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CHARACTERIZATION OF LOW-COST LIPASE BY SOLID-STATE FERMENTATION OF PALM KERNEL CAKE USING CANDIDA CYLINDRACEA

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ABSTRACT

This study aimed to identify the properties as well as the application of *Candida cylindracea* lipase produced from palm kernel cake (PKC) by solid-state fermentation (SSF). Characteristics of PKC-lipase have been investigated which include: thermal stability, pH stability, stability in organic solvents and metal ions solutions. The enzyme assays were conducted using colorimetric method and the activity was expressed as residual activity. It was revealed that the optimal temperature and pH were 37°C and 8.0, respectively. The enzyme was stable at temperature ranges of 25-45°C and pH 7.0-8.0 respectively. $Zn^{2+,}$ Mg^{2+} and Ca^{2+} stimulated the enzyme while Fe^{3+} inhibited its activity. The results showed that 1.0% of EDTA enhanced the lipase activity while incubation in SDS and Tween-80 resulted in the reduction of its activity. Moreover, the produced lipase exhibited some levels of stability at low concentrations of organic solvents (methanol, ethanol, 2-propanol, acetone, hexane and toluene). The application of the PKC-lipase showed promising results in hydrolysis whereas 50.48% and 45.98% of canola oil and olive oil were converted to free fatty acids (FFA), respectively. The knowledge obtained from this study can give an insight on understanding the PKC-lipase properties in order to be used in industrial and biotechnological applications, particularly in biodiesel production.

Keywords: lipase, *Candida cylindracea,* palm kernel cake, enzyme stability, residual activity, hydrolysis.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are important enzymes due to their various industrial applications [1]. The ability of lipases to catalyze both hydrolytic and synthetic reactions [2] has directed research towards seeking better understanding of their properties as well as their potential applications. Specific but robust characteristics are needed for certain reactions. For example, biodiesel production requires lipase to be stable in alcohol solvent at 45°C [3] while hydrolysis required mild conditions. On the other hand, lipases used in detergents are required to be stable in alkaline pH and temperature up to 60°C, exhibit low substrate specificity and resistance to other components [4]. Despite the great industrial potential of lipases, the high production cost of lipases limits their industrial applications. Recently, lipases have been produced using microbial sources along with agro-industrial wastes to reduce the production cost especially employing solid-state bioconversion for the process. Palm kernel cake (PKC) is one of the solid wastes obtained after oil extraction from palm kernel seeds [5]. By the end of 2012, production of PKC in Malaysia reached approximately 2.4 million tons [6]. The nutritive value of PKC directed studies towards utilization of this substrate in order to produce enzymes, in this case, lipase. PKC is a rich source of carbohydrates as well as protein and minerals [7] which makes it suitable for microbial fermentation.

It has been well documented that lipases from different sources have different characteristics. Many reports described the production and characterization of lipases lipases utilizing submerged fermentation but very scarce reports are found for lipase production and characterization obtained through solid-state bioconversion (SSB). For instance, a lipase from *Aspergillus niger* using wheat bran exhibited optimal pH and temperature at 6.0 and 40°C, respectively [8]. Using agro-industrial residues, Coradi, *et al.* [9] reported 25% lipolytic activity of *Trichoderma harzianum* lipase in organic solvents. Extracellular lipase produced from *Penicillium crustosum* using soybean bran as a solid substrate showed more enhanced properties [10]. In our earlier work, the process conditions for the yeast, *Candida cylindracea* were optimized to produce lipase upto 400U/gds (unit per gram dry substrate) by SSB. The purpose of that enzyme production was to obtain a low-cost product from abundant raw materials to be used for biodiesel production after enzyme immobilization. The present study aimed to identify the characteristics of the lipase produced by *C. cylindracea* in terms of optimal pH and temperature as well as stability, effects of metal ions, organic solvents and detergents in addition to its application in lipid hydrolysis.

