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THE ISOLATION AND CHARACTERIZATION OF LIPASE FROM *CARICA PAPAYA* LATEX USING ZWITTERION SODIUM LAUROYL SARCOSINATE AS AGENT

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ABSTRACT

Most of industrial lipases are derived from microbial sources, following by a wide variety of plants. Among plant lipases, lipase from *Carica papaya* latex has been the focus of intense and growing research due to low cost, easy acceptance by consumers and its unique characteristics. This enzyme has been successfully applied for lipid modification and synthesis of some organic compounds. However, research for its molecular structure has been limited due to the difficulty to isolate the enzyme from the latex matrix. In this study, we suggested a modified approach using sodium lauroyl sarcosinate to solubilize the latex, then the protein was precipitated by ammonium sulphate. We also carried out the characterization of the lipase obtained from *Carica papaya* latex. The results showed that freeze-drying the fresh latex could improve significantly lipase activity of latex powder in comparison with sun-drying or oven-drying. The zwitterion sodium lauroyl sarcosinate could solubilize nearly 50% of the latex and the achieved supernatant exhibited great lipase activity. There was no need to use an organic solvent to delipidate the latex prior to solubilization with sodium lauroyl sarcosinate due to possible denaturation of enzymes. The proteins which were fractionally precipitated with 50 – 60%, 60 – 70% and 70 – 80% ammonium sulphate saturation showed lipolytic activity. The fraction from 50 – 60% saturation with the greatest mass was subjected to ion exchange chromatography, SDS electrophoresis and kinetic parameter determination. The results showed the presence of two proteins with molecular mass ranging from 35 kDa to 55 kDa and both presented lipase activity. The K_m and V_{max} of the lipase fraction from 50 – 60% saturation was 1.12 mM and 1.2 x 10^{-6} mM.min^{-1}.mL^{-1} respectively. So, the freeze-drying of papaya latex could help to preserve its lipase activity and the usage of sodium lauroyl sarcosinate could improve the isolation of the lipase from the papaya latex and pave the way for research on the molecular structure of *Carica papaya* latex lipases.

Keywords: lipase; papaya; enzyme isolation; enzyme purification

INTRODUCTION

Lipases are the most applied enzyme in organic synthesis due to their broad substrate acceptance and availability. They are serine hydrolase defined as triacylglycerol acyl hydrolase (E.C.3.1.1.3) and should be distinguished from esterase by the nature of their substrates. While lipases possess the ability to hydrolyse long-chain acylglycerols (>10 carbon atoms), esterases are capable of hydrolysing short-chain ones (<10 carbon atoms) **(de María et al., 2006; Casas et al. 2012).** Lipases in the industry are mainly isolated from microorganisms and partly from plants. Plant lipases with the advantages of low cost, easier acceptance in food and medicine products by consumers and unique characteristics have attracted more and more attentions in research, especially lipases from *Carica papaya* latex (CPL) **(Campillo and Tovar, 2013)**. The *Carica papaya* latex was well known to contain various proteases and chitinases long time ago **(Azarkan et al., 2003)**, however the lipolytic activity of *Carica papaya* was discovered by Frey-Wyssling until 1935. The more information about this hydrolase emerged in the early 1990s mostly on their characterization and utilization **(de María et al., 2006)**. It has been reported that the papaya latex contains several hydrolases which could hydrolyse triacylglycerol of both short- and long-chain fatty acids with preference to the short-chain ones. Despite of the great success in the discovery of possible utilization of CPL lipase in industry, research in the molecular structure of CPL lipase has been limited because of the "naturally immobilized nature" of the enzyme, i.e. the enzyme is contained in a complex matrix of the papaya latex, which is difficult to dissolve. There were some unsuccessful researches to solubilize the enzymatic activity of this latex fraction **(Abdelkafi et al., 2011)**. In 2009, **Abdelkafi et al. (2009)** used zwitterion CHAPS and sonication to solubilize the latex and then separated protein fractions with lipolytic activity. The obtained enzymes were determined to be esterase rather than lipase and therefore latex lipase could lose its activity during separation**.**

In this study, we applied the freeze-drying to the latex as soon as possible to limit solidification of papaya fresh laticifer. The modified separation procedure from **Abdelkafi et al. (2009)** were used to isolate lipase from CPL latex and to characterize the obtained lipase.

