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Biochemical Properties of Coconut (*Cocos nucifera* L.) Lipase

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Ubiquitous in nature, lipases represent an example of enzymes with high versatility. Plant seeds are potential sources of lipase, and they are attracting more attention for specific purposes. In this study, coconut lipase was isolated from germinating coconut seed. Biochemical characterization of coconut lipase was undertaken to reveal its substrate specificity and its subunits properties. By using various chromogenic ester of fatty acids, it was demonstrated that lauric acid is the most preferred substrate for coconut lipase esterase reaction. Calcium ions enhance its activity, whereas other metal ions such as magnesium, nickel, sodium, and potassium reduce it. Electrophoresis under native conditions showed that coconut lipase is a single protein. Since electrophoresis under denaturing conditions revealed four subunits, coconut lipase is likely a complex enzyme. It was further revealed that all subunits are active, as evident in an in-gel hydrolysis assay. However, they also hint that they do not have an equal catalytic rate against the 16-carbon-length palmitate derivative. This finding, thus, opens up a notion that those subunits have different substrates specificity yet to be determined.

Keywords: coconut lipase, in-gel assay, lipase subunits, native electrophoresis, substrate specificity

INTRODUCTION

Fatty acids are widely used in modern life and, hence, are of critical industrial concerns. The utilization of fatty acids spans from essential ingredients in many industries, such as in coating (Rajput *et al.* 2014), surfactants (Semblante *et al*. 2009), lubricants (Ruths *et al.* 2008), essential fatty acids, nutraceuticals production (Sande *et al.* 2018), personal care products (Tavares *et al.* 2018), and bioremediation (Melani *et al.* 2019). Several methods achieve fatty acid production from fats, such as the mechanical separation process, alkaline chemical hydrolysis, and enzymatic process. Mechanical separation requires high pressure and temperature that causes the process costly. Likewise, alkaline hydrolysis also offers a practical method. However, efforts are needed to separate unwanted products (Sande *et al.* 2018). In contrast,

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enzymatic hydrolysis operates at mild conditions (low temperature and pressure). It offers ease in the recovery process (Jain and Mishra 2015) and product loss due to minimized overheating (Barros *et al.* 2010).

Lipases or glycerol ester hydrolases (EC 3.1.1.3) are enzymes that can perform hydrolysis, esterification, and transesterification reactions under mild conditions. Which reaction takes place largely depends on the reaction environment (Tavares *et al.* 2018). Lipases act on different ester compounds, with acylglycerols become their principal substrates. All oilseed plants have significant amounts of lipases. Plant-based lipases are increasingly become the researcher's interest due to low production cost and high specificity (Tavares *et al.* 2018; Villeneuve 2003). They also have an easy pharmacological acceptance due to their eukaryotic source (Seth *et al.* 2014). Essential sources of plant-based lipases are plant seeds, especially the seeds in their germinating phases. Examples are lipases from *Carica papaya* (Campillo-Alvarado and Tovar Miranda 2013), *Pentaclethra macrophylla* (Enujiugha *et al.* 2004), linseed (Sammour 2005), and coconut (Ejedegba *et al.* 2013). However, significant lipase activity from non-germinating seeds also exists, such as in castor beans (Eastmond 2004; Tavares *et al.* 2018).

Coconut trees grow almost in every region in the tropics, mainly along coastal areas of the tropics. The physical appearance of coconut fruits is very distinct and easy to handle. As a consequence, their utilization as lipase sources is foreseeable. The focus of coconut exploration in lipase research was limited to the use of coconut as a medium for lipase-producing fungi (Benjamin and Pandey 1997), immobilization study of other lipases (Brigida *et al.* 2007), and to the potential of coconut as a substrate for lipase reaction (Ibrahim *et al.* 2008). In contrast to its potential, biochemical characterization of coconut lipase has not been sufficiently reported, thus limiting its applications. In this context, the present study investigates the biochemical characterization of coconut lipase. The work includes the analysis of coconut lipase substrate specificity and the property of its subunits. A thorough understanding of the biochemical properties of coconut lipase will lead to its application.

MATERIALS AND METHODS

Materials

Golden coconut (local: *gading* coconut) was obtained from a local garden in Lombok Island of Indonesia. Reagents for buffer and electrophoresis of pro hy grades were obtained from major chemical suppliers. Virgin coconut oil (VCO) was purchased from a local vendor. The artificial lipase substrates were *p*-nitrophenyl butyrate, *p*-nitrophenyl octanoate, *p*-nitrophenyl-decanoate, *p*-nitrophenyl dodecanoate, *p*-nitrophenyl myristate, and *p*-nitrophenyl palmitate, and *p*-nitrophenol (Merck/Sigma-Aldrich). Reagents for in-gel lipase assays were Fast Blue B salt (Sigma-Aldrich) and alpha-naphthyl palmitate (Santa Cruz Biotechnology, USA). Assay buffer contained Arabic gum (Sigma-Aldrich) and sodium deoxycholate (Sigma-Aldrich). Protein determination used a bicinchoninic acid (BCA) kit from Thermo scientific. The Prism 7 tool (GraphPad) and Image-J were used graphical preparation and dye density calculation, respectively.

