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Physical Characteristics of Structured Lipid Synthesized by Lipase Catalyzed Interesterification of Coconut and Palm Oils

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ABSTRACT

The physical characteristics of lipids are important determining factors for appropriate food applications. The objective of this research is to study the physical characteristics, especially thermal (melting and crystallization) behavior, smoke point and solid fat content (SFC), of structured lipids (SLs) produced by interesterification of coconut and palm oils catalyzed by two commercial lipases (Thermomyces lanuginosa imobil/TLIM and Novozyme 435). The results showed that SLs produced by 5 hours of interesterification had physical characteristics differing from the original blended lipids. SLs obtained by both enzyme systems exhibited a higher and wider range of melting temperature (higher enthalpy value, ΔH), lower and wider range of crystallization temperature (higher ΔH), lower smoke point, and lower SFC at 0°C and 10°C than those of their blended lipid counterpart. Furthermore, TLIM lipase produced SLs with a higher and wider range of melting temperature, lower and wider range of crystallization temperature, lower smoke point and SFC at 0°C and 10°C compared to those produced by Novozyme

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435. The SLs produced have the potential to be used as an ingredient in refrigerated foodstuffs and more plastic than the original oil.

Keywords: Coconut oil, crystallization, enzymatic interesterification, palm oil, physical properties

INTRODUCTION

Coconut and palm are two important oil plants found in the tropical region. Both plants have become the source of leading vegetable oils for food and nonfood applications due to their unique qualities and properties. Coconut oil is rich in medium chain fatty acids and is suitable as a raw material for food, medical, or other functional products (McCarty & DiNicolantonio, 2016). Palm oil, especially from its olein fraction, has a high content of unsaturated fatty acids, such as oleic and linoleic acid, the specific functional properties of which are to increase high density lipoprotein (Mba et al., 2015). The use of these vegetable oils is increasing along with the development of the food processing industry. Furthermore, lipids application in food processing technology is also developing very rapidly. However, direct application of native lipids are not always suitable due to their limited properties. Therefore, there is a need to modify native lipids by synthesizing structured lipids (SLs) with specific properties (Rohm, 2018).

The enzymatic interesterification technique can be used to modify physicochemical properties of lipid substrates to produce a new type of lipid suitable for special food product applications. Enzymatic interesterification results in redistribution of fatty acids within the glycerol backbone, at which the position of fatty acids are interchanged (Norizzah et al., 2015; Smith, 2015). Consequently, interesterification of lipids may produce SLs having physical properties different from those of the original lipids due to rearrangement and/or redistribution of their fatty acids on the glycerol backbone (Farmani, 2015). However, this rearrangement does not change the degree of unsaturation or isomer configuration of the fatty acids (Pande & Akoh, 2013; Mba et al., 2015). SLs with different characteristics are expected to increase the added value of native lipids.

The use of lipase allows the reaction to have a specific affinity to produce a specific structured triacylglycerol (TAG). This allows the control of thermal properties and other physical characteristics of the SLs products. Many researchers have attempted to synthesize SLs by using lipases (Bornscheuer, 2014; Smith, 2015; Wirkowska-Wojdyła et al., 2016). The obtained SLs exhibit different physical properties compared to those of the original native lipids. Such physical properties include thermal properties (i.e., melting and crystallization behavior), smoke point, and solid fat content (SFC) (Danthine et al., 2014; Li et al., 2010; Sharma & Lokesh, 2012). Those physical properties are very unique and have a profound effect on certain plasticity behaviors of lipids, which are important for food product applications.

Thermal characteristics, an important aspect of oil and fat physical properties, can cause changes in the solid-liquid and liquid-solid phases, such as melting and crystallization behaviors. The thermal properties are determined based on the enthalpy profile during the crystallization and melting processes, and these properties can be analyzed by DSC (Saberi et al*.,* 2011; Menczel & Prime, 2014; Srivastava et al*.*, 2017; Oliveira et al*.*, 2017). Changing of crystallization conditions will affect the crystal type, crystal size, and crystal amount, which will influence the product's character (i.e. texture, consistency) (Zhang et al*.*, 2013; Tan & Man, 2002; Azis et al., 2011). Meanwhile, smoke point is a vital analytical measurement for any lipid used for high temperature applications, such as the frying process in which a higher smoke point is desirable. Therefore, smoke point is an important consideration when selecting oil for this application. Another important physical property of oil is the SFC profile, which provides a complete melting profile of the lipid at various temperatures (Gunstone, 2011). The SFC profile provides information not only on the functional properties of lipids but also on controlling the crystal structure and polymorphism of a certain product, such as plasticity in bakery products (Xu et al*.*, 2017; Mayamol et al*.,* 2009). The SFC of lipids indicates product characteristics such as general appearance, simplicity of packing, organoleptic properties, and convenience of spreading. SFC can also be used to study fat compatibility by determining its changes in the percentage of solid phase against different fat proportions (Karabulut et al., 2004; Cheong et al*.*, 2009). Time domain nuclear magnetic resonance (TD-NMR) is a tool used to determine SFC for the production and processing of fats and oils. Hindered diffusion is characterized by motion of molecules within the droplets phase of the emulsions (Cobo et al*.*, 2017).