Scientific hypothesis

The hypothesis was that drying method would affect the solidification of latex laticifer and the entrapment of lipase. Using freeze-drying to obtain papaya latex powder could improve the lipase isolation. The zwitterion sodium lauroyl sarcosinate was expected as an effective agent in latex solubility to produce protein fractions with high lipase activity.

MATERIAL AND METHODOLOGY

Chemicals

Sodium lauroyl sarcosinate (purity 97%), *p*-nitrophenyl palmitate (purity 98%), *p*-nitrophenol (purity 99%), NaCl (purity 99.5%), *n*-hexane, *2*-propanol (purity 99.8%) and ammonium sulfate (purity 99%) were purchased from Sigma–Aldrich, Singapore; Tris base (purity 99.9%) and Triton X-100 were provided by Bio Basic, Canada. All other chemicals used were of analytical grade.

Preparation of CPL powder

Papaya latex was collected between 6 am and 8 am from unripe fruits grown in Quang Nam province, Vietnam. The 4 – 6 incisions were made along with the fruits by stainless steel knife and the latex was let drain into plastic bottles and stored in cold box with ice and frozen within 4 hours for freeze-drying. The CPL powder was obtained after grinding of dried matter. The latex was also sun dried and dried in an oven at 40 °C until constant weight and then ground into CPL powders. These powders were compared for their specific lipase activity and the most active powder was used for other experiments.

Preparation of crude CPL lipase

The 3 grams of CPL powder were weighed into 100 mL of distilled water and mixed for 3 minutes. The solution was centrifuged at 6000 rpm at 4 °C in 20 minutes using Hettich universal 320R centrifuge (Germany). The supernatant was disposed, and the precipitate was collected. These steps were repeated for 3 times and the final precipitate was freeze-dried in Alpha 1-2 LDplus freeze dryer (Germany) at -43 °C, 24 h to obtain crude CPL lipase.

Solubilisation of crude CPL lipase

One gram of crude CPL lipase was suspended in the 50 mL of the mixture of n-hexane and 2-propanol (1:1 v/v) and shaken in 30 minutes at 4 °C to extract lipid from the latex. It was then centrifuged at 7500 rpm in 20 minutes. The solvent residue was eliminated from the precipitate in vacuum rotary evaporator to obtain the lipid-free CPL lipase. This residue was then suspended into 50 mL solution of Tris–HCl 0.1M pH 8, prior mixed with the 0.5% (w/v) sodium lauroyl sarcosinate. The suspension was shaken for 30 minutes and then centrifuged at 7500 rpm in 20 minutes at 4 °C to obtain both supernatant and precipitate. These 2 fractions were used in other experiments.

Separation of lipolytic fractions from partially purified CPL lipase

Protein in supernatant collected after solubilizing crude CPL lipase in SLS was fractionally precipitated by using ammonium sulfate crystal. The precipitation was separated by ammonium sulphate saturation in the range of 0 – 90% (v/v). Precipitation was allowed to occur at 4 °C for 1 h and followed by centrifugation at 7500 rpm for 20 mins to obtain protein precipitate. All of the precipitate samples were dissolved in the solution of Tris HCl 0.1M, pH 8 and lipase activity was measured **(Burgess, 2009)**. The fraction with the highest activity was subjected to dialysis using cellophane to eliminate salts at 4 °C for 48h. The obtained solution was used for chromatographic and electrophoretic analysis.

Chromatographic separation of lipase

Samples after dialysis were loaded on the HiTrap Q Sepharose Fast flow column (5.0 mL, 1.6 cm × 2.5 cm) of the flash protein liquid chromatography system (GE Akta Purifier 100, Sweden). The column was firstly equilibrated with starting buffer of Tris-HCl pH 9. The sample was then eluted with an increase of NaCl concentration from 0 to 1 M at rate 0.1 M NaCl.min^{-1}. The flow rate was set at 1 mL.min^{-1}. The eluent was measured for absorbance at 280 nm and collected in fraction tubes of 1 mL volume.

SDS polyacrylamide electrophoresis

The molecular mass of the purified lipase was determined by SDS-PAGE as described by **Minaev and Makhova (2019)** using 15% acrylamide gel, prestained dual colour protein molecular weight marker (10 – 170 kDa).