Methods

Coconut germination, crude extract preparation, and protein determination. The coconut fruits were pickup from coconut the tree after they turned dry, as indicated by the brown color of their shell. The condition was typically reached by the fruits at the age of 11–12 mo. To observe the germination, the outer shell of the fruit was partially removed (Figure 1a). Germination of coconut fruit was attained by storing coconut fruit in the direct sunlight protected open-air condition in our region with an average humidity of above 80% and temperature between 23–28 °C. The humid environment was kept by watering the fruit every day. The coconut shoot's appearance showed germination after c.a. a month of storage (Figure 1a). The germination process was accompanied by the development of haustorium inside coconut fruit (Figure 1b). As the coconut flesh is the primary food storage, coconut lipase was isolated only from the part. Nevertheless, literature reported that all parts of germinating coconut have lipase activity, with the shoot being the most active part (Su'i and Suprihana 2013). The coconut of average size resulted in c.a. 200 grams of meat.

The flesh was shredded and resuspended in 5 mM phosphate buffer, pH 7.0. The suspension was filtered by using a filter cloth. The resulted in coconut milk was centrifuged at 3,000 rpm for 20 min at 4 °C. The floating cream was removed from a 50-mL conical centrifuge tube. The skim fraction was decanted and further subjected to freeze-drying to reduce water content. The resulting 15-mL concentrated coconut lipase was stored at –20 °C for further analysis. Protein concentration was determined using the BCA kit according to the manufacturer's instruction. The developed color was measured at 562 nm by a spectrophotometer (MultiSkan GO, Thermo Scientific).

Enzyme assay. Coconut lipase activity was assayed for its hydrolytic activity against VCO as a substrate (Khor *et al*. 1986). The reaction mixture consisted of 5 g VCO, 2.5 mL n-hexane, 5 mL 100 mM phosphate buffer pH 7.5, and 1 mL of the enzyme. The mixture was incubated

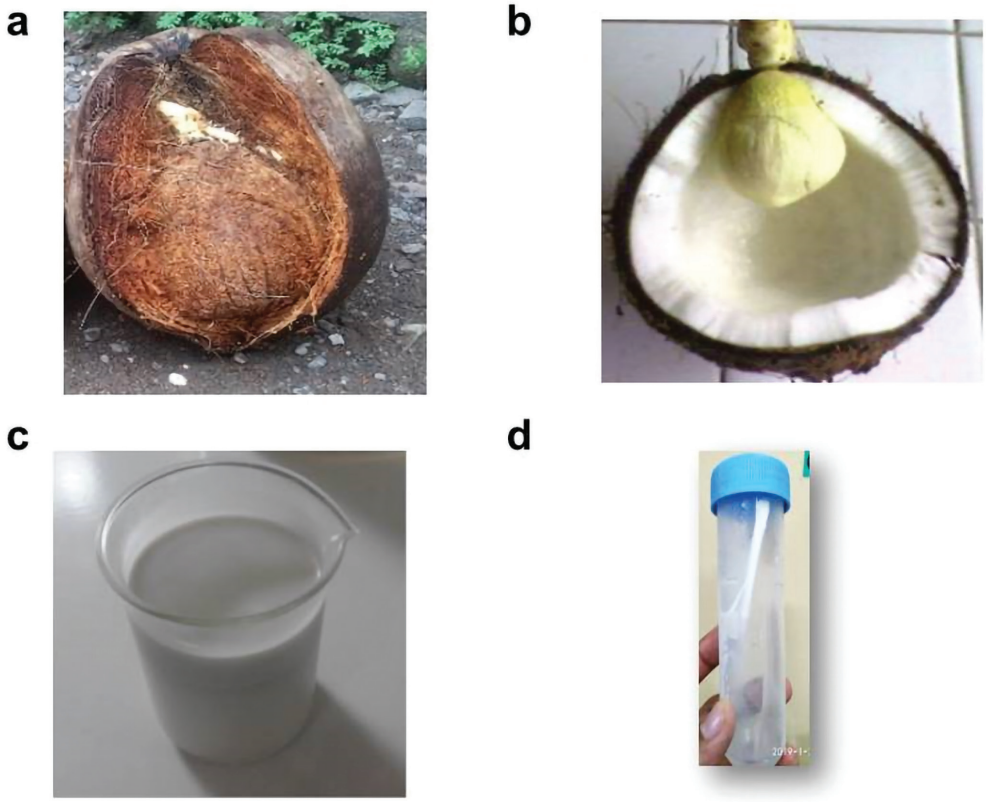

Figure 1. Preparation of coconut lipase from the germinated coconut fruit. a) Coconut shoot appears after a month of germination. b) inside the hard shell, haustorium is developing. Mucilage or coconut flesh was removed and further used as the source of coconut lipase. c) Coconut milk prepared by suspending shredded coconut flesh in 5 mM phosphate buffer, pH 7.0. d) Following centrifugation, the cream fraction was removed. The clear fraction of coconut milk was decanted and stored for electrophoresis and enzyme assays.