MATERIAL AND METHODS

The main substrate in this study, palm kernel cake (PKC), was collected in clean autoclave bags, from West Oil Mill, Sime Darby Sdn. Bhd. (Supercritical Fluid Extraction Unit) in Carey Island, Banting, Selangor, Malaysia. A sample of PKC was ground to 1.0 mm and dried at 60°C in an oven for 72 h to reduce the moisture

content to approximately 3.0% [7]. All chemicals and consumables used in this research were of analytical grade and commercially purchased from Oxoid Ltd. (England), Merck Sdn. Bhd. (Germany), Fisher Scientific (UK), R and M Chemicals (UK), MHM Global (Germany), Bumi Pharma Sdn. Bhd. and Nano-life Quest (Malaysia).

Sub-culturing and inoculum preparation

The microbial strain, *Candida cylindracea* (ATCC 14830) used in this study was purchased from the American Type Tissue Culture, USA. *C. cylindracea* was grown on PDA plates at 28°C for four days in an incubator (Incucell, Germany) and sub-cultured every two weeks. Each plate was washed with 10.0 ml sterile distilled water and the suspension was used to prepare the inoculum in the appropriate medium [11]. An average count of 2.0×10^7 cells/ ml was used for the inoculum. The cell counting was performed using a hemocytometer.

Lipase production by SSB

Experiments were carried out in 250 ml Erlenmeyer flask where 6.0 g of PKC was moisturized up to 70% using sterile distilled water and supplements to a total weight of 20 grams (inclusive of liquids added). The optimized media from our previous study [12] consisted of: 1.5% grams per total weight (w/w) of yeast extract, 0.5% volume (in ml) per total weight used (v/w) olive oil and 2.0% (v/w) Tween-80. The initial pH was adjusted to 6.0-7.0 using 1.0 M (molar) NaOH. After autoclaving at 121°C for 15 min, 7.0% (v/w) of the inoculum was added into each flask and incubated at 30°C for 72 h. The crude enzyme from the fermented media was recovered using a simple extraction method. The fermented material was mixed with 50 ml sterile distilled water and agitated for 2 h at room temperature (30±2ºC) on a rotary shaker (MaxQ-4000) at 150 rpm to facilitate the enzyme extraction (room temperature was determined using a thermometer and n case of changes, alternatively, incubator shaker was used at 30ºC) [12]. The suspension was then centrifuged at 5,000 rpm, 4°C for 20 min and the resulting clear filtrate was used for lipase assay [7, 8].

Lipase colorimetric assay

Analysis of lipase was carried out according to the method described by Gopinath et al. [13] using *p*-nitrophenyl palmitate (*p*NPP) as the substrate with slight modifications. Twenty microliters of the enzyme [11] (diluted to appropriate concentration with deionized water) was added to 2.4 ml of freshly prepared substrate solution and incubated for 15 min at 37°C. The absorbance was measured using a micro-plate reader (Infinite M200, TECAN) at 410 nm against an enzyme free control. One unit of lipase activity was defined as the amount of lipase that releases 1 µmol of *p*-nitrophenol per minute under assay conditions [13]. Enzyme activity was expressed as units/gram of the initial dry substrate (U/gds). Assays were carried out in triplicates and the average values were calculated. Standard curve of *p*-nitrophenol was prepared using different serial dilutions.

Characterization of PKC-Lipase

In this step, effects of several factors that influence the lipase activity and stability were studied. These factors included temperature, pH, metal ions and organic solvents that can enhance or inhibit the activity. Application in lipid hydrolysis was also investigated.

i. Effect of pH on lipase activity and stability

The optimal pH for enzyme activity was determined by incubating the enzyme-substrate solution at different buffers: 50 mM phosphate-citrate buffer (pH 4.0, 5.0 and 6.0), 50 mM phosphate buffer (pH 7.0 and 8.0) and 50 mM Tris-HCl buffer (pH 8.0 and 9.0) using *p*NPP as the substrate. Effect of storage pH on lipase activity was also investigated at pH 4.0 to 9.0 for 6, 12 and 24 hours. Residual activity was measured at pH 8.0 and expressed in percentage taking the control as 100%. Control was incubated in 50 mM phosphate buffer, pH 8.0.