Determination of K_m and V_{max} of purified CPL lipase

The Lineweaver–Burk plots were used to determine the Michaelis–Menten constant (K_m) and the maximum velocity for the reaction (V_{max}) of lipase for *p*-nitrophenyl palmitate at pH 8.0, using a spectrophotometric method. Briefly, assays with lipases were performed in 0.1M Tris–HCl buffer, pH 8.0 at 40 °C with increasing concentrations of *p*-nitrophenyl palmitate from 0.397 mM to 3.973 mM, to calculate K_m and V_{max} **(Holme and Peck, 1998)**.

Lipase activity measurements

Lipase activity was measured spectrophotometrically using an assay based on the enzymatic hydrolysis of *p*-nitrophenyl palmitate (pNPP) to form *p*-nitrophenol (pNP) whose absorbance was measured at 410 nm (Jenway 6305, UK) **(Palacios et al., 2014)**. One activity unit (U) of lipase was defined as 1 µmol *p*-nitrophenol produced per minute under the assay condition.

Briefly, solution A containing 30 mg pNPP in 10 mL propan-2-ol and solution B containing 180 mL Tris-HCl buffer (0.1 M; pH 8.0), 720 µL Triton X-100 and 180 mg gum Arabic were prepared. The mixture of 4860 µL of solution B and 540 µL of solution A were incubated at 40 °C, then added with 0.0015 g enzyme for the reaction to take place. The absorbance was measured spectrophotometrically at 410 nm after 10 minutes and the

amount of hydrolysed *p*-nitrophenol was determined from the standard curve prior built with pNP standard.

Proximate analysis of papaya latex

Proximate composition of papaya latex was analysed using appropriate methods: AOAC 927.05 for moisture, AOAC 425.06 for ash, AOAC 2011.04 for protein and AOAC 948.15. for lipid.

Statistical analysis

Experiments were done in triplicates and the results were expressed as mean values. ANOVA and the Duncan Test was applied to assess significant difference at a significant level of α 0.05 using software Minitab 16.0.

RESULTS AND DISCUSSION

The impact of drying method on lipase activity of latex powder

Papaya latex collected from the fruit was analysed for its proximate composition. The results were shown in Table 1.

Table 1: Proximate analysis of papaya latex.

Moisture (%)	Ash (%)	Protein (%)	Lipid (%)
77.53	2.07	14.6	1.86

Table 2: Effect of drying method on lipase activity of papaya latex powder.

Drying method	Sun-drying	Oven-drying	Freeze-drying
Moisture (%)	11.8	11.3	9.13
Specific activity ($mU.g_{-1}$)	27.68	18.22	120.35

Table 3 Lipolytic activity of fractions from crude CPL lipase after delipidation and centrifuging.

Samples	Crude lipase	Precipitate	Supernatant
Mass/ Volume	1.0120 (g)	0.5632 (g)	45 (mL)
Specific activity	108.55 ($mU.g_{-1}$)	17.30 ($mU.g_{-1}$)	0.19 ($mU.mL_{-1}$)
Total activity	109.85 (mU)	9.74 (mU)	8.55 (mU)

Table 4 Lipase activity of fractions from crude CPL lipase after centrifuging without delipidation.

Samples	Crude lipase	Precipitate	Supernatant
Mass	1.016 (g)	0.498 (g)	97 (mL)
Specific activity	115.07 ($mU.g_{-1}$)	20.83 ($mU.g_{-1}$)	50.16 ($mU.mL_{-1}$)
Total activity	116.91 (mU)	10.37 (mU)	4865.52 (mU)

The fresh latex contained a high amount of water, protein and low lipid. High protein content could be linked to the presence of various enzymes and the high water content may explain for its flowability. This result was in accordance with **Macalood et al. (2013)**.

However, the papaya latex tends to solidify quickly in the air, and this may entrap lipase and affect the lipolytic activity of the latex. In this study, the papaya latex was dried in 3 different methods until its constant mass as described in 2.2.1. The dried latex was ground to powder and measured for their lipolytic activity. The results were presented in Table 2.

