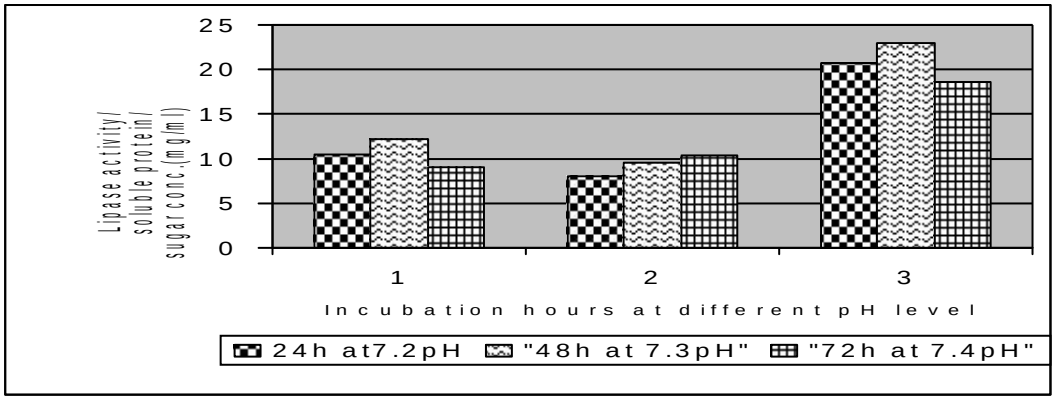


Table-3 Effect of various carbon sources on lipase production by *Aspergillus awamori*

Sl.No	Carbon sources	24h	48h	72h
1	CMC	11.40	26.16	20.08
2	Dextrose	12.87	18.67	14.35
3	Fructose	9.86	22.26	14.97
4	Starch	9.89	28.34	23.21
5	Lactose	11.54	15.87	12.34
6	Maltose	15.87	26.72	21.35
7	Sucrose	13.68	26.06	23.23
8	Mannitol	12.7	21.54	13.54
9	Glycerol	11.65	15.76	17.10

Figure -3 Effect of various carbon sources on lipase production by *Aspergillus awamori*

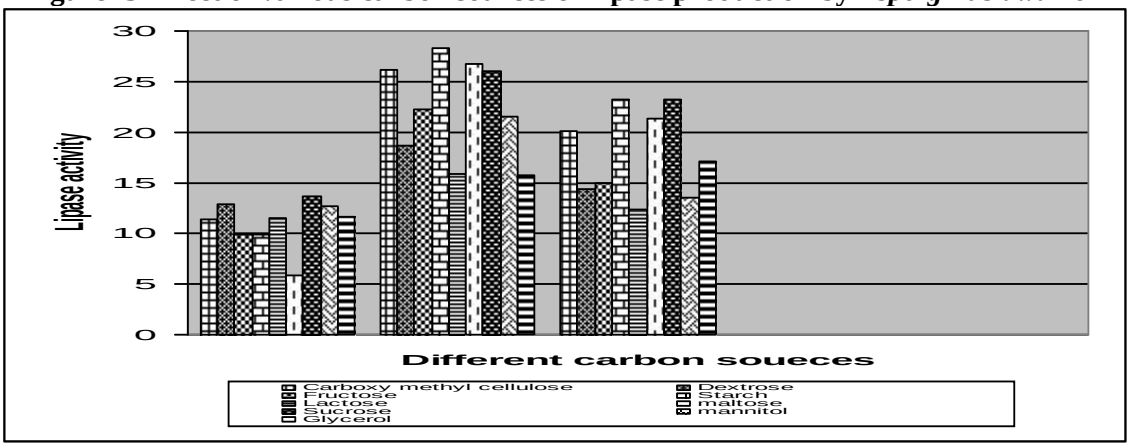


Table- 4 Effect of various Nitrogen sources in Groundnut oil on lipase production by *Aspergillus awamori*

Sl.No	Nitrogen sources	24hrs	48hrs	72hrs
1	Urea	11.10	17.63	14.70
2	Beef extract	16.60	17.10	17.36
3	Malt extract	07.16	21.06	18.30
4	Tryptophane	18.76	21.83	16.66
5	Yeast extract	12.13	18.06	12.40
6	Sodium nitrate	13.93	15.36	10.46
7	Ammonium nitrate	09.00	12.80	12.20
8	Ammonium chloride	07.30	14.06	13.03
9	Peptone	09.23	24.46	1 5.70

