

SYNTHESIS OF LAURIC-RICH MEDIUM-CHAIN TRIGLYCERIDES FROM PALM KERNEL OIL AND LAURIC ACID BY ENZYMATIC ACIDOLYSIS

AGNES IMELDA MANURUNG¹; ELISA JULIANTI^{1*}; JANSEN SILALAH² and DONALD SIAHAAN³

ABSTRACT

Oil palm fresh fruit bunches contain two types of oil: Palm kernel oil (PKO) from the palm kernel and crude palm oil from the fruit flesh. Enzymatic acidolysis of PKO and lauric acid can produce medium-chain triglycerides (MCT), which offer nutritional and health benefits. This study aimed to synthesise laurate-rich MCT through enzymatic acidolysis. The reaction used an immobilised NS 400190 lipase catalyst (Novozyme). Variables included reaction time (0–48 hr), temperature (44°C–104°C), substrate ratio of PKO to lauric acid (1:1–1:11, molar basis) and an enzyme load of 7% w/w. The MCT yield (%) was analysed by determining the triglyceride's fatty acid composition using gas chromatography (GC). Results indicated that lauric acid incorporation increased with treatment time. GC analysis revealed that the highest lauric acid triglyceride profile occurred at 24 hr, 94°C and a substrate ratio of 1:9, achieving 81.4% lauric acid incorporation. The enzyme demonstrated thermophilic properties, with an optimal temperature of 94°C. The NS 400190 lipase enzyme exhibited significant operational stability, with a half-life of 1,517 hr in the acidolysis reaction.

Keywords: acidolysis enzymatic, lauric acid, medium-chain triglycerides, palm kernel oil.

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INTRODUCTION

Native lipids, more than 80%-85% of lipids, are in the form of triglycerides (TG) with specific properties and characteristics (Kim & Akoh, 2015). The characteristics of natural lipids are not always suitable for producing lipids with desirable added value, such as the synthesis of specific TG, known as structured TG. Specific TG with optimal properties results from modification of the fatty acid composition or the positional of the acyl groups on the glycerol backbone (Devi et al., 2008; Huang & Akoh, 1996; Willis & Marangoni, 2002).

Medium-chain TG (MCT) themselves are a type of modified TG. The uniqueness of MCT is metabolised differently compared to the other saturated oils, suggesting the potential benefits of MCT. TG's nutritional value and biochemical properties are measured by the composition of fatty acids and their position in the TG molecule (Hasanah & Warnasih, 2020).

MCT consists of medium-chain fatty acids (C8-C12) esterified on its glycerol skeleton. MCTs are quickly metabolised to produce energy in the body, and the energy produced by MCTs is twice that of carbohydrates and proteins (Nainggolan & Sinaga, 2021). Due to the uniqueness of MCTs in their physicochemical and nutritional properties, MCTs are widely used as nutraceuticals in food and pharmaceuticals, for example treating malabsorption syndrome, cystic fibrosis, epilepsy, improving protein and fat metabolism, premature infant food formulations, increasing stamina, reducing and controlling body weight, improving cognitive function, reducing allergenicity, antiviral therapy

¹ Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia.

² Faculty of Pharmacy, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia.

³ Indonesian Oil Palm Research Institute, Kp. Baru, Medan Maimun, Medan 20158, Indonesia.

* Corresponding author e-mail: elisa1@usu.ac.id

drugs, antibacterial agents (synergy between free fatty acids and monoglycerides) MCTs (Dayrit, 2014; Lee et al., 2012, 2022).

Palm kernel oil (PKO) is an oil primarily made up of medium-chain fatty acids (MCFA), which is more than 50%. Of which, lauric acid (C12) is the major fatty acid (46%–52%), caprylic acid (C10) and capric acid (C8) are the less prominent component (Dayrit, 2014; McCarty & DiNicolantonio, 2016; Silalahi et al., 2018). As lauric acid is the most pervasive medium-chain fatty acid in PKO, it is a key representative of the oil's properties. The metabolic and physiological properties of lauric acid increasingly play important roles in food processing technology, pharmacology and clinical nutrition (Dayrit, 2014; Enig, 1996; Lieberman et al., 2006; Ubgogu et al., 2006).