The results showed that long time of air exposure at high temperature had a significant negative impact on the lipase activity (p <0.001). The freeze-drying gave the highest quality powder, which achieved four-time higher of specific activity than those from sun-drying or Oven-drying. Freeze-drying was also confirmed to be a good method for preserving activity of lipase from *Yarrowia lipotica* **(Darvishi et al., 2012)**.

Partial purification of crude CPL lipase

Partial purification of crude CPL lipase including delipidation

The crude CPL lipase was partially purified according to method described by **Abdelkafi et al. (2009**) using hexane:propanol (1:1) to delipidate. After centrifuging, 45.0 mL of supernatant and 0.5632 g of the precipitate was measured for lipolytic activity, which is illustrated in Table 3.

The data showed that sodium lauroyl sarcosinate could solubilize about 44.3% of the crude lipase mass. Both supernatant and precipitate possessed lipolytic activity, but their specific activities were apparently reduced by approximately 80.6% in comparisons with the origin of crude lipase. The reason for this loss could be the denaturation of lipases in precipitate or in supernatant caused by solvent during the lipid extraction step.

This result suggested that the zwitterionic SLS could solubilize nearly half crude CPL lipase and extraction lipid step might be ineffective and unnecessary.

Partial purification of crude CPL lipase without delipidation

The previous procedure was repeated with the modification of omitting lipid extraction step. The results are presented in Table 4. The data show that precipitate and supernatant contained lipolytic activity. The activity remained in precipitate was just a small portion of the origin activity in crude lipase. However, the total lipolytic activity in the supernatant was 40-fold increased, compared to the activity of crude lipase. This sharp increasing suggests that a significant amount of lipase in the latex was isolated from the matrix by zwitterionic SLS to dissolve part of the latex matrix. The enzyme was more active in free form than in the immobilized form in the matrix and might not be negatively affected by the zwitterionic nature of SLS at used concentration.

Effect of sodium lauroyl sarcosinate concentration on the extraction yield of lipolytic activity from crude CPL lipase

Crude CPL lipase was suspended in zwitterionic SLS in 30 minutes at different concentrations. Figure 1 presents the

effect of SLS concentration on supernatant lipolytic activity.

It could be confirmed that SLS concentration affected significantly on the lipolytic activity of the obtained supernatant (p <0.005). The higher specific lipolytic activity was achieved with the increasing SLS concentration from 0.25% to 0.5% but then decreased with concentration ranging from 0.5% to 1%. SLS is a surfactant with ionic nature.

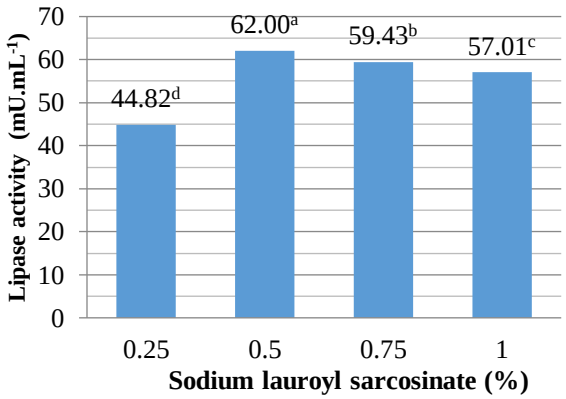

Figure 1 Effect of SLS concentration (%) on lipolytic enzyme extraction.

Table 5 Lipolytic activity of fractions from crude CPL.

Saturation (%)	Precipitate (g)	Lipase activity
10 – 40	0.000	-
40 – 50	0.050	-
50 – 60	0.300	+
60 – 70	0.102	+
70 – 80	0.031	+
80 – 90	0.000	-

"+": present; "- ": absent

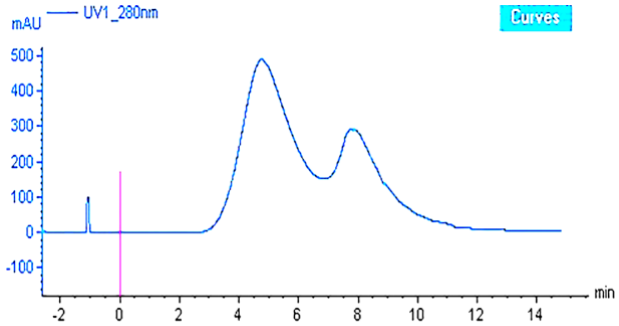

Figure 2 Chromatograph of 50 – 60% fraction precipitate.

