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Oil cakes as substrate for improved lipase production in solid state fermentation

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Abstract: *The plan of work is to estimate the potential of oil cakes and study the properties of enzyme production in SSF after partial purification. Oil cakes as substrate used for improved lipase production in solid state fermentation. The optimum enzyme activity of groundnut oil cake after 96hr was found to be was 7.89mg/L and protein content 30.9 mg/ml while activity of teesi oil cake was found to be 6.24mg/L and protein content 27.5mg/ml. Groundnut and Teesi oil cake possessed good efficiency as a substrate for high yields of lipase under SSF. Optimum fermentation resulted in an increased in enzyme yields by Rhizopus oryzae indicating excellent capacity of fungal strain in lipase production under SSF. The maximum lipase production has increased diverse applications in medicines (digestive enzymes), food additives (flavor-modifying enzymes), clinical reagents (glyceride-hydrolysing enzymes), and cleaners (detergent additives) and for synthesis of biopolymers and biodiesel.*

Keywords: lipase, oil cake, *Rhizopus oryzae*, solid state fermentation,

I. Introduction

Oil cakes /oil meals are byproducts obtained after oil extraction from the seeds. Oil cakes are of two types, edible and non edible. Edible oil cakes have a high nutritional value; especially have protein content ranging from 15% to 50%. Their composition varies depending on their variety, growing condition and extraction methods. Due to their rich protein content they are used as animal feed, especially for ruminants and fish, non-edible oil cakes such as Castor cake, Karanja cake, Neem cake are used as organic nitrogenous fertilizer, due to their N, P, K content. Some of these oil cakes are found to increase the nitrogen uptake of the plant, as they retard the nitrification of soil. They also protect the plants from soil nematodes, insects and parasite; there by offer great resistance to infection [1].

Oil cakes, in particular, edible oil cakes offers benefits when utilized for fermentative production of enzymes and antibiotics etc[2]. Fungal lipases are known to be commercially used in various biotechnological industries, lipases are reported in various microorganisms, plants and they broke down lipids so they are used for various biotransformation reaction, catalysis industries and other Industries and other industries, for eg: detergents, dairy foods, bakery and beverages and health foods, pharmaceuticals. Lipase hydrolyses triglycerides into diglyceride, monoglyceride and fatty acids. Interest in these enzymes has increased markedly over the last decades, in view of their diverse applications in medicines (digestive enzymes), food additives (flavor-modifying enzymes), clinical reagents (glyceride-hydrolysing enzymes), and cleaners (detergent additives) and for synthesis of biopolymers and biodiesel. Lipase catalyzes reverse reaction such as esterification and transesterification. However SSF is most appropriate process due to its various benefits and bioconversion parameters [3-7].

Crop residue as bran, husk, bagasse and fruit seeds are utilized as a potential raw material in bioprocesses as they provide an excellent substratum for the growth of organism supplying the essential nutrients to them [8-16]. Their application in bioprocesses is more advantageous in bioremediation and biological detoxification of hazardous compounds. Their application in the field of fermentation technology has resulted in production of bulk chemicals and value added products such as amino acids, enzymes, mashrooms, organic acids, single cell protein (SPC), biologically active secondary metabolites etc[17-21]

R. oryzae strains are often used in Asia for food fermentation to manufacture alcoholic beverages, ragi and the strains are generally regarded as safe. R. oryzae is ubiquitous in nature and found on decaying organic material. It is able to grow on a wide range of carbon sources, eg. Glycerol, ethanol, lactic acid, glucose, mannose, fructose, sucrose, xylose, cellubiose, fatty acids and oils [22-26]. All mentioned sugars have been shown to be a substrate for l-(+)-lactic acid or fumaric acid production. Moreover, R. oryzae has aminolytic[27], pectinolytic[28] and cellulytic[29-31] catabolite, enabling the conversion of polymeric agricultural residues. It is able to grow well at a wide temperature range (up to 40^0C) and pH range (from 4 to9), indicating a robust behavior and widely applicable potential. Lipases widely occur in bacteria, yeasts and fungi [32-34]. Fungi are broadly recognized as one of the best lipase sources and are used widely in the food industries. Most of the lipase research focuses on the production of extracellular lipase through a wide variety of microorganisms. The technique of solid state fermentation (SSF) involves the growth and metabolism of microorganisms on moist