Figure -4Effect of various Nitrogen sources in Groundnut oil on lipase production by *Aspergillus awamori*

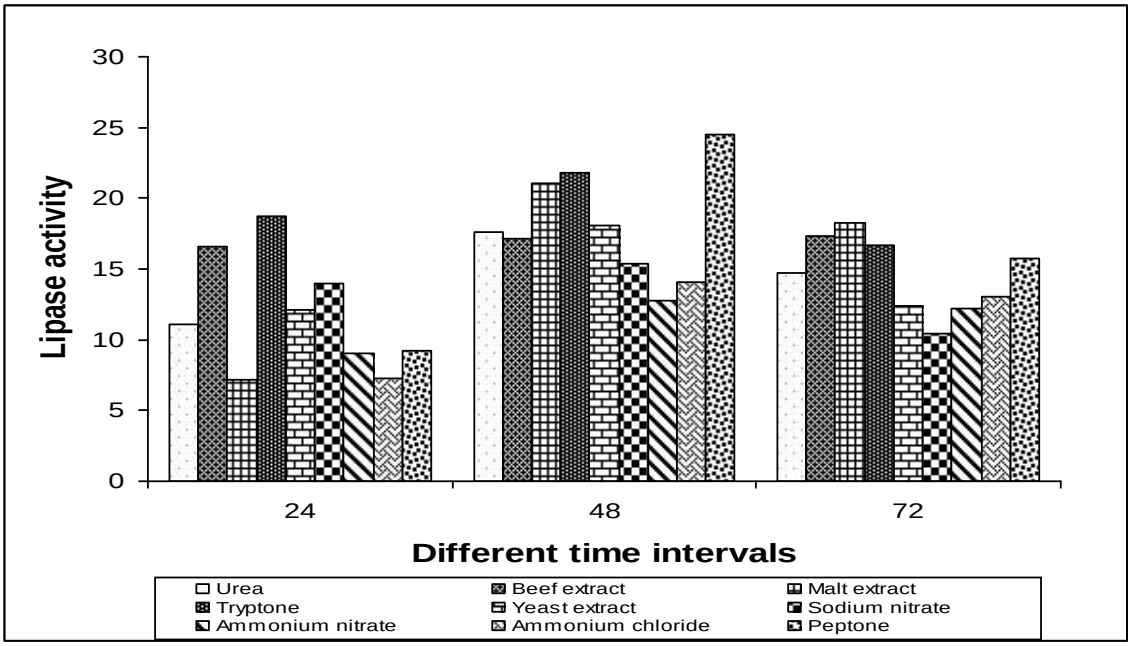


Table -5 Effect of different inducers in Groundnut oil on lipase production by *Aspergillus awamori*

Sl.No	Inducers	28hrs	48hrs	72hrs
1	Coconut oil	07.10	14.63	15.00
2	Olive oil	13.60	25.10	10.36
3	Neem oil	09.16	14.06	13.30
4	Caster oil	09.76	16.83	09.66
5	Palm oil	10.56	24.36	20.40
6	Groundnut oil	11.93	22.36	05.46
7	Gingillic oil	09.00	17.80	12.20

Figure -5Effect of various inducers in Groundnut oil on lipase production by *Aspergillus awamori*