The increasing concentration until 0.5% could solubilize the latex matrix to liberate lipase to increase the activity of the supernatant. However, the higher concentration than 0.5% of this zwitterion might cause denaturation of lipase, leading to the decrease of lipolytic activity of the supernatant. The highest lipolytic activity of 62.00 mU.mL^{-1} in the supernatant was achieved with 0.5% SLS solution.

Isolation of lipase from crude CPL lipase

Fractional precipitation of CPL lipase with ammonium sulphate

The ammonium sulphate was used at different saturation levels to precipitate fractionally the CPL lipase from the supernatant. Table 5 presents the weights and lipolytic activity of different precipitates.

No precipitate was visible after adding $(NH_4)_2SO_4$ to 40% of saturation. All proteins were sedimented in ammonium sulphate solution with saturation ranging from 50% to 90%. Only the fractions by 50 – 60%, 60 – 70% and 70 – 80% saturation showed lipolytic activity.

This result complied rather well with the work of **Paques et al. (2008)**, regarding to saturation level of ammonium sulphate used for precipitation of lipase from papaya seed and skins. Lipases were also found in fraction of 40-80% saturation.

As the first attempt in this study, the fraction achieved by 50 – 60% saturation presented good lipolytic activity and the highest weight was examined for further purity by ion exchange chromatography, electrophoresis and kinetic determination.

Chromatographic analysis of isolated lipase fraction 50-60%

The Figure 2 illustrates the chromatograph of lipase fraction 50 – 60%. It showed the presence of at least 2 proteins in the sample.

The first peak was eluted from 2.58 min to 6.87 min, following by the second peak appeared from 6.87 min to 11.29 min. Accordingly, the first and the second peak fractions had the lipase specific activity of 0.316 mU.mL^{-1} and 0.338 mU.mL^{-1} respectively.

Electrophoretic analysis of isolated lipase fraction 50-60% from $(NH_4)_2SO_4$ precipitation

The proteins from fraction 50 – 60% were also subjected to SDS electrophoresis. The result in Figure 3 showed the presence of 2 bands from all 3 triplicate samples of dialyzed 50 – 60% fraction, suggesting there were 2 proteins in every sample. One protein had a molecular weight in the range of 35 – 40 kDa, while this value in the second protein was 40 – 55 kDa. This result complied very well with the previous result of chromatographic analysis. It can be concluded that there were 2 lipases with a molecular weight ranging from 35 – 55 kDa in the fraction 50 – 60%.

The molecular weights of these papaya lipases are similar to lipases of some microbial and plant lipases, such as 40 kDa of purified lipase from *Microbacterium* sp. **(Tripathi et al., 2014)** or 40 kDa of lipase from Tunisian *Euphorbia peplus* latex **(Lazreg et al., 2014)**. Meanwhile the native molecular weight of lipase from *Raphia* mesocarp was 35 kDa **(Okunwaye et al., 2015)**.

Determination of K_m and V_{max} of lipase fraction 50 – 60%

To study the enzyme–substrate affinity, the kinetic parameters of the lipases to *p*-nitrophenyl palmitate were determined. The K_m and V_{max} values for lipase activity with *p*-nitrophenyl palmitate were calculated from Lineweaver–Burk plots constructed by using activity values depending on substrate concentrations. The Lineweaver–Burk plots

were linear and indicated that hydrolysis of *p*-nitrophenyl palmitate by the tested lipase followed Michaelis–Menten kinetics (Figure 4).

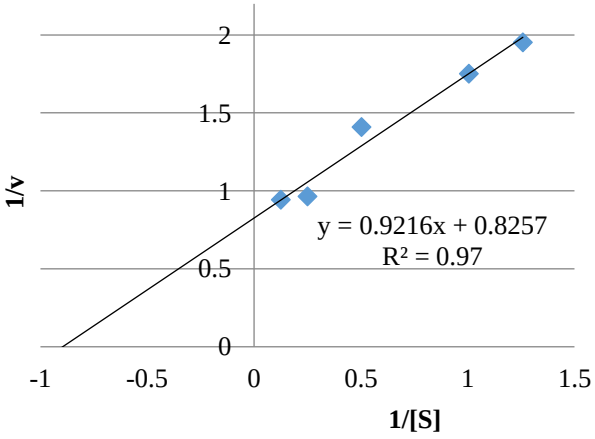

Figure 4 Lineweaver-Burk plot for determination of K_m (mM) and V_{max} (mM.min^{-1}.mL^{-1}) of papaya lipase.