solids without any free flowing water. Oil cakes are rich in fiber and have high concentration of non-starch polysaccharides (NSP). Their chemical composition varies due to the difference in the extraction methods of oil. Oil cakes such as palm carnel cake, seasam oil cake and coconut oil cake contain 14-20% of crude protein. However, groundnut oil cake contains 40-50% of crude protein. Fat content of the oil cakes is also depending on the oil extraction method. They generally have less than 2-3% fat[35]. The aim of this to evaluate the potential of oil cakes and also the study the properties of enzyme production in SSF after partial purification.

Ingredients	In percentage
Dry matter	92.6%
Crude protein	49.5%
Crude fiber	5.3%
Ash	4.5%
Calcium	0.11%
Phosphorus	0.74%

Table no -1: Composition of oil cake:

II. Materials and method

Groundnut and Teesi oil cake, Peptone, Sodium chloride, Calcium chloride (0.05M), Magnessium chloride , Substrate- Tributyrene, Phosphate Buffer, Bovine serum albumin, Alkaline copper sulphate solution, Folin's reagent, minerals salt solution (2.5gm sodium nitrate, 1 gm dipotasium hydrogen phosphate, 0.5gm potassium dihydrogen phosphate, 0.5 gm magnesium sulphate, 0.1gm potassium chloride, 0.01 gm calcium chloride, 0.01 gm ferrous sulphate)

Solid state fermentation:

Rhizopus oryzae was grown on Potato-Dextrose-Agar (PDA) incubated at 28^0C for 7 days and further it was stored at 4^0C. Two low cost available oil cakes obtained from local oil mill viz. GOC (Groundnut oil cake) and TOC (Teesi oil cake) were used as substrate for solid state fermentation. The production medium was prepared using mineral salt solution (20ml) with peptone (15g/L), NaCl (5g/L), CaCl (1g/L) and oil cake (5g/L) as substrate. This was inoculated with 1ml of *Rhizopus oryzae*. This was further incubated for 7 days at 30^0c.

Enzyme extraction:

To the fermentation product 50 ml of 0.1M phosphate buffer pH 7.5 was added, stirred and mixed properly and enzyme was extracted by filtering the solution through whattman filter paper. The culture filtrates obtained were centrifuged at 3000×g for 20 min and clear supernatant was collected and used as enzyme source. The enzyme activity as amount of enzyme required liberating one micromole equivalent fatty acid per ml/min, was measured by titrimetric method using phenolphthalein as indicator. Study for lipase activity and protein estimation by Folin Lowry's method.

Effect of incubation time on solid state fermentation:

To optimize the incubation time, the fermentations were carried out using groundnut and teesi oil cake. Temperature was maintained at 30^0C. Samples were withdrawn every 24hr and extracted. The extract was assayed for lipase and soluble protein contents.

Effect of incubation temperature solid state fermentation:

Solid state fermentation was carried out at different incubation temperatures ranging from 20^0C to 50^0C. The samples were extracted after 7 days. The extracts were assayed for lipase and protein contents on fermentation

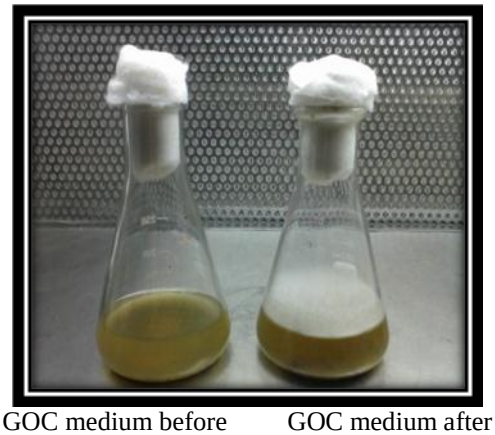
Effect of inoculum size on solid state fermentation:

A master spore suspension was made from a PDA slant and varying levels of inoculation size were used. Solid state fermentation was carried out with sample that was inoculated with 0.5, 1, 1.5, 2 ml of spore suspension. Fermentation's were carried out at 30^0C for 7 days. Samples were centrifuged and the supernatant was assayed for lipase and protein contents.

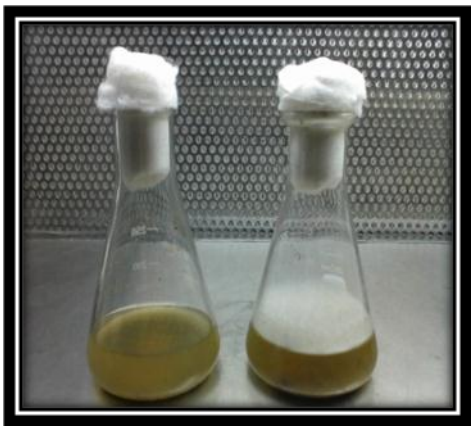
III. Observation and Result

Rhizopus oryzae was able to produce lipase by solid state fermentation with low value oil cakes GOC and TOC upon incubation at 30^0C for 7 days. Among the two substrates used crude enzyme extracted from GOC medium showed highest activity. Activity of enzyme extracted from medium containing GOC & TOC was assayed to be 6.90 & 1.44 mg/L respectively. Total protein of the crude enzyme extracted from the different

medium was estimated by Folin Lowry's method. Total protein content of extracts from GOC and TOC medium were 31.2mg/ml and 26.4mg/ml.



GOC medium before Fermentation GOC medium after Fermentation



TOC medium before fermentation **TOC medium after fermentation**

1] Effect of incubation time on solid state fermentation :

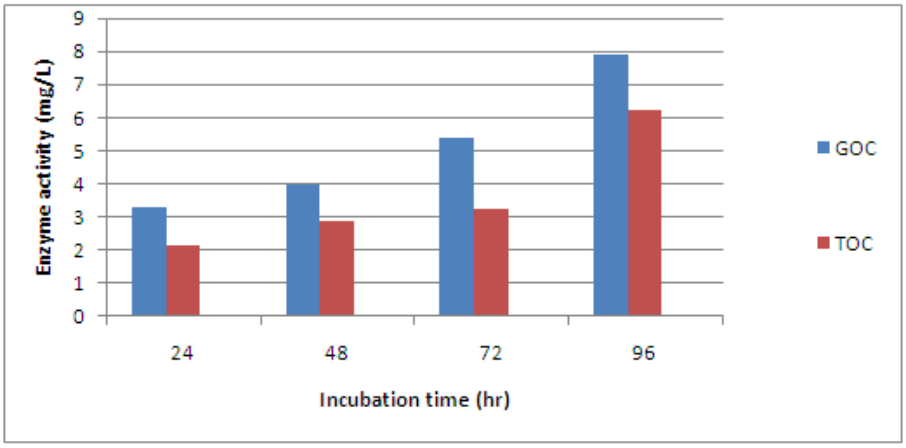
[1] Lipase activity: The optimum enzyme activity was found to be after 96hr.

