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Extraction and partial purification of lipase from coconut seeds

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ABSTRACT

Lipases are widespread in nature and have been largely found in animals, higher plants and microorganisms. Lipase is the main enzyme that breaks down dietary fats in the human digestive system, converts triglyceride substrates found in ingested oils to monoglycerides and two fatty acids. Lipase was extracted from dried coconut and characterization of the enzyme was studied. The lipase extracted from the coconut was found to have optimum pH and temperature of 7.5 and 35°C respectively. Lipase is an enzyme which is economical and can be easily extracted from natural sources at low cost. It also has a wide application in various industries and is very much in need of current lifestyle. Therefore, in the present study, lipase enzyme was extracted from coconut and enzyme activity was measure at various temperature and pH condition and the exact molecular weight of the enzyme was determined using SDS-PAGE technique. Furthermore, studies can be conducted to attain its complete application in various field.

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INTRODUCTION

The coconut belongs to subfamily Cocoidae, order Palmales and family Arecaceae. It is consisting of three celled ovaries, an outer fleshy part surrounds an endocarp provided with three germ pores while the fruit is only one seeded (Smit, 1970). The hydrolysis of fatty acyl esters and other esterase's requires an oil-water interface for normal activity are catalysed by the enzyme lipases. The enzymes hydrolyse the carboxyl ester bonds and therefore are named carboxyl esterase (Sonnet, 1988). The enzyme is most commonly purified by hydrophobic interaction and also by some modern approaches. Lipases can act in a wide range pH and temperature. They are serine hydrolyses and have high stability in organic solvents. Lipases can be extracted also from castor bean, palm seeds, and also dormant seeds (Abigor *et al.,* 1985, Handerson and Osborne, 1991). Various lipase extracted from different sources have a specific function such as flavouring, egg white whipping properties. The vast use of lipase has increased its demand for growth and culture. There have been many plants in which no activity of lipase is reported. Lipases have potential applications in food, leather, detergent, pharmaceutical, textile, cosmetic and paper industries. Among these food, detergent and pharmaceutical are found to be the most important industrial applications (Jooyandeh *et al.,* 2009).

The chemo, regio and enantio specific characteristic of lipase tends to be more focused in the current trends. Lipases are also found to create new ingredients and more functional products such as cocoa butter and fat milk. In recent days it also found to produce bio-diesel which could be more favourable and functional. The coconut lipase had an optimum pH of 7.5 which is mildly alkaline. The activity of enzyme regarding the pH was found to be studied from many different sources. The optimum pH when compared to other studies shows either alkaline or acidic. This confirms those lipases are of both types acidic and alkaline. Acidic lipase ex-

amples are castor bean at pH 4-4.2 (Ory, 1969), Aspergilus with 6.5 (Fu *et al.,* 1995). Alkaline lipase examples are germinating rape seed at 8.5 (Wetter *et al.,* 1957), rice bran with 7.5-8.0 (Funatsu *et al.,* 1971), *Hibiscus Canabinus* with 7.0 (Kausar and Akhtar, 1979), C. Bonducella seeds at 7.0 (Pahoja *et al.,* 2001). Coconut belongs to the alkaline type of lipase class. Hence this study aims to extract, partially purify and observe the lipolytic activity of lipase present in coconut seed.

MATERIALS AND METHOD

Extraction of proteins

Homogenize the coconut material (about 2 g) thoroughly in Tris- HCL buffer pH 7.2 (about 8-12 ml) using sterile glass powder in an ice bath. Centrifuge the homogenate at 10,000 rpm for 10-15 minutes at 40°C. Collect the supernatant. Precipitate the proteins using 10% TCA (150-200µl). Keep it at-200°C for one hour. Collect the protein precipitate by centrifugation at 10,000 rpm for 10 minutes at 4°C. Wash the precipitate once with 5ml of 80% acetone. Repeat the centrifugation step. Dissolve the pellet in 500µl of tris-HCl, pH 7.2.

Assay of lipase

10ml test solution is mixed with 5ml extract and incubated at 300°C for 60 min, with gentle shaking. The reaction is terminated by adding ethanol (40ml). The mixture is then titrated with alcoholic 0.02 N KOH against phenolphthalein. The unit of enzyme activity is the amount of enzyme which liberates 1.0 mg of oleic acid per minute.

Precipitation of protein

The precipitation of desired protein was done dissolving in water to make a solution of this protein. This procedure results in a signification purification and concentration of large quantities of protein. Ammonium sulfate, $(NH4)_2SO4$, is commonly used salt for salting out proteins because its large solubility in water, its relative freedom from temperature effects, and it has no harmful effects on most of the proteins.

Protein dialysis

After washing the dialysis tubing, calculate the length of tubing needed to contain the volume of protein sample. Pipette the protein sample into the tubing and close the other end of the tubing with another closure or with two tight knots. Try to avoid having too many air bubbles inside the tubing. Insert the dialysis tubing containing the protein into dialysis buffer taken in a large beaker. The volume of the buffer should be at least hundred times the original volume of the protein sample. The buffer should also be pre-chilled if the protein is labile. Stir the buffer slowly with a stir bar on a magnetic stir plate for at least a few hours. Dialysis for a few hours or overnight. Pipette the dialyzed protein solution into tubes.