Through the interesterification process in a mixture of coconut oil and palm oil, it is expected that SLs can be produced with superior specific characteristics, both chemical and physical. In this study, the physical characteristics of SLs from a mixture of coconut oil and palm oil were examined. SLs were catalyzed by two commercial immobilized lipases with different specificity. *Thermomyces lanuginosa* imobil /TLIM is an sn-1 and sn-3 specific lipase and Novozyme 435 which is a nonspecific lipase. The physical characteristics were then analyzed, specifically thermal (melting and crystallization) behavior, smoke point and SFC.

MATERIALS AND METHODS

The main materials were coconut oil obtained from PT. Barco, olein fraction of palm oil with 60 iodine value obtained from PT. Salim Ivomas Pratama Tbk, immobilized lipases *Thermomyces lanuginosa* imobil (TLIM) and Novozyme 435 (Novozyme A/S, Bagsvaerd, Denmark), 4A molecular sieves, and chemical materials obtained for analysis purposes. The equipment used were an orbital shaker, differential scanning calorimetry (DSC-60

Shimadzu), and TD-NMR mqone, Bruker. The research was conducted in Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University, Indonesia; and Indonesian Oil Palm Research Institute (IOPRI) Medan, IPB, Sumatera, Indonesia.

Synthesis of SLs from Coconut and Palm Oils

SLs were synthesized using the enzymatic esterification method referred to by Yang et al. (2014) by mixing 10 g of coconut and 10 g of palm oils into a 50 mL Erlenmeyer flask in a free solvent system. The mixture was then combined with 6% (w/w) enzymes (TLIM lipase or Novozyme 435 lipase) and left to react for 5 hours using a rotary shaker (200 rpm, 55°C). At the end of the reaction time, the immobilized enzyme was removed from the mixture by vacuum filtration using Whatman ® Grade 4.

Blending of Coconut and Palm Oils

Blending the coconut and palm oils was conducted by mixing coconut and palm oils in a ratio of 1:1 (w/w) to a total of 500 ml of oil, which was then homogenized using a magnetic stirrer for 15 minutes. The blended oil (without interesterification) was used as a reference to determine the effectiveness of interesterification, catalyzed by two types of lipases, in synthesizing SLs by comparing their respective physicochemical characteristics.

Analysis of Thermal Behavior

The thermal behavior of the oils was determined by using DSC as described by Saberi et al. (2011). As much as 10 mg of sample was added to an aluminum DSC sample pan. The exothermic curve was obtained by maintaining the sample at 80°C for 10 minutes, and continued with cooling until the temperature reached -50°C at a cooling rate of 5°C/minute. To obtain an endothermic curve, the sample was maintained at -50°C for 10 minutes, then gradually heated to 80°C at a rate of 5°C/minute. The crystallization process is indicated by peaks at the beginning of the cooling process stage, whereas the melting process is indicated by peaks produced at the end of the heating process stage.

Analysis of Smoke Point

The smoke point was determined by heating the oil sample using a Cleveland open-cup apparatus. The temperature at which smoke began to appear was taken as the smoke point (AOCS, 2011).

Analysis of Solid Fat Content (SFC)

The SFC was determined using NMR by the standard method of AOCS (Official method Cd 16b-93, AOCS, 1989). The melted oil sample was inserted into 12 tubes for SFC analysis

with a sample height of 4±1 cm. Samples in sealed tubes were heated inside a water bath at 100°C for 15 minutes, then later moved to a 60°C water bath for 5 minutes. Six pairs of sample tubes were immersed in a water bath at 0, 10, 20, 30, 40, or 50°C for 30 minutes. The SFC of each sample at each temperature was measured by inserting the holder to the TD-NMR unit by using the Non Stab AOAC method.