in a 35 °C water bath shaker for 45 min and, after this period, 25 mL of acetone/methanol (1:1) was added. The liberated free fatty acids were determined by titration. Sodium hydroxide of 0.01 M was used for the titration following the addition of a few drops of phenolphthalein. Sodium hydroxide was previously standardized against sodium oxalate. Lipase activity was calculated as follows:

$$\text{Lipase activity (U/mL)} = \frac{(V_{sample} - V_{blank})\, x\, [\text{NaOH}]\, x\, 1000}{V_{enzyme\ x\ t}}\ \text{(U/mL)}$$

where:

V_{sample} = titrant volume for sample

V_{blank} = titrant volume for blank

V_{enzyme} = coconut lipase volume

[NaOH] = sodium hydroxide concentration

Coconut lipase activity in the presence of metal ions. Coconut lipase activity was assayed against VCO, as previously described, in the presence of several metal ions. Magnesium, calcium, sodium, potassium, iron, copper, and zinc ions were added to each lipase reaction mixture to a final concentration of 10 mM.

Substrate specificity of coconut lipase. In order to allow kinetics analysis of coconut lipase, a suitable assay condition was first determined. It was performed by hydrolyzing the artificial substrate *p*-nitrophenyl palmitate by serial dilution of coconut lipase. The intensity of the liberated *p*-nitrophenol was measured at 405 nm. Hydrolysis profiles of *p*-nitrophenyl palmitate were recorded every 5 min with lipase dilution range from 1:3,000 to 1:100,000.

For different *p*NP-fatty acids, an 8-min reaction with 1:100,000 dilution of lipase stock was further employed. For each reaction, the *p*NP-fatty acid substrates were prepared as follows: 2 mL of 8 mM *p*NP-fatty acid in n-propanol was added to 18 mL of an emulsifier solution. The emulsifier contained 20 mg of Arabic gum and 41.4 mg sodium deoxycholate in 50 mM Tris-Cl buffer pH 8.0. The substrate solution was kept in the dark before

use. The final concentration of *p*NP-fatty acid in the substrate solution was 0.8 mM. Hydrolysis assay was performed by pre-incubating of 2.7 mL substrate solution at 37 °C for 5 min before the addition of 0.3-mL diluted lipase. The yellow color formation was recorded after 8 min at 405 nm. The coconut lipase specificity was tested against *p*-nitrophenyl butyrate, *p*-nitrophenyl octanoate, *p*-nitrophenyl decanoate, *p*-nitrophenyl dodecanoate, *p*-nitrophenyl myristate, and *p*-nitrophenyl palmitate. One unit activity (U) is defined as micromole(s) of *p*-nitrophenol released upon hydrolysis by 1 mL enzyme at 37 °C under assay conditions (Kanwar *et al*. 2005).

SDS-PAGE and native PAGE. SDS-PAGE was undertaken according to the method initially developed by Laemmli (1970) on 12.5% separating gel and 5% focusing gel. Briefly, a sample containing 50 µg of coconut lipase was precipitated by the addition of an equal volume of cold absolute-ethanol. The resulted precipitate was resuspended in a 5X sample buffer and boiled for 2 min prior to electrophoresis. Electrophoresis was accomplished by applying 150 V of electric current in a mini gel (MiniVe SDS-PAGE apparatus, GEHealthcare) for c.a. 2 h. The resulted gel was stained with Coomassie Brilliant Blue (CBB). For native-PAGE, coconut lipase was subjected to electrophoresis under non-denaturing conditions, *i.e.* by omitting SDS from the gel and running buffer. The sample buffer was also void of 2-mercaptoethanol or dithiothreitol. Ammonium sulfate fractionation was undertaken according to Sana and coworkers (2004). Briefly, ammonium sulfate threshold of 0–30, 30–45, 45–60, 60–75, and 75–90% saturation was added to the protein sample. The excess of salt was removed by dialysis from each fraction. The resulted fractions were subjected to both SDS and native PAGE.