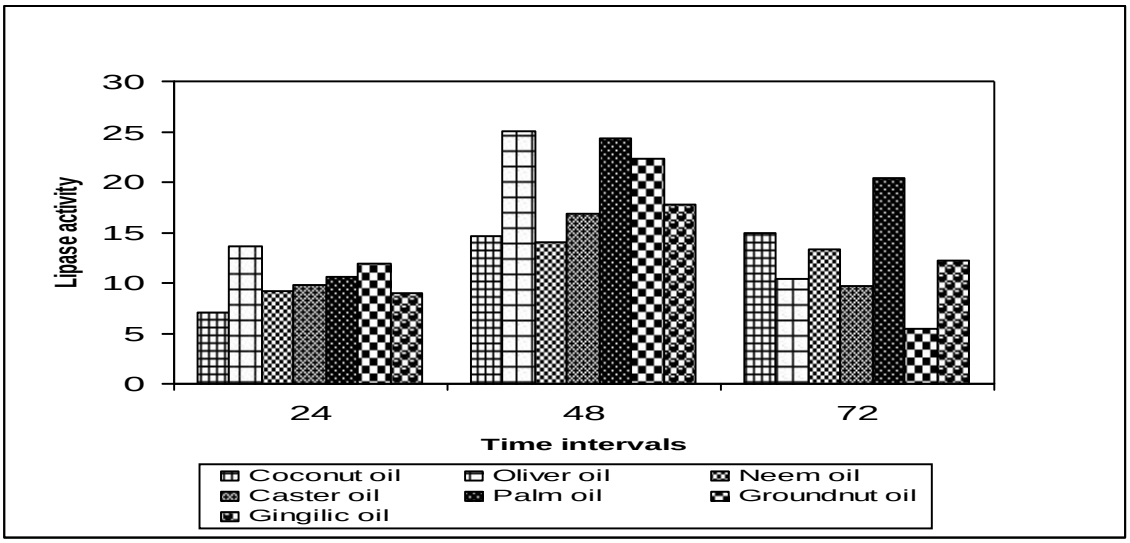


Table-6Effect of various salts in Groundnut oil on lipase production by *Aspergillus awamori*

Sl.No	Salts	24h.	48h.	72hr.
1	KCl	07.80	14.66	15.86
2	$MgSO_4$	08.23	15.36	17.60
3	$CaCl_2$	10.03	17.79	15.10
4	$ZnSO_4$	08.58	13.90	11.98
5	$MnSO_4$	11.55	15.24	08.21
6	$FeSO_4$	11.25	21.54	23.98
7	$MgCl_2$	12.76	13.56	08.67
8	$CuSO_4$	07.78	13.67	12.56

Figure -6 Effect of various salts in Groundnut oil on lipase production by *Aspergillus awamori*

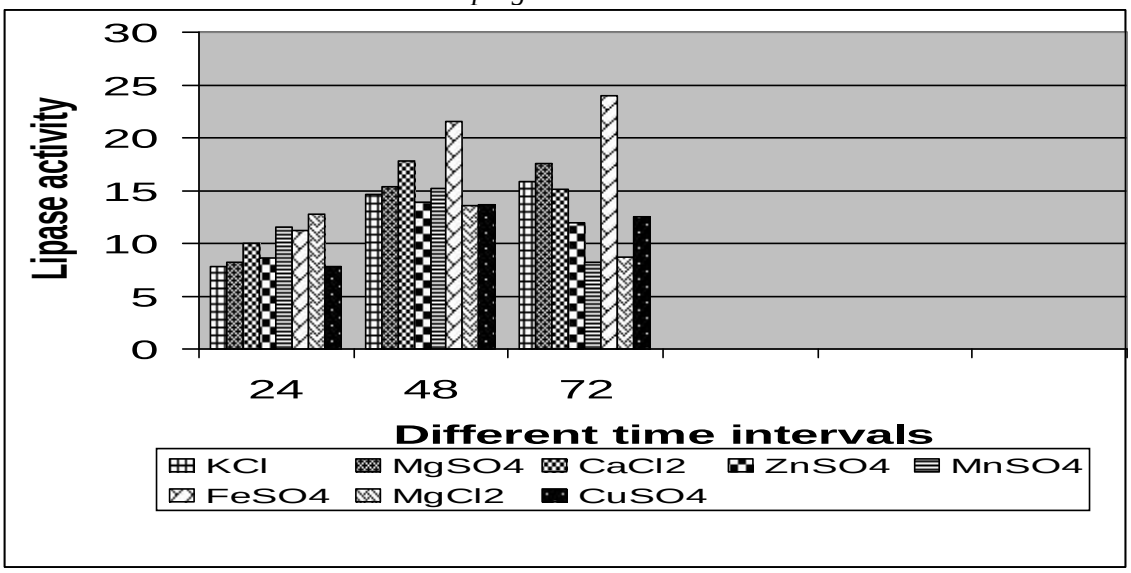


Table -7 Effect of Inoculum concentrations in Groundnut oil on lipase production by *Aspergillus awamori* in shake flask culture