MCTs are not found naturally and must be synthesised (Liang et al., 2019). Enzymatic synthesis of MCTs is more advantageous than chemical as it is an environmental-friendly reaction, taking place at lower temperatures, little or no reaction by-products, ease of product recovery and controlling the reaction and low-risk factors for consumer health (Nunes et al., 2012). Several papers have reported on the enzymatic synthesis of MCT, such as interesterification (Feldes et al., 2009) and esterification (Langone & Sant'Anna, 2002; Nandi et al., 2005; Wong et al., 2000). However, only a few studies reported the acidolysis of medium-chain fatty acid with lipids catalysed by non-specific enzymes (Gökçe et al., 2013; More et al., 2018; Sousa et al., 2018). Non-specific enzymes have catalytic interesterification properties, such as chemicals where the interesterification produces a complete randomisation of acyl groups in TG so that it will produce the highest yield of MCT (Huang & Akoh, 1996; Osborn & Akoh, 2002).

Furthermore, the important objective of this research is to produce a novel type of MCT that contains rich-lauric acid by enzymatic acidolysis using immobilised non-specific lipase NS 400190 with variation in time, temperature and substrate ratio treatments and the operational stability of the enzyme-based on half-life value was carried out.

MATERIALS AND METHODS

Materials

Crude palm kernel oil was obtained from PT. Perkebunan Nusantara IV. Immobilised nonspecific enzyme NS 400190 was provided by Novozymes Group Entity, Denmark. Lauric acid (> 99% purity) and Standard TG mix were purchased from Sigma Aldrich, Germany, for gas chromatographic (GC) analysis using

Series 2010 Plus (Shimadzu, Japan). Thin-layer chromatography (TLC) and TLC silica gel 60 F254 Glass plates were also purchased from Sigma Aldrich, Germany. For chemicals such as N-hexane, 1-octanol, NaOH, Whatman filter paper No. 4, diethyl ether, glacial acetic acid, and other analytical chemicals were obtained from Sigma Aldrich, Germany and PT. Smart Lab Indonesia.

Experimental Methodology

Lipase-catalysed acidolysis reaction. The enzymatic acidolysis reaction used was a modified method (Abigor et al., 2003). A total of 10 g of the substrates (PKO and lauric acid with a molar ratio of 1:1 to 1:11) was transferred into a 50 mL Erlenmeyer flask. The substrate mixture was then added with NS 400190 lipase as much as 7% (of the total weight of the substrate). The reactions were conducted in a solvent-free system. They used seven different temperatures: 44°C, 54°C, 64°C, 74°C, 84°C, 94°C and 104°C. The reactions also varied across ten different time intervals: 0, 4, 8, 12, 16, 20, 24, 32, 40 and 48 hr. A heater shaker was used at a speed of 200 rpm. After the acidolysis reaction, the resultant mixture was separated from the enzyme using Whatman filter paper No. 4. The mixture was stored in a freezer (T, -4°C) for further analysis. Each reaction in this research was carried out in duplicate. The residual activity of the recovered enzyme was calculated to assess its operational stability.

Separation and analysis of triglycerides using (TLC). This procedure was done using a modified method based on Nunes et al. (2011). The triglycerides, which are the primary products of the acidolysis reaction, were analysed through TLC silica gel 60 F254 20 x 20 cm glass plate. The TLC plates were developed with a solution of hexane: diethyl ether: glacial acetic acid (80:20:1, v/v/v). Iodine gas was used to visualise the spots on the TLC plate. TG were identified by comparing them with the standard. Spots corresponding to each lipid type were scraped from the plate and dissolved in hexane. The hexane fraction was used for fatty acid composition analysis using GC.

Fatty acid composition analysis by GC. The fatty acid composition was analysed based on MPOB (Kuntom, 2005) by GC capillary column with flame ionisation detector (FID) under operating conditions: Crossbond capillary column (carbowax polyethylene glycol) of 25 m length, 0.25 mm diameter, 0.25 µm film thickness. The initial column temperature of 120°C and maintained for 7 min. It was then increased to 240°C at a rate of 3°C min⁻¹ and held at this final temperature for

26 min. The detector temperature was also set to 240°C. Helium was used as the carrier gas with a flow rate of 0.8 mL min⁻¹, while nitrogen was used as the make-up with a flow rate of 30 mL min⁻¹. The injection mode was set to split with a ratio of 1:100. Fatty acid composition was identified by comparing the retention time of the sample peak with the standard solution. The concentration of fatty acid in triglycerides was calculated by using Equation (1).

$$\text{The concentration of fatty acid} = \frac{\text{Area of fatty acid}}{\text{Total area}} \times 100\% \quad (1)$$

Residual activity of NS 400190 lipase enzyme.