In-gel hydrolysis assay. The activity of lipase subunits was assayed after lipase was separated in 12.5% gel SDS-PAGE. In this case, the sample was not boiled to keep the protein intact. After separation, SDS content was washed by soaking the gel in 50 mM phosphate buffer pH 7.0 and 2.5% (v/v) Triton X-100. It was done with gentle shaking for 30 min. The washing step was repeated twice. The gel loaded with lipase was incubated in a developing solution for 30 min in a dark container to allow hydrolysis to proceed. The developing solution contained alpha-naphthyl palmitate and Fast Blue B salt. Unbound dye was removed by three-time washing in aquadest, 10 min each. The hydrolysis of alpha-naphthyl palmitate corresponded to the lipase subunit activity. The active subunit released a yellow color of alpha-naphthol (Zienkiewicz *et al.* 2014) that appeared on the gel. An identical gel stained by CBB was prepared for comparison.

RESULTS AND DISCUSSION

In this study, lipase was isolated from germinating coconut and its biochemical properties were investigated. Since many biochemical properties of coconut lipase remain unclear, coconut lipase's biochemical characterization is necessary, and the results will facilitate further utilization of coconut lipase.

VCO was used as the substrate for coconut lipase hydrolysis activity instead of using popular olive oil since it offers a more comprehensive composition of fatty acids ester from various chain lengths. VCO has also been investigated in the optimation of many lipases, such as immobilized *Mucor mihei* lipase (Chua *et al*. 2012), *Candida rugosa*, and porcine pancreas lipases (Nguyen *et al*. 2018). The isolated coconut lipase has an activity of 0.56 U/mL corresponding to a specific activity of 0.30 U/mg protein. These results resemble those reported by Su'i and Suprihana (2013).

Figure 2 shows that coconut lipase has a very high esterase activity against artificial substrate *p*-nitrophenyl palmitate. Sample dilution by factors of 6 and 50 thousand times would lead to immediate saturation curves after 5 min of incubation. Sample dilution by a factor of 100 thousand times showed a delayed saturation curve, namely after 20 min of reaction. This dilution factor was used for the specificity assay below since it met the requirement of first-order kinetics in its initial reaction. The high lipase activity from various germinating seeds has been reported (Barros *et al.* 2010) with castor bean (Eastmond 2004), and egusi melon seed (Bege *et al*. 2015) are only a few exceptions as their ungerminated seeds also show significant lipase activity.

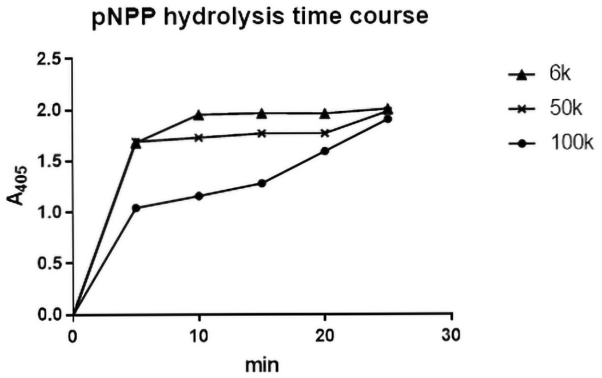

Figure 2. Coconut lipase activity at different dilutions. The activity of coconut lipase of different dilutions was assayed at different time points. The yellow color showed lipase activity as a result of *p*-nitrophenyl palmitate hydrolysis. After ten minutes of reaction, the saturated curve is observed by 6,000 times diluted lipase. For the more diluted lipase, saturation is delayed. In the substrate specificity assay, 8 min of incubation times were chosen, with the sample diluted by 100,000 factors.

Many lipases have their activity altered in the presence of specific metal ions. Here, the effect of several metal ions on the esterase activity of coconut lipase was tested. Figure 3 shows that calcium ions act as coconut lipase activators. A literature survey also suggested that calcium ions activate many plant lipases, such as those from white melon kern (Eze and Ezema 2012). Calcium ion is a well-known activator for different sources of lipases, presumably by stabilizing the three-dimensional structure of lipase during catalysis (Rosenstein and Gotz 2000). On the other hand, Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mg^{2+} – as well as alkali ions K^+ and Na^+ – decreased the esterase activity of coconut lipase (Table 1). It suggests that those ions induced different conformational levels of the lipase that unfavored esterase activity (Hertadi and Widhyastuti 2015), although a deep structural study is necessary to understand the effect of various metal ions. To our knowledge, only a few lipases are inhibited by magnesium ions, such as rice bran (Bhardwaj *et al.* 2001), almond seed (Yesiloglu and Baskurt 2013), and Africa bean seed (Enujiugha *et al.* 2004) lipases (Table 1). Coconut lipase adds a new member to the relatively short list of plant seed lipases inhibited by magnesium ions.