SDS PAGE

The proteins in sample were fractionated using SDS-PAGE electrophoresis (Laemmli 1970). 30 µg protein samples was run on12% reduced SDS-PAGE. After the run, gel was fixed in staining solution for 30 min at room temperature. Subsequently the gel was destained for 45 min and then photographed. In order to find out approximate molecular weight of partially purified lipase enzyme, SDS-PAGE was run with protein marker. All fractions marked intense band in the same region.

RESULTS AND DISCUSSION

The optimum pH and temperature was found to be 7.5 (Figure 1) and 35°C (Figure 2) respectively. On gel images, it was seen that the approximate molecular weight (Mr) of the partially purified lipase enzyme is between 43 kDa and 29 kDa (Figure 3).

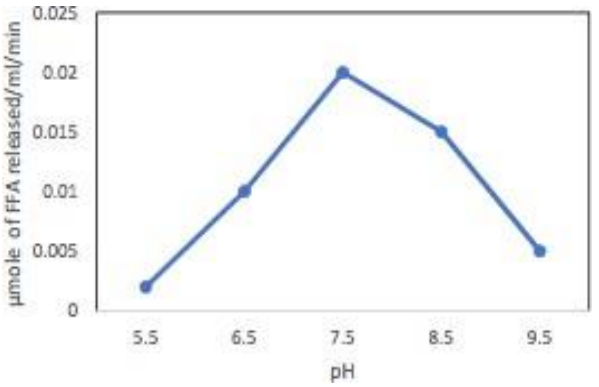

Figure 1: Effect of temp on activity of lipase

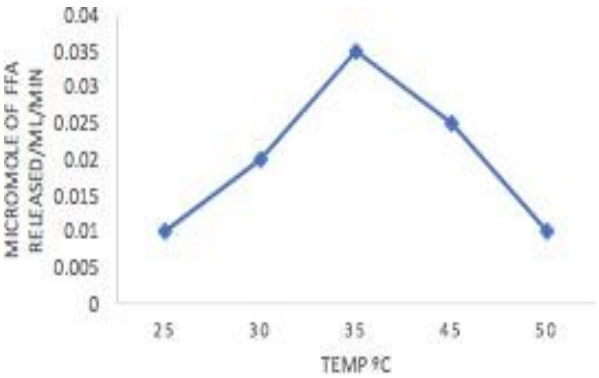

Figure 2: Effect of pH on activity of lipase

Lipases have wide variety of applications in different industries for alcoholysis, acidolysis and aminolysis. Among them food and agro are the most important. Increase in oil yield and better appearance in vegetable oil is given by lipase enzyme (Mala and Dahot, 1995). They enhance the aromatic notes in baking and dairy industries. Egg processing sector is one of the most essential functional regarding the food industry. Breakdown of

milk and characteristic flavour to the cheese is given by the lipase enzyme (Kausar and Akhtar, 1979). When the milk fatty acids are hydrolysed, free fatty acids produce the flavour. Both animal and plant lipase are used for the purpose. Animal lipase are obtained from calf, lamb etc.

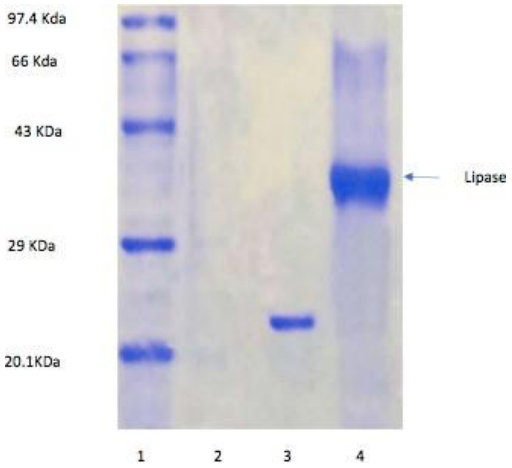

Figure 3: Molecular weight of purified lipase enzyme by SDS-PAGE

Lipases (phospholipases) can use as emulsifier in baking industry through destruction of wheat lipids to produce emulsifying lipids (Pahoja *et al.,* 2001). They also enhance the flavour of bakery products. This is done by the liberation short chain fatty acids through esterification. Among the detergent sector the lipase are the second most common and important group of enzymes which contribute to oil and stain. Lipases can also be used to help in production of polymers such as polyesters (Jooyandeh *et al.,* 2009). They are also used to produce active drug components due to their high specificity level (Robert, 2015). There are also used to remove the undesirable triglycerides and therefore help in the production of papers. These are also used in cosmetic and fragrant industry for synthesis of various useful chemicals. These are also added in the waste/sewage treatment to eliminate thin layers of fat formed at the surface of the reservoirs. One of the most advancing research in the application of lipase is the biodiesel production (Nielsen, 2016). This takes place in two reactionary steps hydrolysis of ester bond and esterification with secondary substrate. This method is generally known as transesterification (Fan, 2012). Bharadwaj et al research concluded that lipase plays a crucial role in causing infections (Bharadwaj and Gopinath, 2016). Lipases can also be detected by other means using plate assay containing trioleoylglycerol and fluorescent (Valli and Gopinath, 2016).

This study characterized the properties of lipase enzyme from coconut, which would help in further studies. Lipases can be further used in detection of low density lipoprotein in patients with liver problems. Therefore, in future the enzyme can be purified and used in the diagnosis of such problems.

CONCLUSION

The applications of the lipase enzyme are vast such as in food, baking, detergent and bio-diesel products. The characterization of lipase enzyme was done in this study and the optimum pH, temperature and molecular weight was found to be 7.5, 35°C, between 29-43kDa respectively. Thus, the enzyme can be further purified for its wide application.

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