Sr. no.	Incubation time (hr)	Enzyme activity (mg/L)
1.	24	3.3
2.	48	3.96
3.	72	5.40
4.	96	7.89

Table no -5: Lipase activity of **Groundnut oil cake**

Sr. no.	Incubation time (hr)	Enzyme activity (mg/L)
1.	24	2.15
2.	48	2.86
3.	72	3.26
4.	96	6.24

Table no -6: Lipase activity of **Teesi oil cake**



Effect of incubation time on enzyme activity of Groundnut & Teesi oil cake

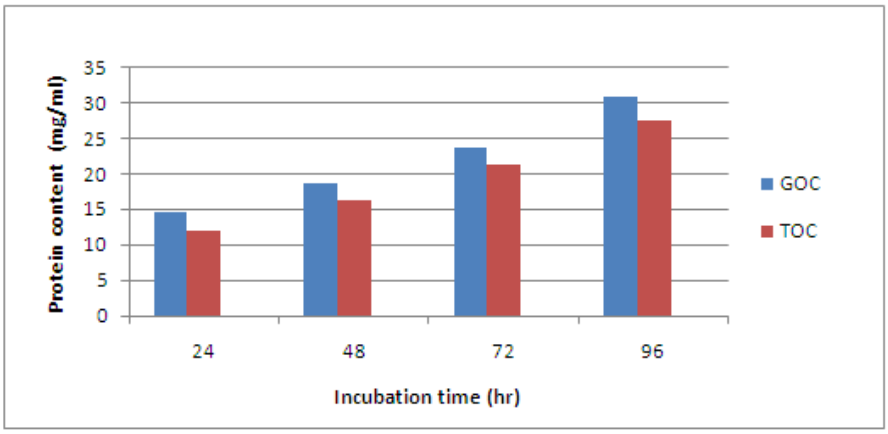
[2] Protein content by Folin Lowry method: The optimum protein content was found to be after 96hr.

Sr. no.	Incubation time (hr)	Protein content (mg/ml)
1.	24	14.7
2.	48	18.6
3.	72	23.8
4.	96	30.9

Table no -7: protein estimation Of **Groundnut oil cake**

Sr. no.	Incubation time (hr)	Protein content (mg/ml)
1.	24	12.1
2.	48	16.2
3.	72	21.3
4.	96	27.5

Table no -8: protein estimation **Teesi oil cake**



Gtaph no -2: Effect of incubation time on protein content of Groundnut & Teesi oil cake

2] Effect of incubation temperature on solid state fermentation:

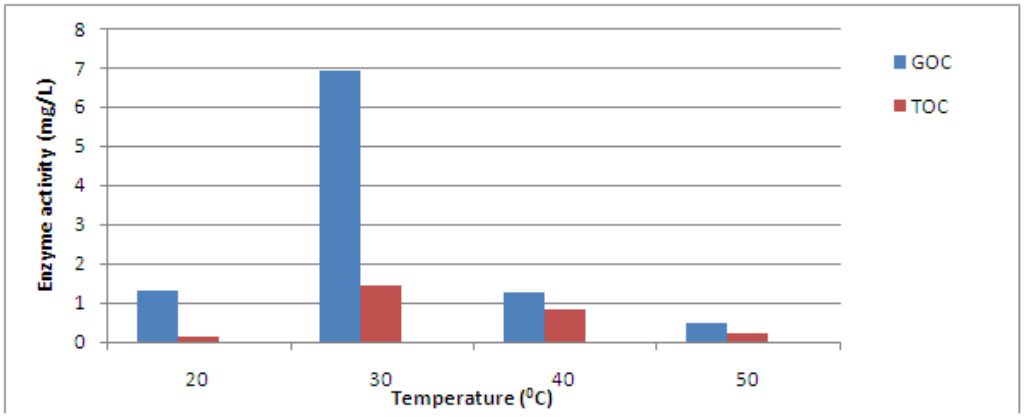
[1] Lipase activity: The optimum enzyme activity was found at temperature $30^{0.}$

Sr. no.	Incubation temperature (^{0}C)	Enzyme activity (mg/L)
1.	20	1.32
2.	30	6.90
3.	40	1.26
4.	50	0.47