RESULTS AND DISCUSSIONS

Lipase-catalyzed interesterification of coconut and palm oils produced SLs as a result of acyl exchange among the TAGs, with observed changes in physical characteristics, such as thermal (melting and crystallization) behavior, smoke point, and SFC.

Thermal Properties

The thermal properties were determined based on thermograms obtained by DSC analysis. Except DSC, other methods that can be used for thermal analysis are thermal gravimetric analysis and differential thermal analysis. DSC is a thermal analysis technique that has been applied in the characterization of thermal behaviors in lipids, especially melting and crystallization curves, and determining the melting temperature, crystallization temperature, and enthalpy value (ΔH). Furthermore, DSC also gives rapid, reproducible results without sample preparation and does not use dangerous solvents (Menczel & Prime, 2014; Chiavaro et al., 2008; Wetten et al., 2015). DSC can be used to determine cooling transition temperatures as well as monitor the extent of crystallization and various thermal parameters of the chemical reaction simultaneously (Tan & Man 2002; Musa & Wong 2013; Menczel & Prime, 2014; Lai et al., 2000; Indartono et al., 2011; Siregar et al., 2011). Figure 1 shows the thermograms of the blended oil and SLs produced from interesterification, whereas temperature and ΔH values are shown in Table 1.

Table 1

Thermal characteristics of blended oil and SLs from coconut and palm oils

Thermal characteristics		Temperature (°C)			ΔH (J/g)
		onset	peak	offset	
Melting	B	11.28	16.17	20.55	-36.29
	N5	10.57	16.56	21.86	-66.43
	T5	10.54	17.50	28.30	-68.39
Crystallization	B	-7.83	-10.59	-13.87	4.71
	N5	-3.65	-9.00	-12.83	8.04
	T5	-4.88	-15.35	-27.85	8.32

Note: B = Blended oil, N5 = SL obtained using Novozym 435 for 5 hours, T5 = SL obtained using TLIM for 5 hours

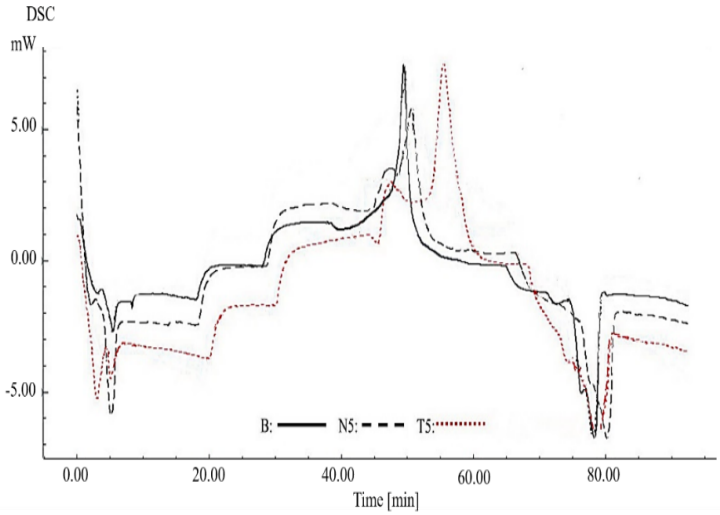

Figure 1. Thermograms of blended oil and SLs from coconut and palm oils

The obtained data was in the form of continuous thermogram representing melting and crystallization phenomena. According to research from Becker et al. (2015) the used of DSC can determined thermal behavior such as melting and recrystallization point, polymorphism, melting/recrystallizing fractions. The thermograms of the melting process of all samples show a negative curve, indicating that the process is endothermic (Chong et al*.,* 2007). The thermograms of the crystallization process have a simpler shape than those of the melting process, attributed to the chemical composition of the oil, not by the crystallization status (Tan & Man, 2000). The crystallization process is marked by the beginning of fat crystal formation, related to the rearrangement of the molecule due to the presence of highly saturated TAG. The ending of crystallization is usually reflected by an aggregation and compaction of the molecule.

Melting point affects the plasticity and simplicity of the melting of fats at human body temperature. The TAG of oil that will first experience transformation into a solid during the cooling process is the one with the longest chain fatty acid (Timms, 2005). Several researchers have attempted to improve the melting properties of fats by the formation of SLs so that the melting profile is more suitable for a specific product application (Fauzi et al*.*, 2013; Zhu et al*.*, 2012).