Sl.No	Inoculum conc.	24h.	48h.	72h.
1	1.0ml	14.06	19.43	15.70
2	1.5ml	19.50	22.51	21.83
3	2.0ml	22.46	27.96	2530
4	2.5ml	30.05	34.50	35.78
5	3.0ml	29.98	37.66	32.76

Table -8 Effect of Gum Arabic concentration in Groundnut oil on lipase production by Aspergillus awamori in shake flask culture

Sl.No	Inoculum conc.	24h.	48h.	72h.
1	0.2%	08.06	15.43	12.70
2	0.4%	12.50	23.51	17.83
3	0.6%	10.46	20.96	15.30
4	0.8%	08.05	17.50	10.78
5	1.0%	07.84	14.66	10.76

Figure -8 Effect of Gum Arabic concentration in Groundnut oil on lipase production by *Aspergillus awamori* in shake flask culture

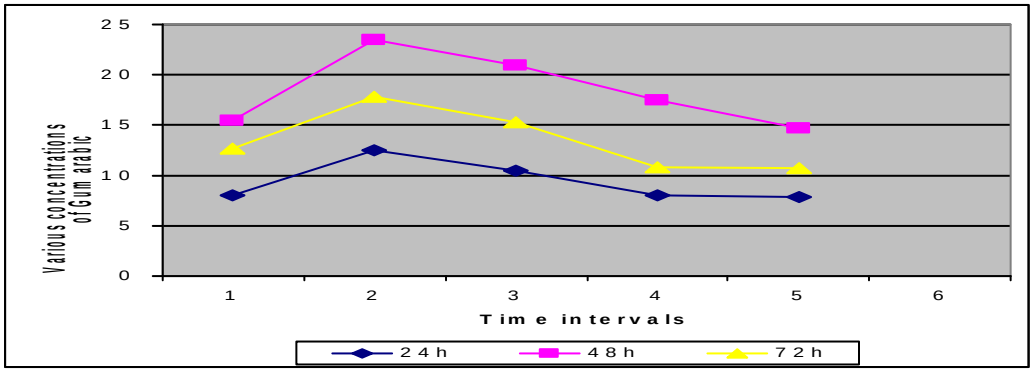


Table-9 Effect of different pH in Groundnut oil on lipase production by *Aspergillus awamori*

Sl.No	pH	24h	48h	72h
1	2	4.10	7.46	5.26
2	3	4.50	7.72	7.73
3	4	6.20	7.20	6.72
4	5	7.23	10.26	11.35
5	6	10.30	13.80	9.52
6	7	12.78	14.26	12.26
7	8	10.24	13.90	10.40
8	9	9.40	11.32	8.40

Figure -9 Effect of different pH in Groundnut oil on lipase production by *Aspergillus awamori*