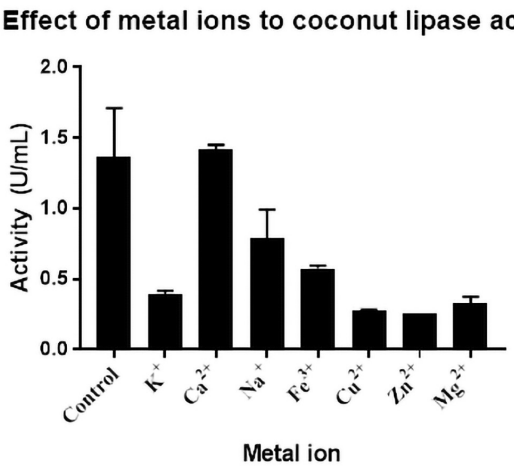

Figure 3. The activity of coconut lipase with the presence of metal ions. The effect of metal ions on coconut lipase activity was assayed by the inclusion of 10 mM of respective metal ions in the assay mixture. The released free fatty acids were titrated by using sodium hydroxide. Control was provided by measuring lipase activity against VCO substrate in the absence of metal ions. All measurements were made in triplicate.

The substrate specificity of coconut lipase was analyzed using various *p*-nitrophenyl fatty acid esters of different chain lengths. Figure 4 shows that *p*-nitrophenyl laurate (C12) gives the highest hydrolysis product in a given time at the initial period of reaction, and the longer fatty acids (C14 *p*-nitrophenyl myristate and C16 *p*-nitrophenyl palmitate) come next. The shorter fatty acids (C4 *p*-nitrophenyl butyrate, C8 *p*-nitrophenyl octanoate, and C10 *p*-nitrophenyl decanoate) give lower hydrolysis products in the same incubation time. Lauric acid (C12) is the predominant fatty acid of coconut that belongs to

Table 1. Properties of some plant-based lipases.

No.	Lipase source	MW (kDa)	Activator	Inhibitor	Reference
1[a]	Rice bran (*Oryza sativa*)	~ 10	Potassium acetate, sodium acetate, NaCl	CHAPS, digitonin, SDS, NP-40, Triton X-100, Mg^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}	Barros *et al.* (2010)
	Rice bran lipase II	33	n/a.	n/a	Aizono *et al.* (1976)
	Rice bran	40	n/a	n/a	Kim (2004)
2[b]	Castor bean (*Ricinus communis* L.)	60	Ca^{2+}	p-chloromercuribenzoic, $HgCl_2$	Eastmond (2004)
	Castor bean	n/a	Slightly activated by Na^+, K^+, and dithiothreitol	Mg^{2+}, Ca^{2+}	Muto and Beevers (1974)
	Castor bean	60	Mn^{2+}, Na^+, K^+, Al^{3+} and Li^+	Zn^{2+}, Co^{2+}, Pb^{2+}, Cu^+	Su *et al.* (2010)
3	Linseed (*Linum usitatissimum*)	42	Mg^{2+}, K^+	Triton x-100, Tween 80	Sammour (2005)
4	Almond seed (*Amygdalus communis* L.)	n/a	Ca^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Ba^{2+}	Mg^{2+}, Cu^{2+}, Ni^{2+}	Yesiloglu and Baskurt (2013)
5	Africa bean seed (*Pentachlethra macrophylla* Benth)	n/a	Ca^{2+}	NaCl, $MgCl_2$, EDTA	Enujiugha *et al.* (2004)
6	Sunflower seed (*Helianthus annuus* L.)	40-50	Ca^{2+}, Mg^{2+}	Hg^{2+}, EDTA	Sadeghipour and Bhatla (2003)
7	Canola lipase (*Brassica napus*)	n/a	Ca^{2+}, Bi^{3+}	Fe^{2+}, Fe^{3+}, Zn^{2+}, Hg^{2+}, Cu^{2+}	Sana *et al.* (2004)

MW – molecular weight; n/a – not available; FA – fatty acid; TAG – triacylglycerol
[a]Different reports of lipases from rice bran
[b]Different reports for lipase from these seeds suggest that they have at least two lipases, *i.e.* the acid and alkaline lipase

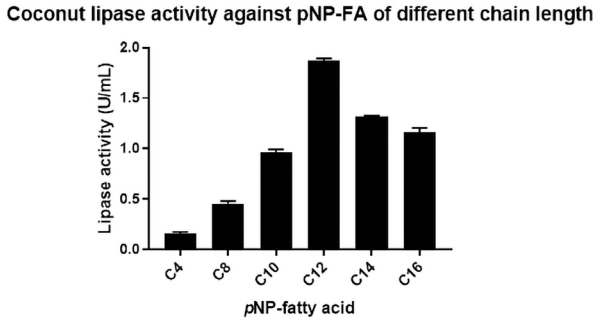

Figure 4. Substrate specificity of coconut lipase. Diluted coconut lipase was allowed to hydrolyze various esters of fatty acids (*p*-nitrophenyl butyrate, *p*-nitrophenyl octanoate, *p*-nitrophenyl decanoate, *p*-nitrophenyl dodecanoate, *p*-nitrophenyl myristate, and *p*-nitrophenyl palmitate) for 8 min of reaction. The released *p*-nitrophenol was measured at 405 nm, and the obtained values were converted to lipase activity. All measurements were made triplicate.

