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Athira VB
PG Scholar, Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Balakrishnan
Professor, Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Karthikeyan
Professor, Center for Post-Harvest Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Marimuthu
Assistant Professor, Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Ganapathy
Professor, Department of Food Process Engineering Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
Athira VB
PG Scholar, Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Investigation on enzyme activity of lipase from papaya (*Carica papaya*) latex

Athira VB, M Balakrishnan, S Karthikeyan, S Marimuthu and S Ganapathy

Abstract
In this study, lipase was extracted from *Carica papaya* latex and its enzymatic activity was evaluated at different time (6-24 h), temperature (35-50 ℃), and enzyme concentration (5-25%). The lipase was extracted from papaya latex by the conventional extraction method. The papaya latex was collected and further processed by centrifugation, washing, and lyophilization to get agglomerated *Carica papaya* lipase powder. The enzyme activity of extracted lipase was evaluated with tween 20 and coconut oil as substrates. The enzyme activity was maximum at the time of 12 h of incubation period, temperature of 45 °C, and with an enzyme concentration of 20% (w/w of substrate). This study revealed that Extracted *Carica papaya* lipase exhibited a powerful lipase activity for catalyzing hydrolysis (esterification and interesterification type reactions). The maximum enzyme activity for both substrates was seen after 12 hours of incubation. The maximum enzyme activity of lipase was 66.87 U/mL and 63.45 U/mL respectively when tween 20 and coconut were used as the substrates for 12 h time period. For both substrates, 45 °C temperature resulted in the highest lipase activity. When using tween 20 as the substrate, the lipase activity of 37.87 U/mL was attained at a temperature of 45 °C. When coconut oil served as the substrate, the enzyme activity peaked at 35.71U/mL at the same temperature. The enzyme concentration with 20% substrate for both substrates produced the highest level of lipase activity. Using tween 20 as the substrate, the enzyme had a lipase activity of 33.54 U/mL at a 20% concentration. At the same enzyme concentration, the lipase activity with coconut oil as the substrate was 30.30 U/mL. The extracted *Carica papaya* lipase can be used for the synthesis of monolaurin from coconut oil by enzymatic glycerolysis.

Keywords: *Carica papaya* lipase, enzyme activity, monolaurin, coconut oil

Introduction
Lipases are stable and belong to the general class of enzymes that break down fat molecules. The catalytic conversion of triglycerides to di-or mono-glycerides, fatty acids, and glycerol is catalyzed by lipase (Stergiou *et al.*, 2013) [13]. They are commonly obtained from animals, plants, or recombinant microorganisms and have a broad range of applications in the food and pharmaceutical industries as significant biocatalysts. The most commercially used lipases are Novozym 435, Lipozyme RM IM, and Lipozyme TL IM. These lipases are costly hence their uses are limited to some extent. Lipase a new source of cheap lipase is one of the keys to achieving a wide variety of reactions like hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification, and aminolysis.

The plant lipases are cheap and easily available. They are generally more accepted for food and medicinal applications. The plant sources of lipases such as *Carica papaya* latex are of considerable interest because of their potential industrial applications (Abdelkafi *et al*., 2011) [1]. The *Carica papaya* tree releases latex abruptly if incisions are made onto the aerial parts, especially from the unripe fruit. Papaya latex is a thixotropic fluid with a milky appearance that contains about 85% water and an insoluble particulate fraction that makes up 25% of the dry matter (Moussaoui *et al*., 2001) [6]. The lipase activity is located in the non-water-soluble fraction of papaya latex since it is naturally bound and immobilized to the non-soluble matrix (Villeneuve, 2003) [17]. In the search for plant-based lipase, *Carica papaya* lipase with high potential enzyme activity can be used for the extraction of monolaurin from coconut oil. The optimized condition such as time, temperature, and concentration can be utilized for the enzymatic glycerolysis of coconut oil for the extraction of monolaurin.

In this study, the enzyme activity of lipase extracted from papaya latex at various temperatures, enzyme concentrations, and time has been investigated.