ii. Effect of temperature on Lipase activity and stability

The optimal temperature for lipase activity was determined by incubating the enzyme-substrate mixture at different temperatures ranging from 15° to 55°C at the best buffer chosen from the previous step. For temperature effects on enzyme stability, enzyme samples were pre-incubated 25° to 75° C, for 15, 30, 45, 60, 75 and 90 min. Residual activity was determined in percentage by taking the initial activity as 100%. Moreover, enzyme stability was monitored for 7 days at different storage temperatures: -20.0°C, 4.0° C, 30±2°C (room temperature) and for three months at -20.0°C.

iii. Stability in organic solvents

PKC-lipase was tested for its stability in organic solvents. Lipase was incubated with 1:1 ratio in different concentrations (25.0 %, 50.0% and 75.0%) of water-miscible solvents (methanol, ethanol, 2-propanol, and acetone), and water-immiscible solvents (*n*-hexane and toluene) for 60 min at the optimal temperature. Control was incubated with 50 mM phosphate buffer, pH 8.0. Relative activity was calculated in percentage by taking the activity of the control as 100% [14].

iv. Effect of metal ions, detergents and other chemicals

One millilitre (1.0 ml) of the enzyme was pre-incubated with 1.0 ml of the following solutions: $CaCl_2$, $CoCl_2$, $MnCl_2$, $MgCl_2$, $FeCl_3$, EDTA, Triton X-100, Tween-80 and sodium dodecyl sulfate (SDS) for 60 min at the optimal temperature and then the residual activity was calculated taking the activity of the control as 100% [14]. The control was incubated in 50 mM phosphate buffer pH 8.0.

Enzymatic hydrolysis of Lipids by the PKC-Lipase

To test the ability of the PKC-lipase to hydrolyze lipids, different oils (olive oil, canola oil and palm oil) were incubated with specified amounts of the lipase and the liberated free fatty acids (FFA) were determined by titration against a base.

To monitor the hydrolysis process, 5.0 g of the oil sample was weighed in 100 ml Erlenmeyer flask. Then, 10.0% (0.5 ml) of the crude lipase (43.5 U/ml) was added to start the reaction while the control sample was left without enzyme loading. The mixture was incubated at 37˚C in an orbital shaker at 200 rpm to ensure a homogenous mixing [15]. The reaction was monitored for 24 h to determine the percentage of FFA. After the incubation, the hydrolyzed sample was dissolved into ethanol, and 2 to 3 drops of 0.5% (w/v) of phenolphthalein (prepared in ethanol) was added. The mixture was titrated with 0.1 M NaOH until permanent (for 30 seconds) faint pink colour appeared [16]. The percentage of FFA can be calculated using the formula:

$$\%FFA = \frac{(V \cdot NaOH) \times (Normality \cdot of \cdot NaOH) \times (28.2)}{Weight \cdot of \cdot sample \cdot in \cdot grams} \quad (1)$$

Where V is the volume of NaOH in (ml) needed to neutralize the acid and 28.2 is the conversion factor of oleic acid [17]. Since oleic acid is the major constituent (45 - 80%) of these oils, the degree of hydrolysis has been expressed as percentage of oleic acid as suggested by Serri, *et al*. [15].

Determination of kinetic parameters

Emulsion of *p*NPP of different concentrations (mM) were used at fixed temperature of 37°C and pH 8.0 to determine the kinetic parameters: maximum reaction rate (V_{max}) and Michaelis-Menten constant (K_m) for the enzyme. The kinetic parameters were determined based on Lineweaver-Burk plot equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (2)$$

where [S] is the substrate concentration in millmolar, V is the initial reaction rate of the enzyme (µM/min) and K_m is Michaelis-Menten constant [14].

Statistical analysis

The results were analysis using Microsoft Excel 2013 and SAS V. 9.1.3.

RESULTS AND DISCUSSIONS

The investigation of some characteristics of PKC-lipase was accomplished during this study as follows:

Effect of pH on PKC-Lipase activity and stability

PKC-lipase was tested in different buffers to determine the optimal pH. The assays were conducted first at different pH buffers and the results showed that the optimal pH for the activity was 8.0. PKC-lipase activity appears to increase by increasing pH until it reached the optimal pH where the activity decreased beyond pH 9.0.Very low activity was detected at pH 4.0 and 5.0.