the medium-chain fatty acid (Manohar *et al*. 2019; Dayrit 2014). The complete hydrolysis of VCO by other lipases reported by Chua *et al.* (2012) and Nguyen *et al.* (2018) confirmed that lauric acid is the predominant fatty acid of coconut. Instead of using complete hydrolysis, the kinetics study reported here took advantage of the use of various *p*NP-fatty acid substrates to allow the investigation at the initial period of reaction, from which the fatty acid preference of coconut lipase can easily be determined. The aforementioned result indicates that coconut lipase – in order of preference – hydrolyzes medium, long, and short-chain fatty acid esters.

SDS-PAGE and native-PAGE of coconut lipase are shown in Figure 5. SDS-PAGE electroforegram reveals at least four protein bands of approximately 54 kDa, 32 kDa, 21 kDa, and 15 kDa. However, native-PAGE reveals only a single band of c.a. 130 kDa. After thresholds of ammonium sulfate precipitation, separation of coconut lipase also shows a single complex band in native PAGE for all fractions (Figure 6). Together, these suggest that coconut lipase is a heteromeric enzyme. In humans, hormone-sensitive lipase – an enzyme involves in the mobilization of lipid storage in adipose tissue – has long been shown to be more active in its ~ 160 kDa dimer. It is 40 times more active than the ~ 85 kDa monomer form (Shen *et al.* 2000). A reverse situation is recently reported for the human lipoprotein lipase, whose 55 kDa monomer has similar activity to its 110 kDa homodimer (Beigneux *et al.* 2019). The fact that coconut lipase consists of several subunits and that it is not universal that all subunits of given lipase are functional highlights the need to dissect whether all coconut lipase subunits are active. To address the above question, an in-gel cleavage assay was performed.

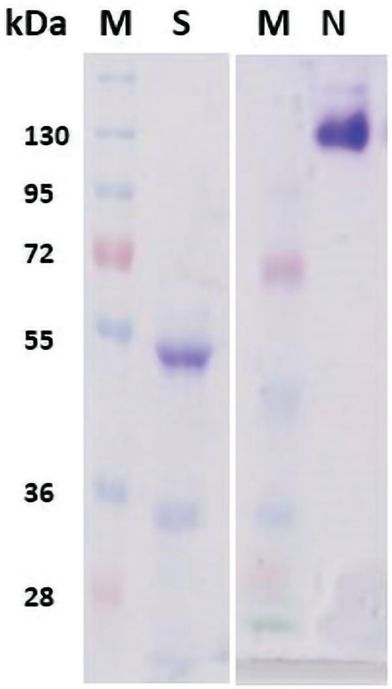

Figure 5. Coconut lipase separation in SDS-PAGE and native-PAGE. Coconut lipase separated on a 10% gel of SDS-PAGE (S) shows different protein bands, *i.e.* 54 kDa, 32 kDa, and 21 kDa. The bands are from a single complex protein of c.a. 130 kDa, as shown in native-PAGE (N). Note: the smallest band of c.a. 15 kDa is not shown here but is obvious on a 12.5% gel (Figures 6 and 8).

In-gel hydrolysis assay allows us to analyze whether a specific protein can hydrolyze fatty acyl ester after the separation of proteins by electrophoresis. An active protein within the gel cleaves the substrate alpha naphthyl palmitate to release a yellow coloring of naphthol (Figure 7), following SDS removal from the gel (Zienkiewicz *et al.* 2015). Figure 8 shows that all coconut lipase subunits can hydrolyze alpha naphthyl palmitate, which indicates that all coconut lipases are active. Two subunits with equal intensity on CBB staining produce different naphthol intensity, demonstrated by the 54 kDa dan 21 kDa subunits (Figure 8). It suggests that the two subunits have a different affinity to alpha naphthyl palmitate, with the latter has a lower affinity. However, this does not nullify the possibility that the 21 kDa subunit has a higher esterase activity for shorter or longer fatty acids. Subashri and coworkers (2018) have identified coconut lipase with a molecular weight between 29–43 kDa , which is comparable to the 32 kDa subunit in the present study. Since Subahsri *et al*. used ester of oleic acid as substrate, one can speculate that the 32 kDa subunit of coconut lipase has a preference for the C18 fatty acid ester. Hence, it is worth testing whether the cleavage of medium-chain and short-chain fatty acids by coconut lipase gives the same