Materials and Methods

Raw materials

Tween 20, tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), and CaCl2 were obtained from commercial sources in analytical grade. Lipase was extracted from *Carica papaya* (lyophilized powder). Virgin coconut oil was purchased from the supermarket. All experiments were carried out under standard ambient temperature and pressure (SATP). All the experiments were performed in triplicate.

Lipase buffer: Lipase buffer was prepared with 20mmol/l Tris-HCl, 80mmol/l $CaCl_2$, and 1% Tween 20.

Preparation of *Carica papaya* Lipase

The Papaya latex was collected from the orchard in Horticulture & Research Institute, Tamil Nadu Agricultural University, Coimbatore. Latex is obtained by cutting the skin of the unripe papaya fruit (70-100 days), then collecting the latex which flows from the cuts, latex collection was done during the morning hours. Tapping the fruit at this time is conducive to getting higher yields of latex. Two or three vertical cuts 1-2 mm deep are made which meet at the base of the fruit. The incisions are made using a stainless steel razor blade. The exuded latex was allowed to run down the fruit and drip into the collecting 150× 25 mm petri dish. The collected papaya latex was transferred into a 500 ml glass bottle and stored at -20 ℃ in the deep freezer until it became ready to use. Hard latex was defrosted at room temperature and centrifuged for 15 minutes at 9,500 rpm. The sediments were washed with distilled water. The washed latex was lyophilized to obtain a powder containing more than 95% dry matter (Pinyaphong *et al*., 2012) [10].

The standard method based on Tween 20

The standard method based on Tween 20 for lipase was used for the determination of lipolytic activity. The experiment was carried out with tween 20 and coconut oil as the substrate to determine the lipase activity of extracted *Carica Papaya* lipase. The enzyme activity was determined spectrophotometric ally. Substrates of tween 20 and coconut oil of 1% were added to the buffer and mixed with lipase (200 U) the absorbance of the solution was measured over a time of 6-24 h, and enzyme concentrations of 5-25% of substrates, and temperature of 35-50 °C at a wavelength of 450 nm (Valek *et al*., 2019) [16].

Effect of incubation time on lipase activity

The reaction mixture consisting of enzyme, oil emulsion substrate, and lipase buffer was incubated for 6, 12, 18, and 24 h in a conical flask. The enzyme activity was measured by UV Spectrophotometer (UV1800ENG40V, SOFT, Shimadzu, Singapore, S.N A11635480376) to determine the optimal time for incubation.

Effect of incubation temperature on lipase activity

The optimal incubation temperature for maximal lipase activity was evaluated by incubating the reaction mixture at different temperatures of 35, 40, 45, and 50 ℃, then lipase activity was measured (Haidari *et al*., 2019) [18]

Effect of enzyme concentration on lipase activity

Optimal enzyme concentration which supports maximal lipase activity was evaluated using different concentrations of lipase 5%, 10%, 15%, 20%, and 25% (w/w % of the substrate), then lipase activity was measured (Pinyaphong *et al*., 2012) [10].

Results and Discussion

Effect of incubation time on enzyme activity: Enzyme lipolysis was performed to assess the effect of reaction time (6-24 h) on the enzyme activity of Carica *papaya* lipase by the standard method based on Tween 20.