Table 1 and Figure 1 show that SLs obtained using interesterification catalyzed by both enzymes exhibit a higher and wider range of melting temperature than that of blended oils. This result is in agreement with Oliveira et al*.* (2017), in which the chemical interesterification of palm stearin and patawa oil produced a lipid with an increased melting point. The melting point of fats is highly dependent on the properties of the materials,

especially the types of fatty acids comprising the fat (i.e., chain length and double bonds) (Marikkar et al., 2013; Knothe & Dunn, 2009). The difference in melting points results from the difference in the number of hydrogen bonds in the carboxyl group and the hydrophobic interactions along the hydrocarbon chain of each product.

SLs obtained by TLIM lipase-catalyzed interesterification have a higher melting peak and a longer melting process than those catalyzed by Novozyme 435 lipase. The increase in melting point is caused by the formation of high melting point TAGs and the occurrence of TAG hydrolysis during enzymatic interesterification. The formation of a new TAG species consisting of high melting fatty acids influences the melting point of the TAG itself, thus a high melting point TAG is produced. The TAG also changes to monoacylglycerol and diacylglycerol (Mardani et al., 2015).

Another important thermal property analyzed for SLs is the crystallization profile. The rates of crystal growth and nucleation determine parameters that are directly related to the consistency and characteristics of textures, such as crystal distribution, shape, and size (Saberi et al., 2011; Ribeiro, 2015). A crystallization profile starts with the formation of the fat crystal, which occurs upon the rearrangement of the molecule due to the presence of high saturated TAG and ends with aggregation and compaction of the fat molecule (Tan & Man, 2002). The crystallization peak was calculated based on the DSC crystallization curve. The results in Table 1 indicate that, in general, interesterification of coconut and palm oils produced SLs with a lower and wider range of crystallization temperature than that of their blended oils. SLs obtained by TLIM lipase interesterification had a lower and wider range of crystallization temperature compared to those catalyzed by Novozyme 435. The greater the range of onset and offset temperatures, the wider the range of crystallization temperature. Crystallization temperature may affect the kinetic and physical properties of the crystallization system significantly. Crystallization at lower temperatures is the driving force for the nucleation process and supports the formation of less stable polymorphs (Tran & Rousseau, 2016). Polymorphism characteristics of the end texture for food as complex systems can be determined by thermal treatment. Such characteristics consist of spreadability, microstructure, mouthfeel, and rheology (Rønholt et al., 2012; Rønholt et al., 2014).

The enthalpy value (ΔH) of melting can also be determined from the DSC thermogram, as presented in Table 1. ΔH of melting indicates the amount of energy absorbed by the sample when melting occurs, whereas ΔH of crystallization is the amount of energy released by the sample when crystallization occurs (Tan & Man, 2002). The controlled heating rate of the number of active nuclei and the time for growth affect the rate of crystallization. The crystallization enthalpy is a relative measure of the number of nuclei before the heating scan. Interactions between component TAGs and molecular structures is observable by kinetic crystallization. Increasing crystallization temperature causes the ΔH value to

decrease significantly (Tran & Rousseau 2016). SLs obtained using both enzymes show higher enthalpy values for both melting and crystallization processes, than those of their native blended lipids.

Smoke Point

During lipid heating in a smoke point analysis, the temperature is monitored and the smoke point is determined as the temperature of the lipid where a steady evolution of smoke emerges from the lipid in the cup (Eyres, 2015; Gunstone, 2011). This point indicates that the oil begins to break down to glycerol and free fatty acids, marking the beginning of both flavor and nutritional degradation. The smoke points of the SLs produced in this study are presented in Figure 2.

SLs produced by both enzymes had lower smoke points compared to their native blended lipids. The lower smoke point of the SLs obtained by lipase-catalyzed interesterification might be attributed to the exchange of fatty acid in the oil, which had a higher vapor pressure than TAG in blended oil (Willis & Marangoni, 2002).