As for stability, the activity remained almost the same at pH 7.0 and 8.0 with slight increment of stability at pH 7.0 while 60.0% of the activity retained after 24 h at pH 9.0. At lower pH, the activity of the lipase dropped to around 50.0% as illustrated in Figure.1. Activity of the lipase was not measured beyond pH 9.0 because of the hydrolysis of *p*NPP at pH above 9.0 [18]. The lipase produced in this study showed some levels of stability for 12 h at pH 5.0 and 6.0 (retaining more than 50% residual activity), but more than 50.0% of the activity was lost at pH 4.0.

The optimal pH value was in agreement with many other studies which proved that the optimal pH for yeast lipases was pH 8.0 [7, 14]. Moreover, enzyme stability in different pH was also in agreement with other studies. Zouaoui and Bouziane [19] reported the lipase produced by *Pseudomonas aeruginosa* to be stable at pH 6.0 to 8.0 although 60% of the activity was retained at pH < 6.0 and 70.0% at pH > 8.0 after incubation for one hour at 35ºC.

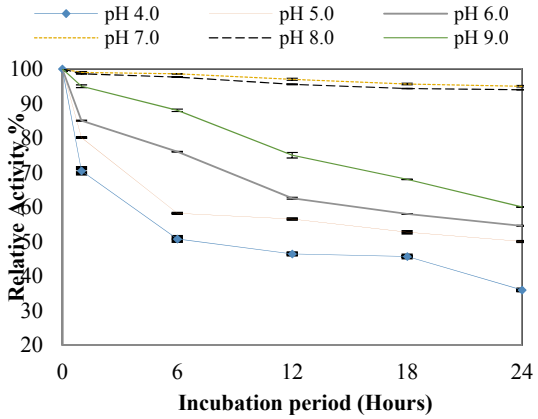

Figure-1. pH stability of PKC-lipase after incubation in different buffers. (Assay was performed at pH 8.0 and 37°C using phosphate buffer and *p*NPP as a substrate). Error bars present the standard deviation.

Effect of temperature on lipase activity and stability

The effect of temperature on the enzyme activity was studied between 15.0 and 55˚C. The PKC-lipase was found to be mesophilic where the optimal temperature was 37˚C. The results obtained revealed that *C. cylindracea* PKC-lipase was stable and retained its full activity at 25 - 45˚C after 90 min of the incubation (Table-1). At 50˚C, 73% of the enzyme activity was retained while 68% of the

activity remained after 90 min of incubation at 55°C. Increasing the temperature resulted in almost complete loss of the activity. At 60°C, 12% of the activity was retained whereas at 65 and 75°C, no lipase activity was detected. The PKC-lipase obtained in this study was stable at 45°C for 90 min with full activity, 50°C and 55°C for 60 min with 95% and 83% of its initial activity, respectively.

Moreover, the stability of the PKC-lipase was investigated after storage at different temperatures: room temperature (30±2°C), 4.0±1°C and -20±1°C. The enzyme retained its full activity when stored at -20°C for 90 days while after seven days, 92.6% of the activity remained when the enzyme was stored at 4.0°C. On the other hand, lipase activity was reduced to 65.2% when stored at room temperature.

The results were subjected to one-way ANOVA analysis and Tukey's test at alpha (α= 0.05) to define the significance of the groups. The statistical analysis listed the three temperatures (25, 35 and 45°C) under the same category (A) which proved that there was no significant difference in the activity across the incubation time (p= 0.1576 and MSD of 1.1996). The degree of freedom was 20 and the error mean square has the value of 1.573655. The results showed same levels for the three values. While for the other groups (50, 55, 65 and 75 °C), the differences were significant and the interaction was significant (p<.0001, MSD of 0.0931 and R^2= 0.999998). Tueky's test (α= 0.05) listed five groups (A, B, C, D and E) with values of means (of activity as the response) as follows: 92.77, 85.69, 55.66, 54.39 and 32.97. Error degree of freedom was 34 and the error mean square was 0.007325.