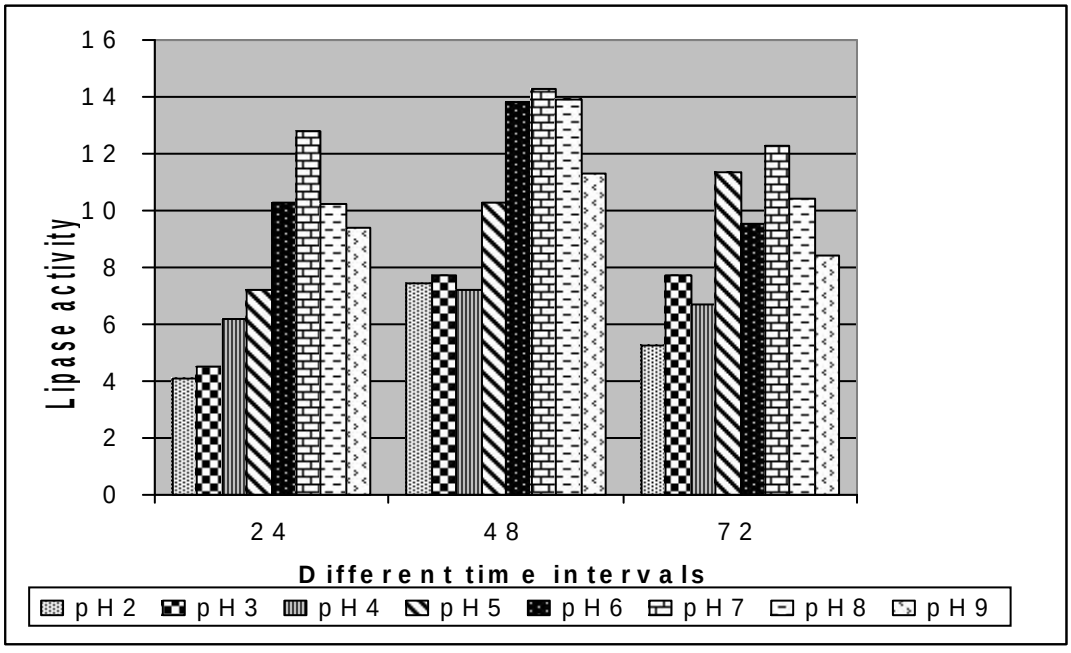


Table-10 Effect of different temperature in Groundnut oil on lipase production by *Aspergillus awamori*

Sl.No	Temperature	24h	48h	72h
1	0ºC	07.45	08.34	08.32
2	28 ºC	08.65	11.76	10.89
3	38 ºC	10.98	14.34	13.32
4	48 ºC	10.32	11.87	12.97
5	58 ºC	06.54	12.87	09.87
6	68 ºC	07.98	08.98	07.54

Figure -10 Effect of different temperature in Groundnut oil on lipase production by *Aspergillus awamori*

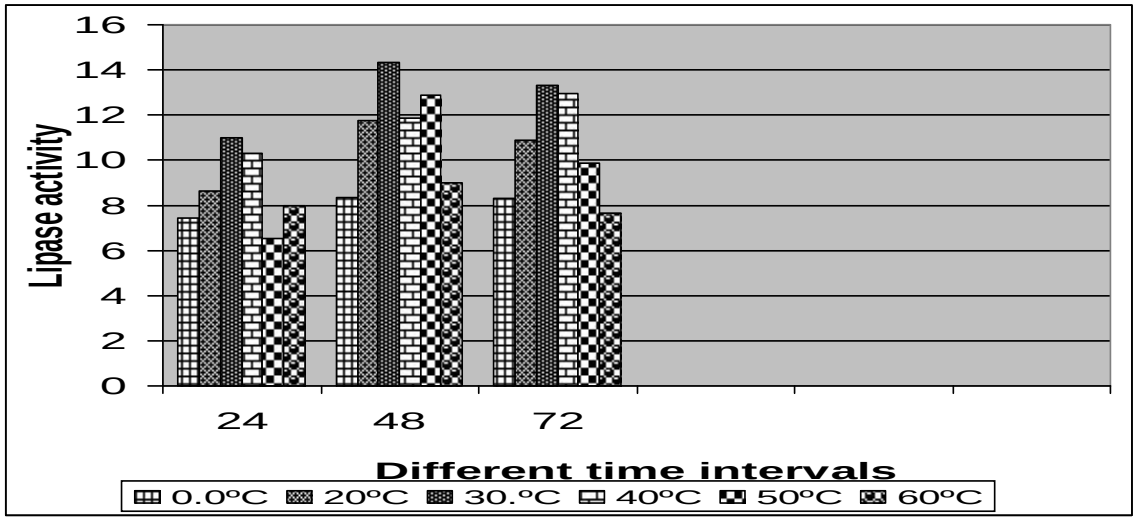


Table-11 Growth curve of *Aspergillus awamori*

Sl.No	Time	Growth rate	Lipase activity
1	12	0.125	07.35
2	24	0.390	12.00
3	36	0.716	15.80
4	48	0.820	16.40
5	60	0.690	11.95
6	72	0.515	08.20