Table no -9: lipase activity of **Groundnut oil cake**

Sr. no.	Incubation temperature (^{0}C)	Enzyme activity (mg/L)
1.	20	0.13
2.	30	1.44
3.	40	0.84
4.	50	0.22

Table no -10: lipase activity of **Teesi oil cake**



Graph no -3: Effect of incubation temperature on enzyme activity of Groundnut & Teesi oil cake

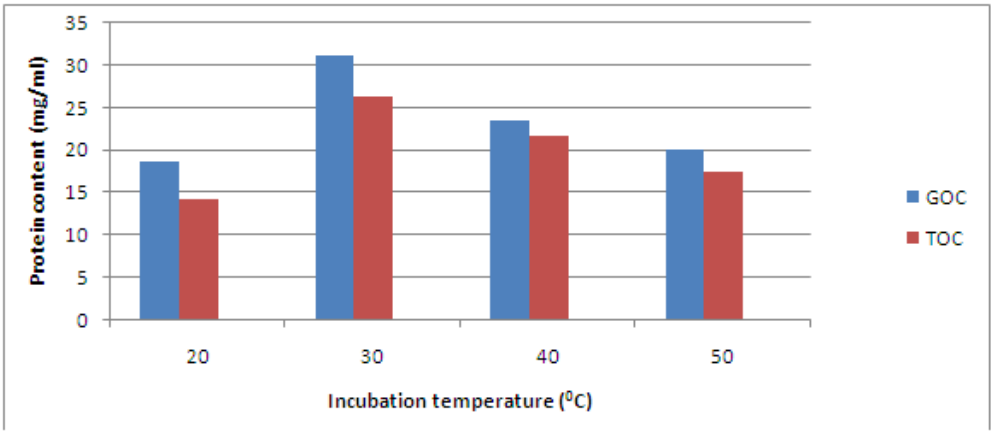
[2] Protein content by Folin Lowry method: The optimum protein content was found at temperature 30^{0C}.

Sr. no.	Incubation temperature (^{0}C)	Protein content (mg/ml)
1.	20	18.7
2.	30	31.2
3.	40	23.5
4.	50	20.1

Table no -11: protein estimation of **Groundnut oil cake**

Sr. no.	Incubation temperature (^{0}C)	Protein content (mg/ml)
1.	20	14.2
2.	30	26.4
3.	40	21.6
4.	50	17.5

Table no -12: protein estimation of **Teesi oil cake**



Graph no -4: Effect of incubation temperature on protein content of Groundnut & Teesi oil cake

3] Effect of inoculum size on solid state fermentation:

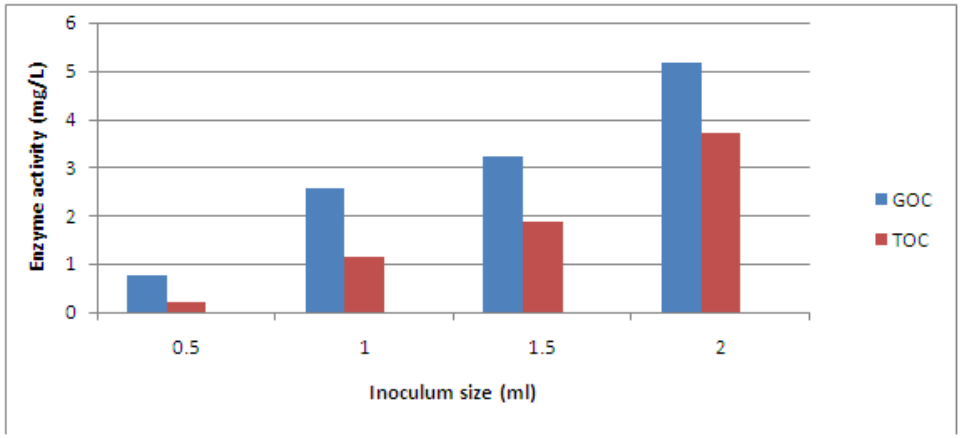
[1] Lipase activity: The optimum enzyme activity was found at inoculum size 2ml.