Determination of the remaining activity of NS 400190 lipase enzyme was carried out by esterification reaction based on the modified method of Kuhn et al. (2010). The esterification reaction was chosen because the analytical method is very simple and gives a rapid response. The used enzyme must first be washed using a modification of the Aguiéiras et al. (2016) method. The washed enzyme was then incubated using 15 mL hexane as a solvent for 2 hr at 25°C. Then the suspension was separated from the supernatant with the enzyme by filtration with Whatman filter paper No. 4. The separated enzyme was analysed for enzymatic activity. Esterification was carried out by reacting lauric acid substrate with 1-octanol with a molar ratio of 1:1 in a 50 mL Erlenmeyer flask. A 5% (w/w of total substrate) amount of lipase NS 400190 was added to the substrate. The reaction was carried out at 50°C for 60 min. After the reaction, the substrate was filtered to separate from the enzyme. The substrate mixture was then titrated with 0.01 M NaOH solution. In addition, a blank sample titration was also carried out which was a mixture of 1-octanol and lauric acid substrates without enzyme. Determination of lipase enzyme activity is based on the amount of lauric acid consumed. One unit of lipase activity (U) is defined as 1 μmol of lauric acid used in the esterification reaction (EA) per min per gram of lipase, according to the following Equation (2).

$$EA = \frac{V_{NaOH} \times M_{NaOH} \times 1000}{W \times t} \quad (2)$$

where V_{NaOH} is the volume difference of NaOH blank and after esterification reaction, M_{NaOH} is the molarity of NaOH, W is the amount of enzyme (g), and t is the reaction time (min). The same procedure was used to calculate the initial esterification activity value of NS 400190 lipase enzyme (reaction hr 0/ EA_0). The remaining activity value of the enzyme ($EA\Delta$) was calculated using

Equation (3), where $EA\Delta$ indicates the esterification activity value of the enzyme after reaction at a certain time:

$$EA\Delta (\%) = \frac{EA\Delta}{EA_0} \times 100\% \quad (3)$$

Statistical analysis. All data in this study were conducted in duplicate. Each data was presented as means ± standard deviation by using MS Excel. One-way ANOVA was performed to determine the difference between the means in the activity of temperature, hours and molar ratio of substrate. Half the lifetime of the lipase was predicted based on the linear regression model.

RESULTS AND DISCUSSION

In this study, enzymatic acidolysis between PKO and lauric acid is expected to produce lauric-rich medium-chain triglycerides with the treatment of time, temperature and substrate ratio. The lipase enzyme used was immobilised NS 400190 lipase

Effect of Reaction Time on Lauric Acid Incorporation into Palm Kernel Oil

Figure 1 shows the chromatogram profile of the fatty acid analysis before and after the acidolysis reaction. The incorporation of lauric acid (C12) before reaction (A) increases after the enzymatic acidolysis reaction of PKO with C12 (B). Peak characterised as C12 (peak #3) with a retention time of 2.653, increasing from 47.83 (area%) to 60.97 (area%).

Figure 2 shows that the reaction time is significantly affected by lauric acid concentration. At the beginning of the reaction (up to 4 hr), there was no change in lauric acid incorporation, which remained steady from 44.15%–44.30%. Between 4 and 8 hr, lauric acid incorporation increased substantially from 44.3%–62.9%, representing an 18.6% rise. In contrast, from 8–24 hr, the increase was modest, with incorporation rising from 62.9%–66.6%, or just 3.7%. This indicates that the reaction period from 4–8 hr is more effective than from 8–24 hr. Extending the reaction time to 48 hr showed no significant difference in lauric acid incorporation between 8–48 hr. Thus, it appears that the reaction has reached an equilibrium state at a reaction time of 8–48 hr. These results indicate that the reaction time influences the concentration of lauric acid incorporation. In this study, the concentration of lauric acid peaked at 66.6% after 24 hr. There was no significant difference in lauric acid concentration between 8–24 hr. During this period, the rate of acyl incorporation into TAGs either slowed down or slightly decreased.