Figure 11 Growth curve *of Aspergillus awamori*

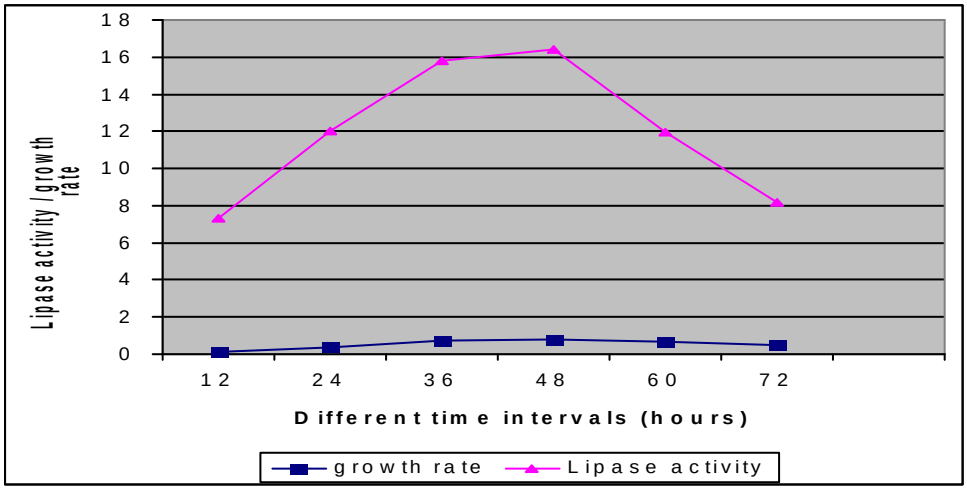


Table-12 Thermostability of Lipase Enzyme

Sl.No	Temperature	30 minutes	1 hour	1.30 minutes	2 hours
1.	30 ºC	15.66	15.67	08.87	04.55
2	40 ºC	17.00	17.89	08.65	04.87
3	50 ºC	12.30	09.00	05.26	04.05
4	60 ºC	07.98	07.78	03.78	01.98
5	70 ºC	04.35	02.80	01.89	01.67
6	80 ºC	01.69	01.18	00.78	00.54
7	90 ºC	00.78	00.65	00.47	00.68

Figure – 12 Thermostability Lipase Enzyme

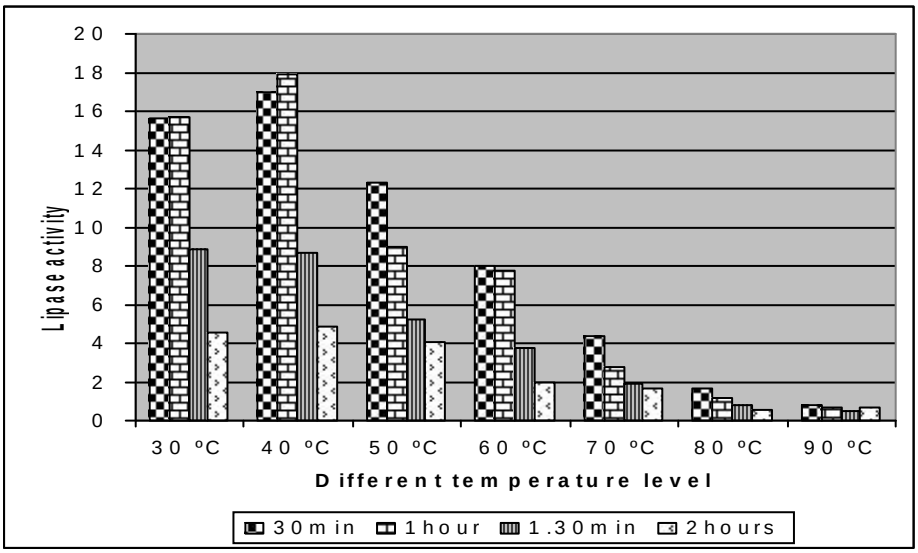
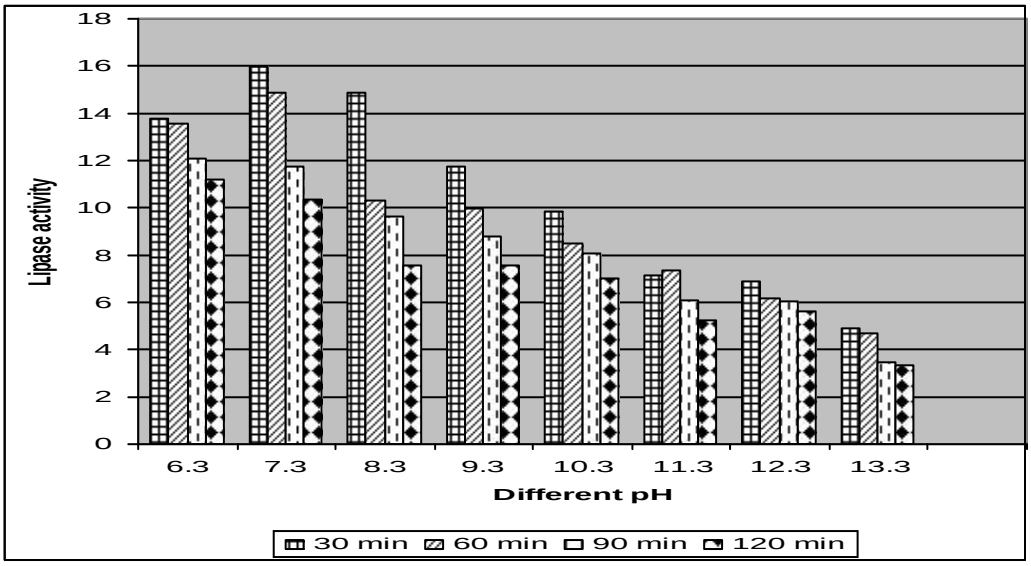