Sr. no.	Inoculum size (ml)	Enzyme activity (mg/L)
1.	0.5	0.78
2.	1	2.56
3.	1.5	3.23
4.	2	5.18

Table no -13: Lipase activity of **Groundnut oil cake**

Sr. no.	Inoculum size (ml)	Enzyme activity (mg/L)
1.	0.5	0.22
2.	1	1.13
3.	1.5	1.89
4.	2	3.72

Table no-14: Lipase activity of **Teesi oil cake**



Graph no -5: Effect of inoculum size on enzyme activity of Groundnut & Teesi oil cake

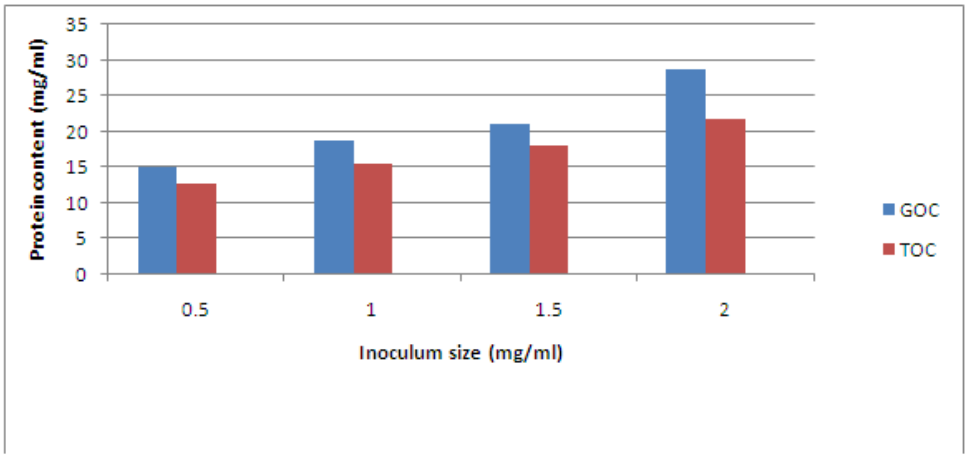
[2] Protein content by Folin Lowry method: The optimum protein content was found at inoculum size 2ml.

Sr. no.	Inoculum size (ml)	Protein content (mg/ml)
1.	0.5	14.9
2.	1	18.6
3.	1.5	21.1
4.	2	28.6

Table no -15: protein estimation of **Groundnut oil cake**

Sr. no.	Inoculum size (ml)	Protein content (mg/ml)
1.	0.5	12.5
2.	1	15.3
3.	1.5	17.9
4.	2	21.8

Table no -16: protein estimation of **Teesi oil cake**



Graph no -6: Effect of inoculum size on protein content of Groundnut & Teesi oil cake

IV. Discussion

Solid state fermentation for lipase production from Rhizopus oryzae, using different low cost available oil cakes GOC (groundnut oil cake) and TOC (teesi oil cake) was carried and it was found that the fungus produced significant amount of lipase utilizing oil cake as substrate. Among the two substrates used crude enzyme extracted from GOC medium showed highest activity. The production medium was prepared using mineral salt solution with peptone, NaCl, CaCl and oil cakes as a substrate. This was inoculated with 1ml of spores of R. oryzae. This was further incubated for 7 days at 30^0C. Both the oil cake shows optimum enzyme activity and protein content was found after 96 hr, at 30^0C and at inoculum size 2ml. Study of effect of incubation time, temperature, inoculum size on fermentation medium was carried out. After fermentation crude enzyme were extracted and assayed for lipase activity and soluble protein content. In the present study groundnut oil cake (GOC) gives more lipase production than teesi oil cake (TOC). The maximum lipase production by GOC after 96hr was7.89mg/L and protein content was 30.9mg/ml and lipase production by TOC after 96hr was 6.24mg/L and protein content was 27.5mg/ml.