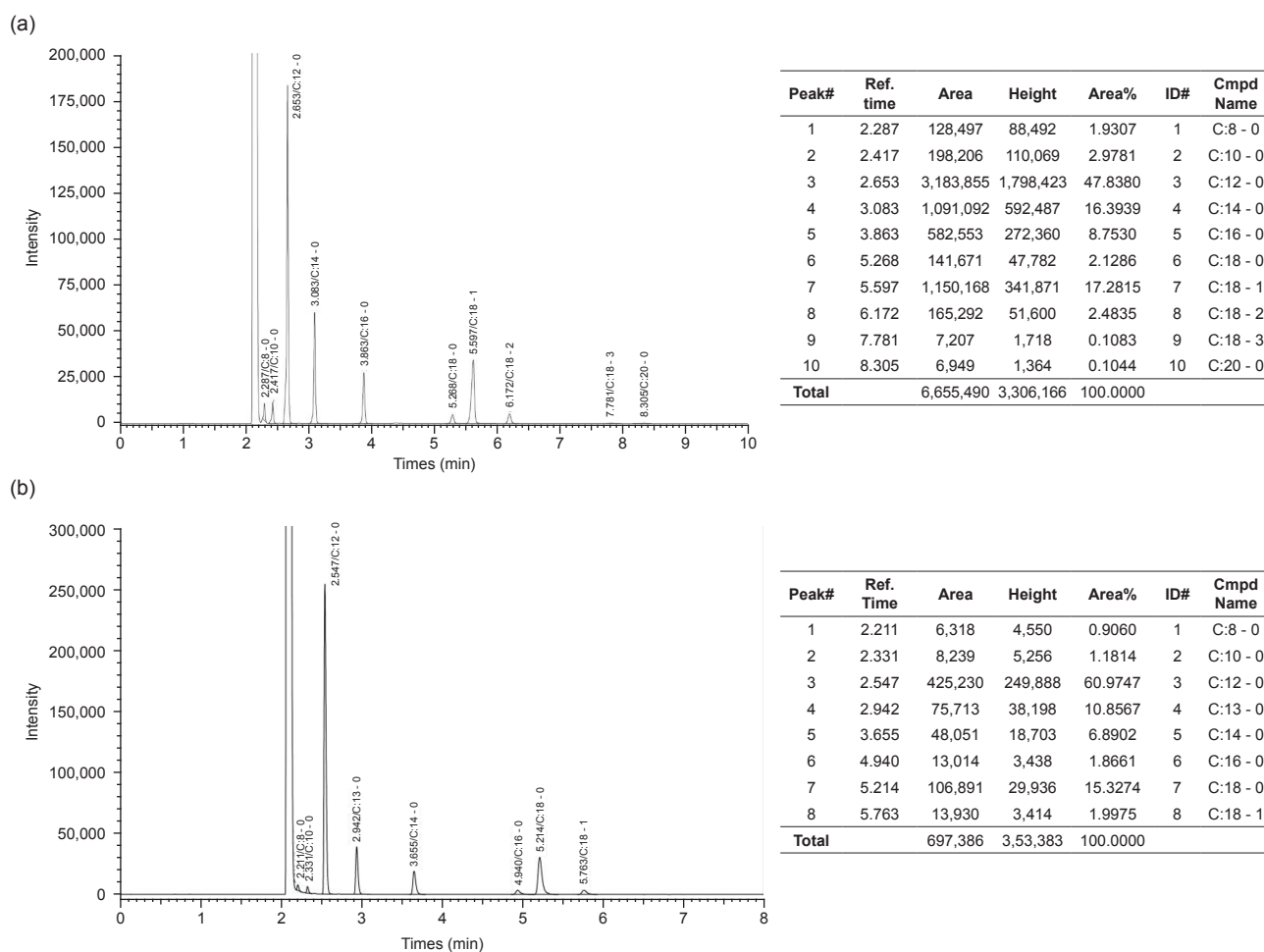


Figure 1. Fatty acid chromatogram (a) before and (b) after acidolysis reaction.

