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Research Article

Extraction, purification and Characterisation of Lipase from *Ricinus communis*

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ABSTRACT

This study focused on the extraction, purification and characterization of lipase from germinated castor beans (Ricinus communis). Among other oilseeds, where lipases are only active during germination, castor bean lipase is present throughout the germination process for both latent and sprouted seeds. Lipase activity was observed to peak on the fourth day of germination, prompting its extraction using sodium phosphate buffer. The enzyme was subsequently purified through a series of steps including salt precipitation, dialysis, ion exchange chromatography, and gel filtration. Protein estimation and enzyme activity assays were conducted at each stage to monitor purification progress. The purified lipase exhibited optimal activity at pH 8 and 30°C. Kinetic studies revealed a positive correlation between substrate concentration and enzyme activity. SDS-PAGE analysis estimated the molecular weight of the lipase to be approximately 48 kDa. The study successfully extracted and partially purified a lipase from a natural source, demonstrating its potential for applications in industries such as flavouring and biodiesel production.

KEY WORDS: Lipase, Purification, Ion-Exchange, Gel-filtration, SDS-PAGE.

Introduction

Lipases are enzymes that catalyse the total or partial hydrolysis of fats and oils, releasing free fatty acids, diacylglycerol, nonglycerol and glycerol. Lipases are the most versatile enzyme widely used in biotechnological applications. The purified enzyme is used in a wide range of food industries, detergent industries, personal care products and cosmetics industries It is also used in biodiesel production, pharmaceutical industry, polyesters industry [and fatty acids production (Alaa M. Dh. Al-Haidari et al. 2019). The lipases play an important role in processing of polyunsaturated fatty acids, a food colorant, g-linolenic acid, methyl ketones of flavour molecules characteristic of blue cheese (Patel, et al 2021).

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Plant lipases are extracted from different parts of the plant, such as fruits, latex and seeds. However, seeds, especially oil-seeds, are the main parts which contain higher concentrations of lipases. The lipases in seeds play an important role in the growth of the embryo, providing it with energy for growth and development. Therefore, lipases are present in higher levels during the period of seed germination. Lipase enzyme is used in synthesis of fine chemicals, agrochemicals and new polymeric materials (Abhijit Ray 2012). The lipase shows a unique behavior and extraordinary characteristics. Among other oilseeds, where lipases are only active during germination, castor bean lipase is present throughout the germination process for both latent and sprouted seeds. (Alka Srivastava et al., 2016).

India, China, and Brazil are the three main producers of castor oil, that is extracted from castor beans annually. (Polyanna Nunes Herculano et al 2016). Castor oil is a thick, pale amber liquid extracted from the seed. (U.G

Akpan et al 2006). castor bean's therapeutic benefits are utilized due to phytochemical compounds including flavonoids, alkaloids and tannin(Abolfazl alirezalu et al., 2021).

The castor plant (Ricinus Communis L.) is a member of the Euphorbiaceae family and found abundantly in warm temperate, tropical, and subtropical regions throughout the world. The castor plant has a red, smooth, spherical stem with transparent sap. Castor leaves have a palmate arrangement, huge (above 10-75 cm), and resemble umbrella. Each leaf has five to eleven finger-like, pointed lobes with thick central veins and serrated edges. Green with white leaves, maroon leaves, black-purplish, green-bronze, and dark green with a reddish tinge are some variants in leaf color. Many researchers reported that lipases activities in plants are varied according to the species under study and the condition of extraction; therefore, the screening process and the determination of the optimum conditions of extraction are essential steps for the selection of plant species with the higher activity of lipases. Lipase enzyme is used in synthesis of fine chemicals, agrochemicals and new polymeric materials .(Abhijit Ray 2012).

Material and Methods

Seed Collection and germination studies: Castor seeds were collected from (Ricinus communis L.) the tree located near Devanahalli village, Bangalore district of Karnataka State, India. The collected seeds were soaked in water for 24 hrs and seeds were tied tightly in a white cloth and allowed for germination. The emergence of radicles was considered as an indication of germination and enzyme assay was conducted every 24 hrs.

Preparation of Plant Extraction: Following germination, 1gm of seed was taken and washed twice with distilled water. Subsequently, the seedcoat was manually removed and the seed cotyledons were homogenized using 10 ml of ice-cold mM Sodium phosphate buffer (all chemicals used were of AR grade and supplied by SDFCL) of pH of 7.2, using mortar and pestle. The resulting solution was then centrifuged at 6000 rpm for a duration of 15 min. The supernatant was used as a crude enzyme for conducting assays related to germination studies, enzyme activity and for partial purification purposes.