In terms of temperature stability on the other hand, the results showed better improvement compared to the results obtained from POME-lipase by Salihu *et al*. [14] where only 60% of the lipase activity was retained after 20 min at 45°C and almost no activity was detected at 55°C. According to Mitchell *et al*. [20], enzymes produced by SSB are more stable and more concentrated compared to submerged fermentation. For example, *Trichoderma harzianum* lipase produced using castor bean remained stable at -22°C and 4°C after 30 days [9]. The results of PKC-lipase obtained by *C. cylindracea* in this study follow the same trend with the reported data. PKC-lipase showed great stability when stored at -20°C with no loss of activity. The PKC-lipase produced in this study did not undergo any purification process which means that yeast cells and other impurities were not completely removed by centrifugation. Thus, it is recommended to store the crude PKC-enzyme at -20°C for further.

Table-1. Thermal stability of PKC-lipase.

Incubation period (min)	**25°C**	**35°C**	**45°C**	**50°C**
Sample	Relative Activity %			
(Control)	100.00			
15	99.55±0.0071	99.40±0.0141	99.98±0.70	99.70±0.905
30	99.20±0.000	99.05±0.0141	99.31±0.0283	98.40±0.0141
45	98.90±0.0071	99.00±0.0071	98.18±0.0071	97.80±0.0141
60	99.10±0.0141	99.20±0.0071	98.50±0.0212	95.00±0.07071
75	98.91±0.0071	99.10±0.0071	98.25±0.1202	85.33±0.0141
90	99.00±0.0354	99.36±0.0283	98.20±0.7637	73.00±0.08485
Incubation peroid (min)	**55°C**	**60°C**	**65°C**	**75°C**
15	96.22±0.0778	95.17±0.062	98.22±0.021	97.00±00.113
30	90.64±0.0637	90.00±0.085	96.15±0.035	32.16±0.096
45	83.33±0.0778	54.00±0.106	75.00±0.085	0.85±0.0021
60	83.17±0.0491	22.00±0.354	9.02±0.0141	0.42±0.0141
75	78.95±0.0494	16.00±0.085	1.91±0.063	0.33±0.0014
90	67.80±0.0494	12.00±0.135	0.39±0.0070	0.04±0.0113

Effect of organic solvents

Different organic solvents were investigated for their effects on the activity of PKC-lipase. The effects of the addition of different concentrations of methanol, ethanol, 2-propanol, acetone, toluene and *n*-hexane are presented in Table-2. PKC-lipase retained 94.25% and

93.43% of its initial activity when incubated in 25% methanol and ethanol, respectively while increasing the concentration to 50% alcohol reduced the activity to 55.4% in methanol and only 8.0% remained in ethanol. Similar results were reported by Salihu *et al.* [14] for POME-lipase by *C. cylindracea*. In contrast, better stability of PKC-lipase was obtained in both 50% and 75% of methanol. At 25% solution, 2-propanol and acetone retained 85.6% and 82% of PKC-lipase activity, respectively. At higher concentrations, all water-miscible solvents showed inhibitory effect, where less than 10% of the lipase activity was detected. Meanwhile at low concentrations of water immiscible solvents (*n*-hexane and toluene), the enzyme retained 52% and 48.18% of its activity, respectively. Unlike other solvents, higher concentrations of *n*-hexane and toluene did not completely inhibit the activity where 38.7% and 37.62% of the activity was detected after one hour of incubation.

From the analysis, the stability in organic solvents can be listed as methanol> hexane>toluene>ethanol>acetone and lastly isopropanol.

Lipases can function in both aqueous solutions as well as in nearly anhydrous solvents [21]. The stability of lipases in organic solvents is advantageous [14] particularly in the reversible reactions which involve synthesis such as esterification [21] and transesterification [22]. It has been documented by Colton *et al*. [23] that in organic solvents, lipase undergoes disaggregation which keeps it in an open conformation providing better stability or activity. Some solvents cause a complete loss in the enzyme activity due to the change in the enzyme structure. Stability in methanol has an important potential for biodiesel production.

Table-2. Effect of various concentrations of organic solvents on PKC-lipase activity.