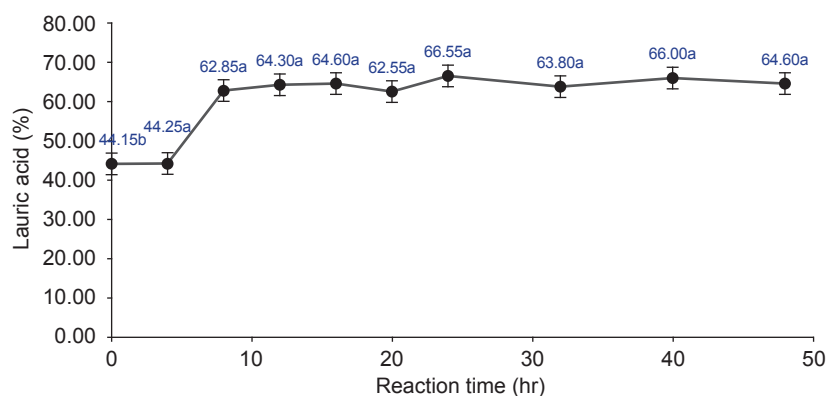


Figure 2. Effect of reaction time on lauric acid incorporation in PKO.

Effect of Reaction Temperature on Lauric Acid Incorporation into Palm Kernel Oil

In Figure 3, the temperature of the enzymatic acidolysis reaction affected the incorporation of lauric acid. The increasing incorporation of lauric acid at a reaction temperature of 44°C–94°C, from 61.8%–71.3%, is about 9.5%, then at a reaction temperature of 104°C, the lipase

NS 400190 began to denature, causing reduced and catalytic activity which was indicated by a decrease in lauric acid incorporation to 49.2% (Kosiyanant et al., 2018).

At 94°C temperature, lauric acid incorporation was the highest at 71.3%, but there was no significant difference between 64°C and 94°C. The same research conducted by Langone and Sant'Anna (2002) showed that the highest trilaurin

was 76% at 80°C–90°C after 26 hr of reaction. The temperatures of 44°C and 94°C showed a significant difference in lauric acid incorporation. The higher the reaction temperature, the solubility of the reactants increases and decreases the viscosity of the solution. It improves the interaction between the reactants and the enzyme’s active sites, leading to increased enzyme activity and a higher rate of fatty acid incorporation (Abed et al., 2017; Kavadia et al., 2018; Langone & Sant’Anna, 2002; Lee et al., 2012; Li et al., 2021). The advantage of this study is that the lipase enzyme is non-specific, such that its activity is like that of a chemical reaction. Based on this study, the lipase NS 400190 is a high-temperature resistant enzyme, and the best reaction temperature is from 44°C–94°C.

Effect of Substrate-Molar Ratio on Lauric Acid Incorporation into Palm Kernel Oil

The substrate-molar ratio is a critical factor affecting the equilibrium point in the reversible reaction to produce optimum medium-chain TG during the enzymatic acidolysis reaction (Hu et al., 2011).

Figure 4 shows the effect of substrate molar ratio on lauric acid incorporation in TG. The results

indicated that substrate molar ratio had a significant effect on lauric acid incorporation. When the molar ratio was increased, the concentration of lauric acid increased from 57.20%–80.00%. With increasing molar substrate, the reaction equilibrium will shift toward the synthesis direction. The concentration of lauric acid incorporation in triglycerides increased (57.2%–80.0%) as the molar ratio of substrate increased (1:1–1:11) (Kosiyanant et al., 2018; Langone and Sant’Anna, 2002). This study showed that the reaction reached equilibrium at a molar substrate ratio of 1:3 to 1:11. However, lauric acid incorporation decreased when the molar ratio shifted from 1:9–1:11. This reduction may be attributed to decreased activity of lipase NS 400190 at higher substrate molar ratio, which affects the equilibrium constant and may also impact the product purification stage (Abed et al., 2017; Li et al., 2021).

Operational Stability of Non-specific NS 400190 Lipase

The operational stability of non-specific lipase NS 400190 was measured by observing the esterification activity and residual activity after being used for a certain reaction time.

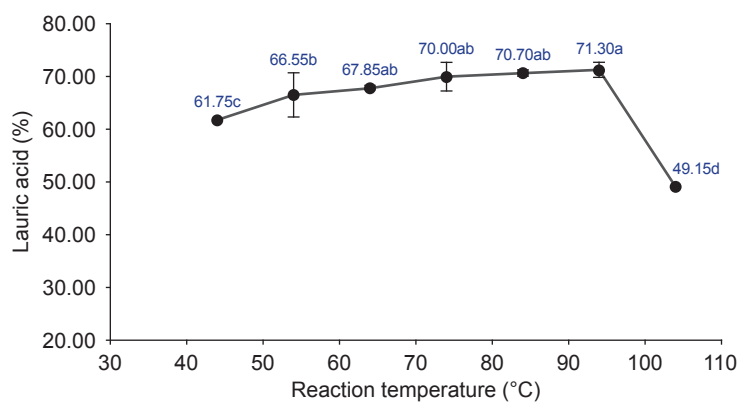


Figure 3. Effect of reaction temperature on lauric acid incorporation in PKO.