Lipase enzyme assay: The lipase activity was determined by the spectrophotometric assay method (Quinn et al., 1982; Shirai & Jackson, 1982). The chemicals used in the experiment were from (Sigma Aldrich and SDFCL). The reagents used were Reagent A, which consisted of a 100 mM Sodium phosphate buffer, 150 mM Sodium Chloride, and 0.25 % Triton X-100 at a pH of 7.2 (adjusted using 1M NaOH) at 37° C. Additionally, Reagent B was composed of 900 µl of Acetonitrile and 10 µl of p-nitrophenyl butyrate, while Reagent C comprised the Crude Lipase enzyme. The reaction conditions were maintained at a temperature of 37°C, a pH level of 7.2, with absorbance readings taken at 400 nm using a UV Spectrophotometer 400 nm with a Light path of 1 cm.

Purification of Lipase enzyme: Purification of Lipase enzyme carried out by three step purification method such as Salt precipitation by 70% ammonium sulphate precipitation followed by Dialysis. After dialysis enzyme sample is separated by Ion exchange Chromatography using DEAE Cellulose and Gel Filtration by Sephadex G 75

Enzyme characterization: The Spectrophotometric lipase activity assay is carried out with in pH, temperature, substrate concentration. Subsequently, a plot is constructed based on the data to determine the values of Km and Vmax.

Effect of pH: In the experimental setup, all reagents were maintained under standard conditions which was adjusted to pH of 5, 6, 7, 8, and 9. For the blank sample, each trial involved mixing 900 µl of Reagent A, 10 µl of distilled water and 100 µl of Reagent C. The absorbance of mixture was measured at 400 nm for a period of 5 min at an interval of 1min Similarly, for the test sample in each trial, 900 µl of Reagent A, 10 µl of Reagent B, and 100 µl of sample were measured at period of 5 mins at interval of 1 min and the enzyme activity was calculated.

Effect of Temperature: The relative activity of castor lipase enzyme at temperature (25-45 0C). The enzyme assay was done for both test and blank as same as the early mentioned procedure by enzyme sample, buffer and PNPB. Absorbance was measured at 400nm.

Substrate specificity: Substate specificity was determined by assaying the lipase activity under the different amount of PNPB from (5 -25). Enzyme assay was done by enzyme sample, buffer and PNPB Absorbance was measured at 400nm.

Molecular weight Determination: The molecular weight of the enzyme was determined using SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), the protocol outlined in (Sambrook & Russell, 2006), to the specific study.

Results and discussion

Lipase estimation assay and germination studies: The germination studies indicated that the highest enzyme activity was observed on the 4th day of germination, as presented in Table 1. Lipase enzyme activity is assessed through the enzyme assay employing the spectrophotometric method, as described in the studies by Shirai & Jackson, 1982 and Quinn et al., 1982.

Taxonomical position of the citrus species

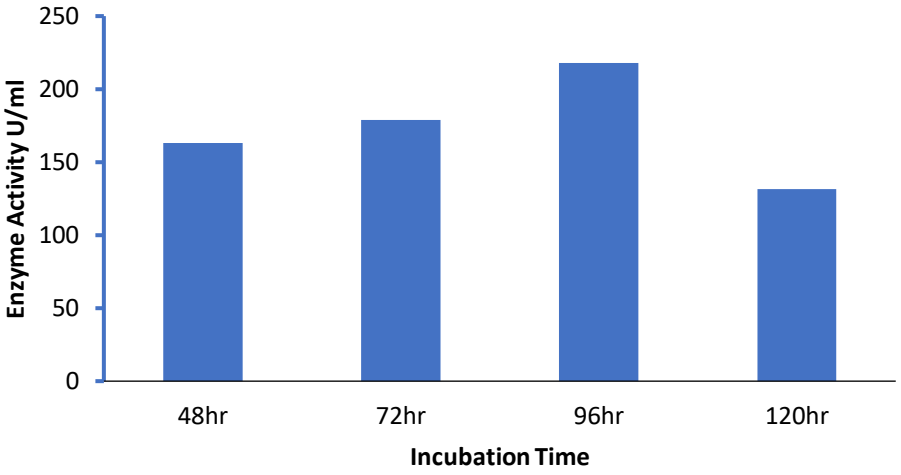

Fig 1: Enzyme activity at different incubation time.

Purification of Lipase enzyme: Cell free solution used for the enzyme activity for the crude was showed 263.23, Salt 312,53, Dialysis 334.16, Ion exchange 392.72 and after gel filtration activity showed 454.16, nearly 5.5 % of Fold purification increased.