Organic solvent	**Concentration** (%v/v)	**Relative activity %**
Methanol	25.0	94.25±0.1767
	50.0	55.37±0.0494
	75.0	13.17±0.00
Ethanol	25.0	93.43±0.308
	50.0	8.01±0.00778
	75.0	6.31±0.14142
2-propanol	25.0	85.62±0.0494
	50.0	4.00±0.0071
	75.0	3.64±0.0636
Acetone	25.0	81.97±0.19092
	50.0	18.05±0.12021
	75.0	3.44±0.05656
n-Hexane	25.0	52.05±0.04243
	50.0	50.41±0.04243
	75.0	38.70±0.04242
Toluene	25.0	48.17±0.04243
	50.0	41.57±0.16971
	75.0	37.62±0.79195
*Control		100.0

Effect of metal ions and detergents

PKC-lipase exhibited changes in activity when incubated with metal ions (Figure-2). All tested cations activated the enzyme except for Fe^{3+} where only 17.0% of the activity is retained after one hour. Calcium and magnesium ions stimulated the enzyme the most while cobalt showed no considerable effect; control and cobalt were placed in the same group (F) by Tukey's test, α= 0.05 and p <.0001. On the other hand, the presence of 10 mM EDTA enhances the activity by 15%, whereas 74% of the activity is retained in a solution containing 1.0% (v/v) of Triton X-100. In 1.0% (v/v) SDS and 1.0% (v/v) Tween-80, only 3.32% and 11.62% lipolytic activity remained after incubation for one hour, respectively. Statistical analysis revealed that except for (F), all groups are significantly different from each other, R^2 has the value of 0.999984, MSD was 1.0437 and error mean square was 0.066548.

It was not surprising for Ca^{2+}, $Mg^{2+}$and other metal ions to enhance the lipolytic activity of lipase because of their contribution as cofactors as well as their role in stabilization of the tertiary structure [24]. The ability of calcium ion to stimulate lipase activity was correlated to its role in the binding process which influences the position specificity on the active site [25].

Lipase from *Rhizopus oligosporus* was activated by Mn^{2+}, Ca^{2+} and Mg^{2+} while Co^{2+} had no effect on the activity. Extracellular lipase from *Staphylococcus* was enhanced by calcium, zinc and copper ions (100 mg/L) while ferrous ions resulted in inhibition of the lipolytic activity [26]. In case of *C. verticillata* lipase, stimulatory effect was observed in the presence of Ca^{2+}, Mn^{2+} and Ba^{2+} [27]. According to Tembhurkar *et al*. [26], lipase produced from *Staphylococcus* was stimulated by the presence of 100 ppm of EDTA whereas 300 ppm decreased the activity. Bussamara et al. [18] stated that lipase produced from *Pseudozyma hubeiensis* lost its activity in the presence of 1.0% SDS. In case of *Penicillium candidum,* all detergents had positively influenced the lipase activity except Tween-80 and SDS that showed negative effect [28].

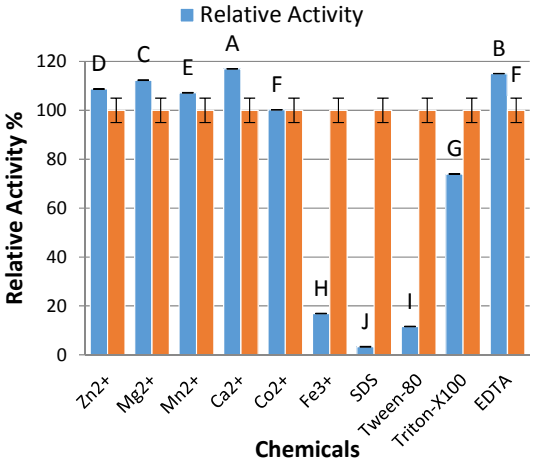

Figure-2. Effect of metal ions and other chemicals on PKC-lipase activity. Labels of different letters showed that the different between groups is significant by HSD (α= 0.05). Error bars present the standard deviation.