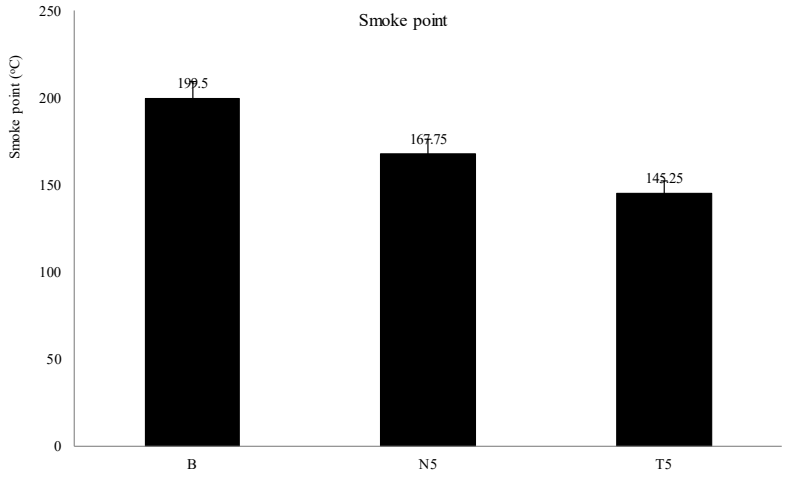

Figure 2. Smoke point of blended oil and SLs from coconut and palm oils

Note: B = Blended oil, N5 = SL obtained using Novozyme 435 for 5 hours, T5 = SL obtained using TLIM for 5 hours

Solid Fat Content (SFC)

Lipid consistency can be determined by SFC, which indicates the amount of solid TAG in the lipid at a certain temperature. The SFC profile of blended coconut and palm oils and the obtained SLs can be seen in Figure 3.

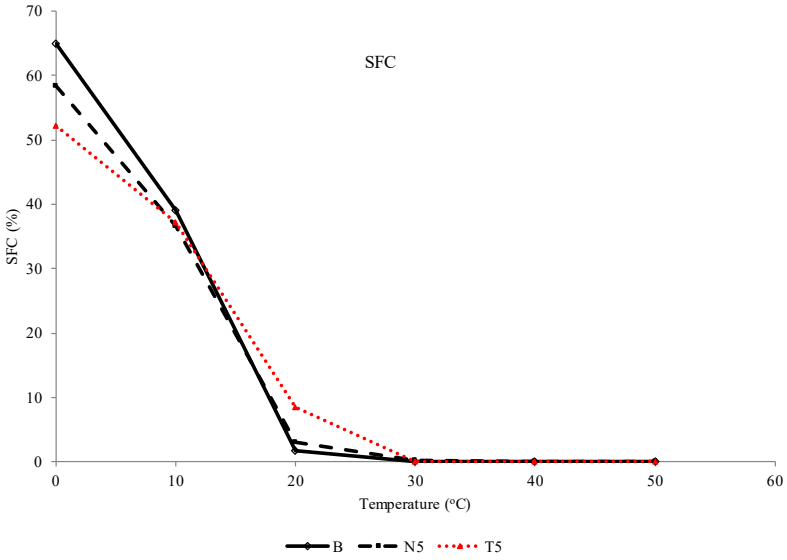

Figure 3. SFC of blended oil and SLs from coconut and palm oils

Note: B = Blended oil, N5 = SL obtained using Novozyme 435 for 5 hours, T5 = SL obtained using TLIM for 5 h

Figure 3 shows that the higher temperature produced lower SFC, because fat crystals would melt when the temperature rose to the fat melting point (Xu et al., 2017; Mayamol, et al. 2009). The SLs obtained by both enzymes had a lower SFC than the blended oil at 0ºC and 10ºC. The SLs catalyzed by TLIM lipase had a lower SFC at 0ºC and 10ºC, resulting in a more sloping curve compared to those catalyzed by Novozyme 435. SFC affects the hardness, melting rate, and flavor release in the mouth. A high SFC tends to give a waxy mouth feeling when the product is consumed. To ensure good melting properties and product temperature stability need a steep SFC profile is desired (Rousseau & Marangoni, 2002). Sloping SFC profiles (low SFC values at low temperatures) indicated more plastic lipids, suitable for spread product. The SFC test results are in line with the melting point test results using DSC. From the thermal and SFC characters, make lipids have plastic properties.

Based on the study of their physical properties, the SLs produced herein have higher melting temperature, lower crystallization temperature, lower smoke point, and lower SFC at 0ºC and 10ºC. Thus, it can be concluded that the structured lipids produced herein have the potential to be used as ingredients in refrigerated foodstuffs.

CONCLUSIONS

SLs obtained from interesterification of coconut and palm oils catalyzed by two lipases

exhibit different physical characteristics compared to their native oils. The SLs produced have a higher melting temperature and wider range of melting temperature, lower crystallization temperature and wider range of crystallization temperature, and higher enthalpy values for both melting and crystallization. The SLs also showed a decrease in smoke point and a lower SFC in the lower temperature region compared to their blended lipid counterpart. Based on the physical properties identified, the SLs produced have the potential to be used as an ingredient in refrigerated foodstuffs and more plastic than the original oil.

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