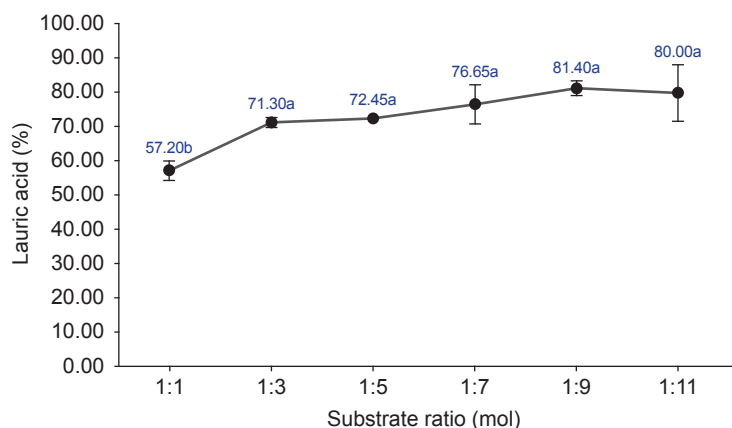


Figure 4. Effect of the molar ratio of PKO oil to lauric acid substrate on lauric acid incorporation into PKO.

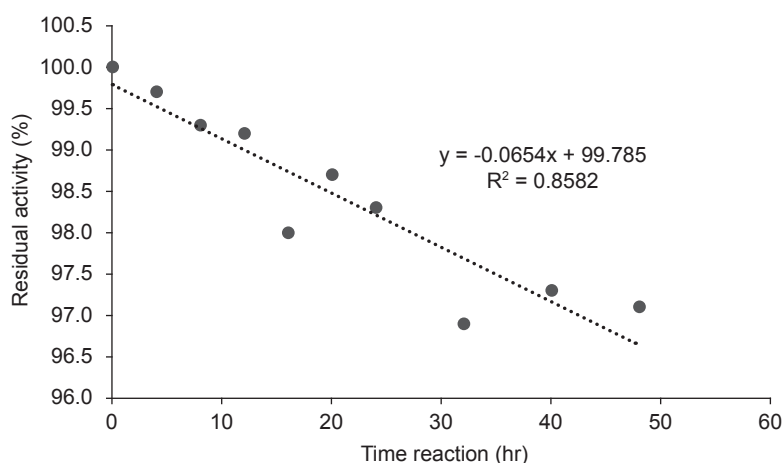


Figure 5. The remaining activity value of the lipase NS 400190 in acidolysis reactions.

In this study, esterification and residual activity of NS 400190 lipase enzymes were observed because of the different interesterification reaction times (acidolysis). Esterification activity shows the esterification ability of the enzyme after being used in the interesterification reaction (acidolysis) during a specific time interval. The remaining activity value is the value of the enzyme esterification activity that remains after being used in the interesterification reaction during a specific time interval. The remaining activity value can be obtained by comparing the esterification activity value of NS 400190 lipase enzyme after the interesterification reaction at a certain time to the initial esterification activity value of NS 400190 lipase enzyme (before being used in the interesterification reaction).

Figure 5 shows the stability value of the non-specific NS 400190 lipase enzymes based on the change of the enzyme's remaining activity value after being used in the acidolysis reaction. In this study, the initial (esterification) activity of NS 400190 lipase was 814.0 U after being used for 48 hr in the acidolysis reaction, the remaining activity value of NS 400190 lipase was 792.5 U. The remaining activity value of NS 400190 slightly decreased from 100%–97.1%. Based on the linear equation, the operational stability value of the non-specific NS 400190 lipase enzyme was obtained based on the 50% half-life time value of 1,517 hr in the acidolysis reaction.

CONCLUSION

The synthesis of lauric-rich medium chain triglycerides can be carried out by enzymatic acidolysis in a solvent free system with the best conditions at 94°C, 24 hr and a palm kernel oil to lauric acid substrate ratio of 1:9 which can incorporate 81.4% lauric acid. These conditions provided operational stability of the immobilised NS 400190 lipase for 1,517 hr in the acidolysis reaction.

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