Table 1: Fold Purification of Lipase enzyme

Sample	Enzyme Activity u/ml	Protein concentration(mg)	Specific Activity (U/mg)	Fold Purification %
Crude	263.23	10/2	25.80	1
Salt	312.53	9,9	31.56	1.22
Dialysis	334.16	9.1	36.72	1.42
Ion Exchange	392.72	6.2	63.34	2.45
Gel filtration	454.16	3.2	141.92	5.5

Effect of pH: pH plays a vital role in the enzyme activity to find out optimum pH. Enzyme assay was carried out different pH such as 5-9. Enzyme activity was carried out was shown at optimum at 8. But less activity was shown at 5. Activity increases up to 8 decreases after 9. The activity of hazelnut seed lipase showed a high dependence on the pH. The optimum pH of the enzyme was found to be 9.0 (Ismail Kilic and Ayten Sagiroglu 2012).

Effect of Temperature: Temperature important a vital role in the enzyme activity to find out optimal temperature. Enzyme assay was carried out different such as 25,30,35,40,45 °C. Enzyme activity was shown at optimum at 40 °C. But less activity was shown at 25°C activity increase upto 40 and decrease after 40 such as 40 °C. The optimal activity of enzyme was observed at 50 °C, when the assayed at pH 7.5, although the enzyme showed the ability to maintain high reactivity at lower temperature.

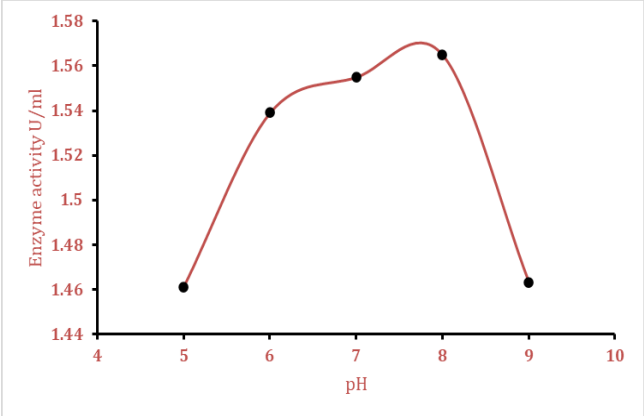

Fig 2: Effect of pH

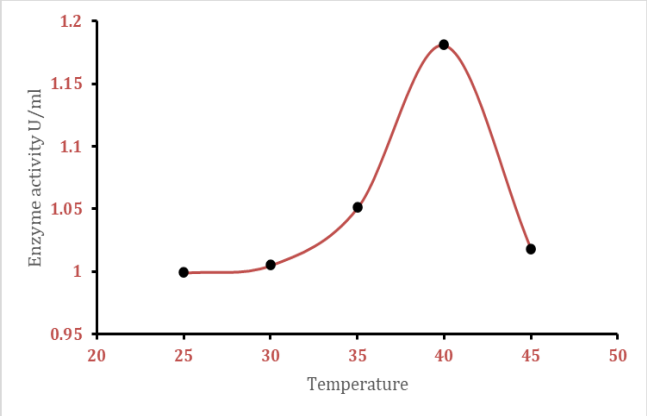

Fig 3: Effect of Temperature

Substrate Specificity: Substrate specificity play an essential role in the enzyme activity to find out optimum specificity. Enzyme activity was carried out in different substrate concentration such as 5-25 enzyme activity was shown at Vmax 250 mM, Km 140 mM.

Molecular Weight Determination: In the SDS-PAGE analysis, determination of molecular weights for the lipase proteins bands at various points along the purification process. Lane 1, designated as marker lane. Within lanes of crude, salt precipitation, dialysis, Ion Exchange, Gel filtration of lipase enzyme is highlighting bands with molecular weight of 48kDa. Overall, the partial purification process successfully eliminates unwanted protein and compounds from the crude enzyme. SDS-PAGE gel analysis reveals the molecular weight of the lipase enzyme to be 48kDa.this suggests that the lipase enzyme is likely a relatively large protein with multiple subunits or domains. The study focuses on the extraction, purification and characterization of lipase from germinated castor seeds.