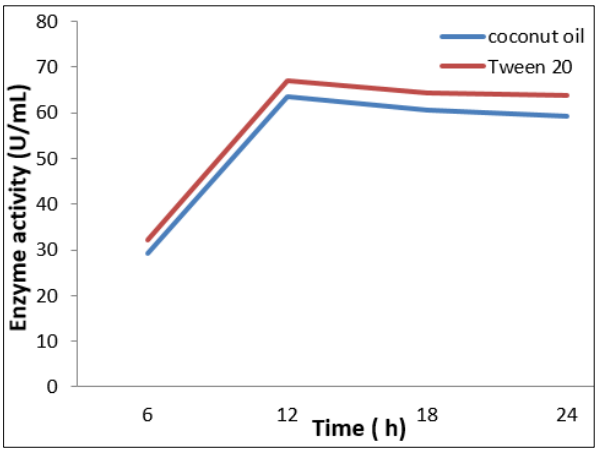

Fig 1: Effect of incubation time on enzyme activity

The rate at which an enzyme catalyzes is critical to a process. Every enzyme has its rate phenomena in the conversion of substrates into products. Enzyme kinetics explains the catalytic behavior of enzymes. Each enzyme functions at a different rate during the conversion of substrates into products (Kaja *et al*., 2018) [11]. The maximum enzyme activity was shown at 12 hours of the incubation period for both substrates. With tween 20 as the substrate for the first 12 hours of the incubation period and coconut as the substrate at the same time, the maximal enzyme activity of lipase was 66.87 U/mL and 63.45 U/mL. The enzyme activity will increases with increase in incubation period up to complete conversion of substrate into product.

Effect of temperature on enzyme activity of lipase

The enzyme activity of the assay mixtures was determined after an hour of incubation at varied temperatures (35-50 ℃) (Enujiugha *et al*., 2004). The optimum temperature for *Carica papaya* lipase with tween 20 and coconut oil as substrate was found to be 45 ℃.

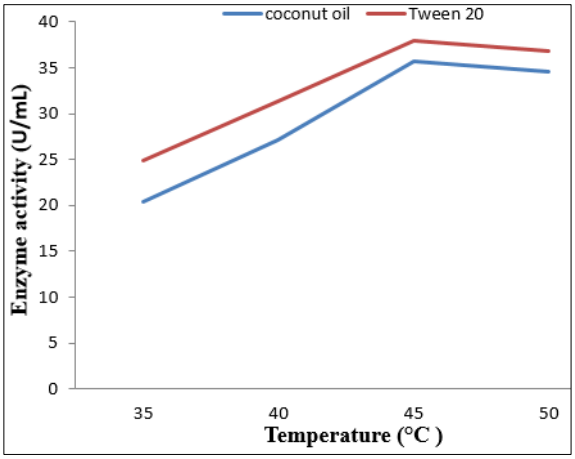

Fig 2: Effect of temperature on enzyme activity

The maximum lipase activity was observed at a temperature of 45 °C for both conditions. Tween 20 was utilized as a substrate, and lipase activity of 37.87 U/mL was obtained at a temperature of 45 °C. When coconut oil acted as the substrate, the enzyme activity was measured at 35.71 U/mL at the same temperature. The enzymatic reactions often depend on temperature. With increasing temperatures, the rate of reaction is accelerated.

However, at a higher temperature, there will be denaturation of the enzyme. This is because the conformation of enzyme molecules is composed of polymers made up of amino acids that are linked by covalent peptide bonds. In the formation of the tertiary and quaternary structure of the enzyme, the peptide bond is supported by an α- helix and β-pleated sheet that is stabilized by a hydrogen bond. Besides, the bonding between enzymes and substrates is usually by van der Waals force and hydrogen bonding. The bonds are broken when the enzyme absorbs additional heat energy from the environment. This heat energy is converted to kinetic energy which drives the reaction faster, but over the limit damages the bonding involved in the conformation of enzyme and results in inhibition (Gusniah *et al*., 2020) [9]. Thus, from this finding, the result suggested that the optimum temperature for *Carica papaya* lipase was 45 °C. Kaja *et al*., (2018) [11] studied the specific hydrolytic activity of all enzymes at different temperatures ranging from 40 °C to 80 °C. Except for lipase immobilized on chitosan using acetone adsorption, all lipases demonstrated the highest activity at 45 °C. Ayinla *et al*., (2017) [3] discovered that the optimum temperature for lipase production by *Rhizopus oryzae* was 45 °C. When coconut oil was treated by enzymatic glycerolysis using *Carica papaya*, a high yield of monoacylglycerol was produced at an optimum temperature of 45 °C (Pinyaphong *et al*., 2012) [10]. Results indicated that the enzyme is less stable at high temperatures since its activity decreases as the temperature increases (Abdelkafi *et al*., 2011) [1].