The result of K_m and V_{max} calculation for purified lipase from papaya latex from Lineweaver-Burk plot were 1.12 mM and 1.2 x 10^{-6} mM.min^{-1}.mL^{-1}, respectively. The value of K_m depends on the type of substrate and on environmental conditions such as pH, temperature, ionic strength and polarity. K_m is also a measure of the strength of the ES complex or the affinity of the enzyme for substrate. The smaller the K_m, the bigger the enzyme-substrate affinity, the faster the reaction rate. The K_m of the purified papaya lipase showed its higher affinity to pNPP than lipase from *Microbacterium* sp. which had K_m of 3.2 mM as determined in the work of **Tripathi et al. (2014)**. However, its ability to form the complex with pNPP was not as good as that from the lipase of the thermophilic *Bacillus stearothermophilus* MC 7, whose K_m was only 0.33 mM **(Kambourova et al., 2003)** or from *Acinetobacter sp.* AU07 whose lipase K_m was determined to be 0.51 mM **(Gururaj et al., 2016)**. In the other work, the K_m and V_{max} of lipase from raphia palm fruit mesocarp were calculated as 0.01 mM and 20.5 µmol.min-1.mL^{-1} respectively **(Okunwaye et al., 2015)**.

CONCLUSION

Papaya latex lipase is so far the only plant lipase used commercially in many applications. However, the molecular characteristics of this lipase are less known due to its immobilized nature in the papaya latex. This study has shown the possibility to liberate substantially lipolytic activity from the latex. It is suggested to freeze-dry the latex as soon as possible to preserve lipase activity and then use sodium lauroyl sarcosinate to dissolve the latex without delipidation step to avoid possible denaturation of lipase. The purified enzyme could be obtained using traditional fractional precipitation of protein with ammonium sulphate. In this study, the concentration of sodium lauroyl sarcosinate determined of 0.5% (w/v) gave the highest lipolytic activity. Two lipases were found in the papaya latex with molecular mass ranging from 35 – 55 kDa.

The K_m and V_{max} of lipase fraction obtained from precipitation using 50 – 60% saturation of ammonium sulphate was 1.12 mM and 1.2 x 10^{-6} mM.min^{-1}.mL^{-1} with *p*-nitrophenyl palmitate as substrate.

Further study should be carried out to isolate lipases from other precipitate fractions with ammonium sulphate and characterize isolated lipase.

REFERENCES

Abdelkafi, S., Ogata, H., Barouh, N., Fouquet, B., Lebrun, R., Pina, M., Scheirlinckx, F., Villeneuve, P., Carrière, F. 2009. Identification and biochemical characterization of a GDSL-motif carboxylester hydrolase from *Carica papaya* latex. *Biochimica et Biophysica Acta*, vol. 1791, p. 1048-1056. https://doi.org/10.1016/j.bbalip.2009.06.002

Abdelkafi, S., Barouh, N., Fouquet, B., Fendri, I., Pina, M., Scheirlinckx, F., Villeneuve, P., Carrière, F. 2011. *Carica papaya* Lipase: A Naturally Immobilized Enzyme with Interesting Biochemical Properties. *Plant Foods Hum Nutr.*, vol. 66, p. 34-40. https://doi.org/10.1007/s11130-010-0206-0

AOAC 927.05. *Moisture Determination*.

AOAC 425.06. *Ash Determination.*

AOAC 2011.04. *Protein Determination.*

AOAC 948.15. *Lipid Determination*.