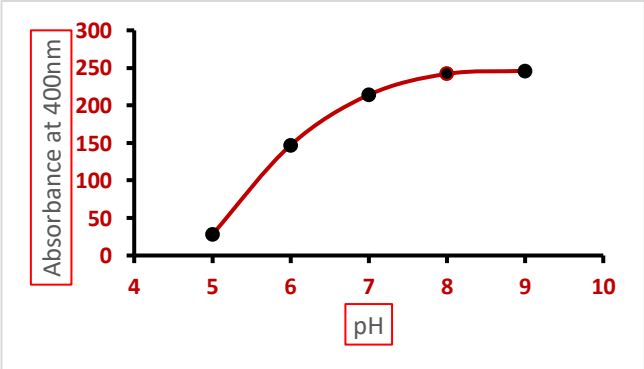

Fig 4: Substrate Specificity

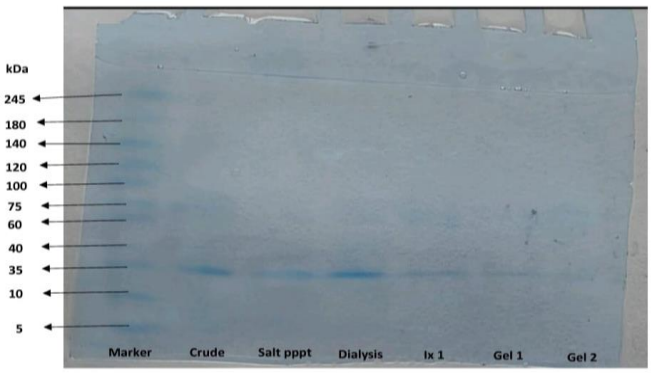

Fig 5: SDS PAGE

Conclusion

The comprehensive study on the extraction, partial purification, and characterization of lipase from germinating Ricinus Cummins L. seeds has yielded valuable insights and potential applications. The observed correlation between seed germination and lipase activity highlights the enzyme's role in utilizing stored triacyl glycerides (TAGs) as an energy source for seedling growth. The kinetic studies have illuminated the enzyme's stability and specific activity under distinct conditions. The temperature and pH profiles indicate the enzyme's adaptation to function optimally within the temperature range of 25°C to 45°C and a slightly alkaline pH environment (pH 7 to 8). These conditions align with the physiological processes of seed germination and early growth. The enzyme's efficient utilization of its substrate, as indicated by the low Km and high Vmax values, underscores its suitability for hydrolysing TAGS.

References

1. Abhijit Ray. Application of Lipase in Industry. Asian J. Pharm. Tech. 2(2): April-June 2012; Page 33-37.
2. Akpan, U.G., Jimoh, A. and Mohammed, A.D. (2006) Extraction Characterization and Modification of Castor Seed Oil. Leonardo Journal of Sciences, 8, 43-52
3. Alaa M. Dh. Al-Haidari et al., (2019) extraction and Purification of Lipases Enzyme from Germinating Seeds of four crop. Iraqi Journal of Science, 2020, Vol. 61, No. 9, pp: 2182-2188DOI: 10.24996/ijs.2020.61.9.4
4. Kılıç, I., & Sagıroglu, A. (n.d.). hazelnut seed lipase: extraction, purification, and characterization.
5. Mirzamohammad, E., Alirezalu, A., Alirezalu, K., Norozi, A., & Ansari, A. (2021). Improvement of the antioxidant activity, phytochemicals, and cannabinoid compounds of Cannabis sativa by salicylic acid elicitor. Food Science & Nutrition, 9(12), 6873–6881.
6. Patel, G. B., Shah, K. R., Shindhal, T., Rakholiya, P., & Varjani, S. (2021a). Process parameter studies by central composite design of response surface methodology for lipase activity of newly obtained Actinomycete. Environmental Technology & Innovation, 23, 101724.
7. Polyanna Nunes Herculano , Kelia , Taiana Souza Porto, Cristina Maria de Souza-Motta, Ana Lucia Figueirdo Porto Aparecida , Moreira , Raquel Pedrosa Bezerra2016) cellulase production by aspergillus japonicus urm5620 using waste from castor bean (Ricinus communis l.) under solid-state fermentation. Applied Biochemistry and Biotechnology, 2011.
8. Quinn, D. M., Shirai, K., Jackson, R. L., & Harmony, J. a. K. (1982). Lipoprotein lipase-catalyzed hydrolysis of water-soluble p-nitrophenyl esters. Inhibition by apolipoprotein C-II. Biochemistry, 21(26), 6872–6879.
9. Sambrook, J., & Russell, D. W. (2006). Isolation of High-molecular-weight DNA from Mammalian Cells Using Proteinase K and Phenol. Cold Spring Harbor Protocols, 2006(1), pdb.prot4036
10. Srivastava A, Mathur R. M, Prakash R, Agrawal S. Studies on Ricinus Lipase Enzyme Isolated from Castor Seeds. Orient J Chem 2016;32(2). Studies on Ricinus Lipase Enzyme Isolated from Castor Seeds.

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