Rao et al. (1993a), Benjamin & Pandey studied Pongamia oil cake was found to be the best substrate for these purpose yielding 98.3U/gm DM followed by coconut oil cake yielding 92.5U/gm DM. among the

selected substrates, coconut oil cake sediment and fish bone produced maximum lipase. The an other substrate supplied, almost in all the substrate, 48hr was found to be the optimum time for maximum lipase production was high when compared to 24hr and 72hr.

T.Selva Moham, A.Palavesam and R.L.Ajithas carried out lipase production by Vibrio sp. At different concentrations of lipidic substrates during various time intervals shown. The result inferred that 1.5% was the optimum concentration for enhancing lipase production in all the tested substrates. Moreover among the selected substrates coconut oil sediment and fish bone produced maximum lipase then other substrates supplied. Almost in all the substrate, 48hr was found to be the optimum time for maximum lipase production and at this period the lipase production was high when compared to 24hr and 72hr. It was reported that, coconut oil is the best and inexpensive substrate for lipase production. Lipids like coconut oil are found to be an inducer of lipase production and this was observed in lipase production by Mucor.

Griseocyanus, Supakdamrongkul et.al also reported that, lipase production by Nomuraearileyi was high, when coconut oil is used as substrate. The effect of different initial medium pH at various incubation periods on lipase production resulted that neutral pH was optimum for enhancing lipase production. This pH supported well for lipase production in all the tested incubation time intervals (24,48 and 72hr). The results inferred that this strain prefers neutral pH for better growth and enzyme production

V. Summary and Conclusion

There are other notable reports on lipase production through SSF using oil cake, purification, statistical optimization and use in industry and other notable reports on immobilizing the enzyme and natural selection for lipase producing microbial strains are available but there are few reports indicating utilizing low value oil cake as substrate mentioned in the present work it was thus reported that groundnut oil cake could be utilize as better substrate over other oil cake (Teesi oil cake) for lipase production from Rhizopus oryzae. This study gives as idea on utilization of waste oil cakes for enzyme production through SSF and adds value addition to oil mill wastes. For the production of lipase groundnut and teesi oil cake used as substrate along with mineral salt solution and other compounds such as peptone, NaCl and CaCl as nutritional supplement. Groundnut and Teesi oil cake serves as an cheapest source of lipase production, as both the oil cakes are economically reliable so that it is used for industrial production of lipase. The maximum lipase production by GOC after 96hr was7.89mg/L and protein content was 30.9mg/ml and lipase production by TOC after 96hr was 6.24mg/L and protein content was 27.5mg/ml.

The optimum enzyme activity of groundnut oil cake was found to be after 96hr was 7.89mg/L and protein content 30.9 mg/ml, at 30^0C enzyme activity was 6.90mg/L and protein content was 31.2mg/ml and at inoculum size 2ml groundnut oil cake gives enzyme activity 5.18mg/L and protein content was found to be 28.6 mg/ml.

The optimum enzyme activity of teesi oil cake was found to be after 96hr was 6.24mg/L and protein content 27.5mg/ml, at 30^0C enzyme activity was 6.90 mg/L and protein content was 26.4mg/ml and at inoculum size 2ml groundnut oil cake gives enzyme activity 3.72mg/L and protein content was found to be 21.8 mg/ml.

Thus it is concluded from the above study that groundnut and teesi oil cake possessed good efficiency as a substrate for high yields of lipase under SSF. Optimum fermentation resulted in an increased in enzyme yields by R.oryzae indicating excellent capacity of fungal strain in lipase production under SSF. The maximum lipase production by GOC after 96hr was7.89mg/L and protein content was 30.9mg/ml and lipase production by TOC after 96hr was 6.24mg/L and protein content was 27.5mg/ml.

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