Azarkan, M., El Moussaoui, A., van Wuytswinkel, D., Dehon, G., Looze, Y. 2003. Fractionation and purification of the enzymes stored in the latex of *Carica papaya*. *Journal of Chromatography B*, vol. 790, p. 229-238. https://doi.org/10.1016/S1570-0232(03)00084-9

Burgess, R. 2009, Protein Precipitation Techniques. In Burgess, R., Deutscher, M. *Guide to Protein Purification*. Cambridge, USA : Academic Press, p. 332-342. ISBN 9780080923178. https://doi.org/10.1016/S0076-6879(09)63020-2

Campillo, A. G., Tovar, M. A. 2013. Recent advances and applications of the lipolytic activity of *Carica papaya* latex. *Journal of Molecular Catalysis B: Enzymatic*, vol. 90, p. 49-60. https://doi.org/10.1016/j.molcatb.2013.01.015

Casas, G. L., Duquesne, S., Bordes, F., Sandoval, G., Marty, A. 2012. Lipase: An Overview. In Sandoval G. *Lipase and Phospholipase-Methods and Protocols*. New Jersey, USA : Humana Press, p. 3-30. ISBN 978-1-61779-599-2. https://doi.org/10.1007/978-1-61779-600-5_1

Darvishi, F., Destain, J., Nahvi, I. 2012. Effect of additives on freeze-drying and storage of *Yarrowia lipolytica* Lipase. *Appl. Biochem. Biotechnol.*, vol. 168, p. 1101-1107. https://doi.org/10.1007/s12010-012-9844-z

Gururaj, P., Ramalingam, S., Devi, N. G., Gautam, P. 2016. Process optimization for production and purification of a thermostable, organic solvent tolerant lipase from *Acinetobacter* sp. AU07. *Braz. J. Microbiol.*, vol. 47, no. 3, p. 647-657. https://doi.org/10.1016/j.bjm.2015.04.002

Holme, P. D. J., Peck, H. 1998. *Analytical Biochemistry*. 3rd ed. Harlow, England : Pearson Education Limited, 507 p. ISBN-0 582 29438-X.

Kambourova, M., Mandeva, N., Derekova, A. 2003. Purification and properties of thermostable lipase from a thermophilic *Bacillus stearothermophilus* MC 7. *Journal of Molecular Catalysis B: Enzymatic*, vol. 22, no. 5-6, p. 307-313. https://doi.org/10.1016/S1381-1177(03)00045-6

Lazreg, H. A., Mosbah, H., Fekih, A., Kenani, A. 2014. Purification and biochemical characterization of lipase from Tunisian *Euphorbia peplus* latex. *J. Am. Oil Chem. Soc.*, vol. 91, p. 943-951. https://doi.org/10.1007/s11746-014-2444-z

Macalood, S. J., Vicente, J. H., Boniao, D. R., Gorospe, G. J., Roa, C. E. 2013. Chemical Analysis of *Carica papaya* L. Crude Latex. *American Journal of Plant Sciences*, vol.4, no. 10, p. 1941-1948. https://doi.org/10.4236/ajps.2013.410240

de María, P. D. D., José, V. S., Tsai, S. W., Andrés, R. A. 2006. *Carica papaya* lipase (CPL): An emerging and versatile biocatalyst. *Biotechnology Advances*, vol. 24, p. 493-499. https://doi.org/10.1016/j.biotechadv.2006.04.002

Minaev, M., Makhova, A. A. 2019. Recombinant metalloprotease as a perspective enzyme for meat tenderization. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, 628-633. https://doi.org/10.5219/1087

Okunwaye, T., Obibuzor, U. J., Okogbenin, A. E. 2015. Purification and biochemical properties of lipase from raphia palm fruit mesocarp. *African Journal of Biochemistry Research*, vol. 9, no. 5, p. 73-80. https://doi.org/10.5897/AJBR2015.0818

Palacios, D., Busto, D. M., Ortega, N. 2014. Study of a new spectrophotometric end-point assay for lipase activity determination in aqueous media. *LWT - Food Science and Technology*, vol. 55, no. 2, p. 536-542. https://doi.org/10.1016/j.lwt.2013.10.027

Paques, F. W., Pio, T. F., Carvalho, P. de O., Macedo, G. A. 2008. Characterization of the lipase from Carica papaya residues. *Braz. J. Food Technol*, vol. 11, no. 1, p. 20-27.

Tripathi, R., Singh, J., Barti, K. R., Thakur, S. I. 2014. Purification and characterization of lipase from *Microbacterium* sp. and its application in biodiesel production. *Energy Procedia*, vol. 54, p. 518-529. https://doi.org/10.1016/j.egypro.2014.07.293

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