Effect of Concentration on enzyme activity of lipase

Carica papaya lipase was added to the mixture with enzyme concentrations of 5%, 10%, 15%, 20%, and 25% of the substrate. An enzyme assay was carried out for lipase activity with an hour of incubation period and the synthesized lipase activity was evaluated with tween 20 and coconut oil as substrates.

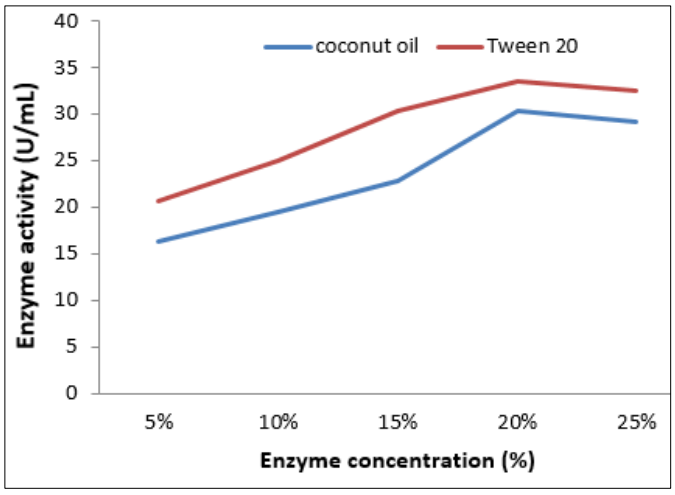

Fig 3: Effect of enzyme concentration on enzyme activity

Maximum lipase activity was obtained at a concentration of enzyme with 20% substrate for both substrates. The lipase activity of 33.54 U/mL at a concentration of enzyme with 20%. When tween 20 was used as substrate. The lipase activity using coconut oil as substrate was 30.30 U/mL at the same enzyme concentration. A high yield of monoacylglycerol was produced at an optimum concentration of 20% when coconut oil was treated by enzymatic glycerolysis using *Carica papaya* (Pinyaphong *et al*., 2012) [10].

The increase in enzyme concentrations increased the rate of reaction (Straathof, 2003) [14]. This is due to the presence of a higher available active site with higher enzyme loading, which encourages more reactions to occur (Gupta *et al*., 2013) [8]. But further increasing lipase concentration does not affect the rate of hydrolysis as all enzyme molecules are saturated with substrate molecules. Increasing the amount of substrate does not always increase the rate of the reaction (Subroto, 2020) [15]. At a high concentration of enzymes, they will tend to agglomerate with each other. According to Marangoni (2003), the decline in the enzyme activity at a high loading of the enzyme is because the substrate is depleted which affects the saturation degrees of the enzyme. The oil substrate may change the physical and chemical characteristics of the lipase enzyme and a higher oil concentration may not be suitable for lipase activity or even becomes inhibitory to the lipase enzyme (Haidari *et al*., 2020) [19].

Conclusion

Carica papaya lipase was extracted from papaya latex and its enzyme activity was assessed at various temperatures, enzyme concentrations, and time. The results suggested that synthesized lipase enzyme activity was optimum at the temperature of 45 °C and with an enzyme concentration of 20% of the substrate. The maximum enzyme activity of lipase was 66.87 U/mL shown during 12 hours of incubation period with tween as substrate and at same time coconut as substrate, 63.45 U/mL of maximum enzyme activity obtained. At a temperature of 45 °C and using tween 20 as the substrate, the lipase activity of 37.87 U/mL was found maximum. At the same temperature, the maximum enzyme activity of 35.7 U/mL was obtained when coconut oil was the substrate. When tween 20 was employed as the substrate, the lipase activity was 33.54 U/mL at a concentration of enzyme with 20%. The lipase activity using coconut oil as substrate was 30.30 U/mL at the same enzyme concentration.

These low-cost lipases can be procured in large quantities for industrial applications like the oil and fat industry for hydrolysis reactions. *Carica papaya* lipases are convenient replacements for microbial lipases and can be used for the extraction of monolaurin from coconut oil by enzymatic glycerolysis.

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