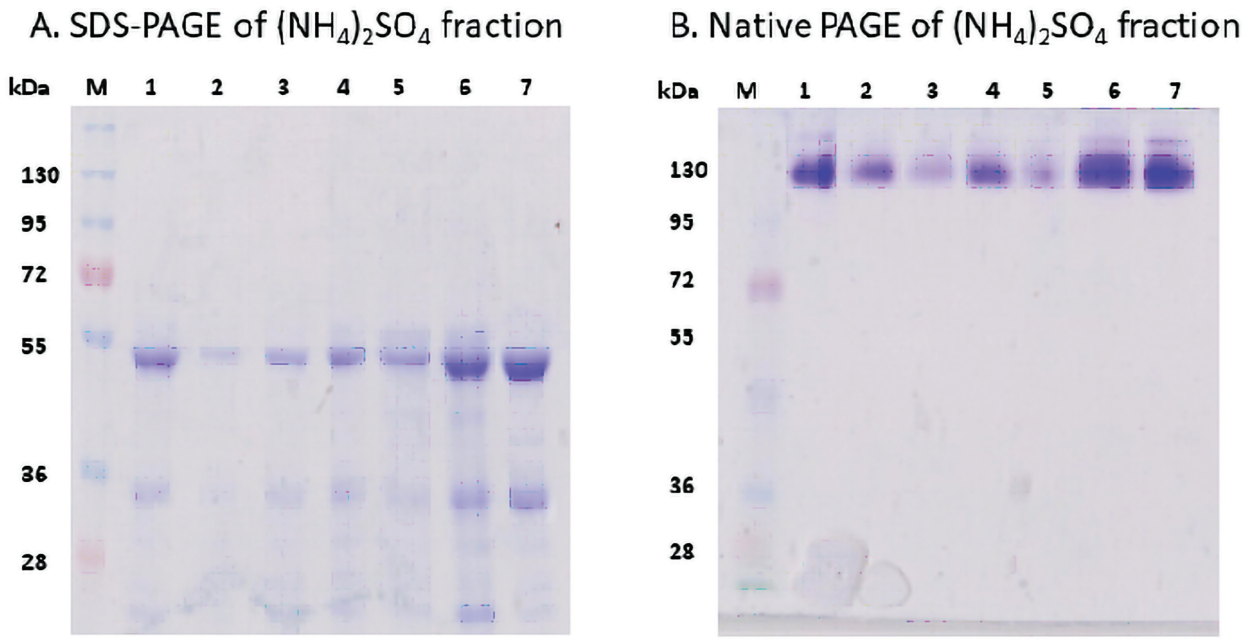

Figure 6. Ammonium sulfate fractions of coconut lipase separated by SDS-PAGE (A) and native PAGE (B). M – protein marker; 1 – crude extract; 2 – fraction 0–15%; 3 – fraction 15–30%; 4 – fraction 30 –45%; 5 – fraction 45–60%; 6 – fraction 60-75%; 7: fraction 75–90%.

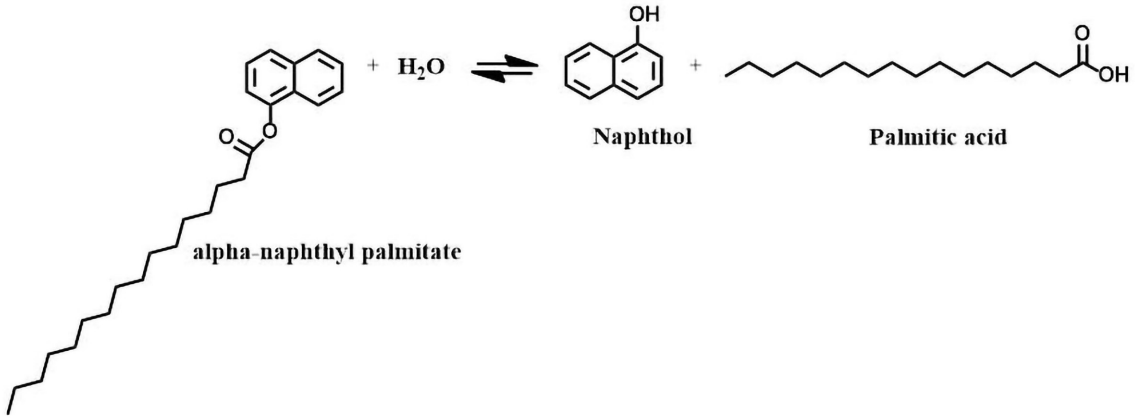

Figure 7. Hydrolysis of alpha-naphthyl palmitate by lipase. An active lipase or esterase cleaves alpha-naphthyl palmitate to release naphthol. The yellow color of naphthol is measured spectrophotometrically at 405 nm.

pattern. There are faint protein bands at c.a. 40 kDa and 45 kDa in addition to the four distinct subunits. We speculate that the two proteins are glycosylated forms of the 32 kDa subunit. Our finding that coconut lipase consists of several active subunits may explain contradictory reports on plant seed lipase activities, such as those from rice *Oryza sativa* lipase (Table 1).