Table-13 pH stability of the lipase enzyme

Sl.No	pH	30 minutes	60 minutes	90 minutes	120 minutes
1	06.3	15.79	13.56	12.07	11.18
2	07.3	15.98	14.87	11.75	10.35
3	08.3	14.87	10.31	09.64	07.55
4	09.3	11.76	09.98	08.80	07.56
5	10.3	09.86	08.50	08.06	07.00
6	11.3	07.15	07.35	06.08	05.25
7	12.3	06.87	06.15	06.05	05.64
8	13.3	04.90	04.67	03.45	03.35

Figure-13 pH stability of the lipase isolated from *Mucor pusillus*



results contradicts with that of the results reported by Prabhakar *et al*., (2002) for *Aspergillus niger*, they reported that 26ºC is favourable for good lipase activity. A temperature of 28ºC is found to be most conductive for the production of lipase by *Bacillus mycoides*, (Achamma Thomas et al., 2003). However the optimum temperature for maximum lipase production is between 22-35ºC for *Aspergillus wentii* (Chand *et al*., 1981), *Mucor heimalis* (Akhtar *et al.,* 1980), *Rhizopus nigricans* (Chand *et al*., 1981), *R.oligosporus* (Nahas, 1988) and *Penicillium aeruginosa* (Gillbert *et al.,* 1991).

Growth curve

Growth and enzyme production were determined at 12hours intervals. The cell growth increased up to 48h, after which it declined. In early log phase growth started and the maximum yield was obtained at late exponential growth phase. A fall in lipase production was observed in stationary and decline phase (Table -11&Figure-11).

Enzyme characterization

Thermal stability

Thermal stability of lipase enzyme isolated from *Aspergillus awamori* was examined at various temperatures, (30 ºC, 40 ºC, 50 ºC, 60 ºC, 70 ºC, 80 ºC 90 ºC) along with various incubation periods, (30 minutes, 1 hour, 11/2 hours, 2 hours). The results are shown in (Table -12 & Figure-12) enzyme was stable at 40 ºC for nearly 60 minutes.

pH stability

The optimum pH of the enzyme was determined using different pH buffer, Tris Acetate (pH 6.0-7.0), and Tris HCl (pH 7.5-9.0). The purified enzyme was incubated in these buffers for 30minutes, 60 minutes, 90 minutes and 120 minutes at 37 ºC and the residual activity was assayed for determining its pH activity. The enzyme was stable for 30 minutes at pH between 6.3 and 7.3. Similar results have been reported by Saxena *et al.*, (1999) for lipase from various fungi (Table-13 & Figure-13).

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