Enzymatic hydrolysis of Lipids by the PKC-Lipase

Since oleic acid is the major constituent (45-80%) of the oils used (palm, olive and canola oils) the degree of hydrolysis has been expressed as percentage of oleic acid as suggested by Serri, et al. [15]. PKC-lipase has the ability to hydrolyse triglycerides into FFAs and glycerol of different oils with different degrees (Figure-3). The highest percentage of hydrolysis (50.48%) after 24 h was observed with canola oil, while the lowest was with palm oil (40.11%). On the other hand, hydrolysis of olive oil is not to be neglected (45.98%). Using ANOVA analysis, the results were significant with a value of $p < .0001$ and R^2 of groups with 0.999897. Tukey's Studentized Range (HSD) test for the data showed 3 significant different MSD of 0.2705 and error mean square of 0.053418. This suggests that the three oils had significantly different degree of hydrolysis across the time interval.

Extracellular lipase from *Penicillium crustosum* produced through SSB showed high specificity toward olive oil [10].

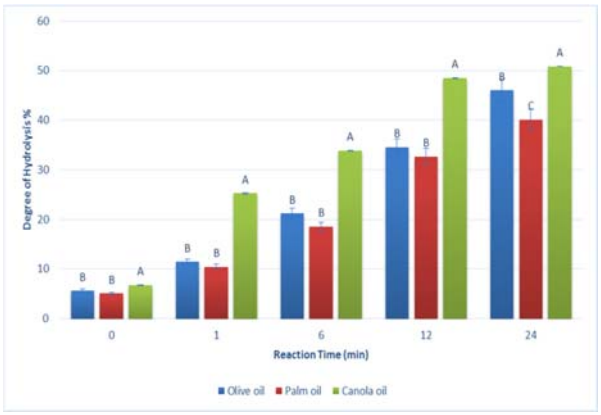

Figure-3. Degree of hydrolysis using PKC- lipase, expressed as % oleic acid in various lipids across time (in min). Groups of different letters are significantly different from each other by HSD (α= 0.05). Error bars present the standard deviation.

Immobilized *C. cylindracea* lipase caused 69.5% of rice bran oil hydrolysis to FFA in 24 h incubation period [29].

Compared to these results, although PKC-lipase can hydrolyse canola, olive and palm oils, the percentage of conversion is quite low. One plausible reason might be because the crude PKC-lipase used in the present study may contain other impurities from the fermentation medium. Thus, the difference in the percentage of hydrolysis would be acceptable.

Determination of kinetic parameters

The two parameters V_{max} and K_m define the kinetics behaviour of PKC-lipase as a function of substrate concentration [S] were: 0.21806 µmol/min and 0.35402 mM respectively as calculated from Figure-4. In the study conducted by Salihu, *et al*. [14], V_{max} and K_m (using *p*NPP as a substrate, at 37°C and pH 8.0) obtained were 0.133 µmol/min and 0.324 mM, respectively. As many cases, it was reported that enzymes such as lipases obey Michaelis-Menten kinetics [30]. K_m determines the affinity of an enzyme for a particular substrate; a low K_m value represents a high affinity [31]. The low K_m obtained in this study denotes the high affinity of the PKC-lipase to *p*NPP substrate.

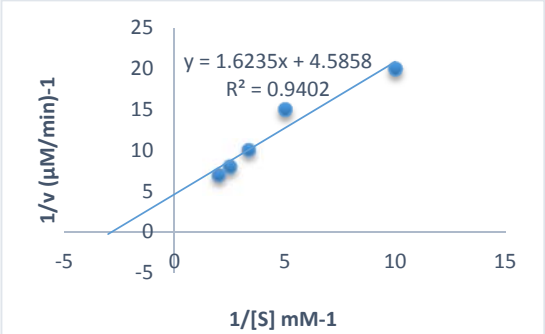

Figure-4. Lineweaver-Burk plot for determination of kinetic parameters V_{max} and K_m using *p*NPP as a substrate.

CONCLUSIONS

Defining the characteristics of the lipase is an important part of the enzyme production process. This is because of the wide range of lipase applications in biotechnology. The enzyme has a notable stability at temperatures up to 50°C as well as in low concentrations of organic solvents. In mild alkaline conditions, the PKC-lipase from *C. cylindracea* showed some levels of stability. Some metals stimulated the enzyme while others resulted in inhibition of its activity. The data obtained from the characterization study could show the efficiency of the enzyme in industrial processes such as applications in biodiesel production.

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