The data presented in this study show that all coconut lipase subunits can cleave fatty acid esters at a different rate of hydrolysis. However, it is worth noting that the regioselectivity and stereoselectivity of coconut lipase remain unclear. To address this issue, the hydrolysis of triacylglycerol substrates of various acyl group lengths will provide the required data. From the present experiment, we expect that coconut lipase has the highest affinity to trilaurin. Hydrolysis study with 1-monolaurin, 1,3-dilaurin, and 2,3-dilaurin substrates will reveal regioselectivity of coconut lipase. Moreover, to obtain details of individual subunits' activity, it is deemed necessary to separate the subunits and test their specificity. Such a study may reveal the contribution of subunits to the coconut lipase as a whole. Furthermore, if cloning and heterologous expression are desired, this can be directed to

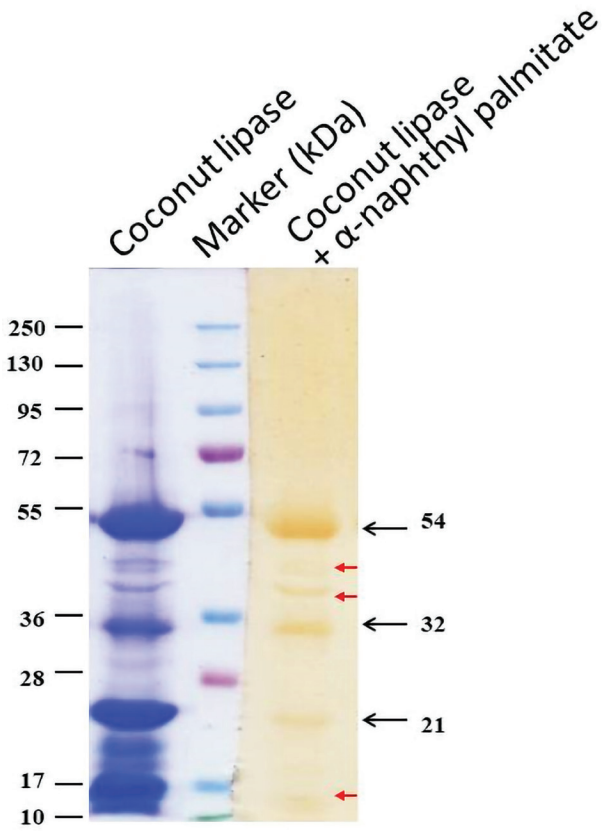

Figure 8. In-gel activity assay of coconut lipase. Coconut lipase is separated in a 12.5% polyacrylamide gel under denatured conditions, except for the boiling step. The gel was cut for CBB staining (left) and an in-gel assay(right). At least four distinct bands are noticed upon CBB staining, including the smallest subunit of c.a. 15 kDa. Additional faint bands are seen above the 32 kDa. The corresponding hydrolysis products by lipase subunits appear as yellow bands. It represents the results of alpha naphthyl palmitate hydrolysis by respective lipase subunits. The pixel density ratio of naphthol to CBB staining for the 54 kDa and the 21 kDa subunits is 77.5% and 43.2%, respectively. Note: several distinct bands above the 32 kDa band (red arrows) are shown to be active in hydrolyzing the alpha naphthyl substrate. It gives a hint that the 32 kDa is glycosylated. We also speculate that a smeary pattern observed in the native gel (Figure 5) indicates the glycosylation of native protein.

the study of individual subunits, especially at the current circumstance when the coconut genome is emerging on the horizon (Xiao *et al.* 2017). Accordingly, biochemical characterization of various subunits (optimum temperature and pH reaction, substrate specificity, metal ions effect, and detergent effect) would provide more detailed information.

CONCLUSION

By using a simple procedure, we have been able to isolate coconut lipase. A direct comparison of SDS-PAGE and native PAGE readily hint that coconut lipase is a complex enzyme. This enzyme consists of four subunits of 54, 32, 21, and 15 kDa. In its complex form, coconut lipase shows the highest preference for lauryl esters. The enzyme is activated by Ca^{2+} ion, whereas Fe^{3+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, K^{+}, and Na^{+} decrease coconut lipase activity. Each subunit can cleave the fatty acid ester bond; hence, this enzyme might be regarded as a cluster of smaller active proteins. Since all coconut lipase subunits are active as esterases, specificity determination of subunits and further biochemical characterization of the subunits are yet to be investigated. We also propose that a similar approach can be applied for the initial study of other plant or seed-based lipases.

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STATEMENT ON CONFLICT OF INTEREST

All authors declare to have no conflict of interest.

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