

Lowering of Palm Oil Cloud Point by Enzymatic Acidolysis

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Abstract: This study was carried out to lower the cloud point value of palm oil by enzymatic acidolysis at different mole ratio of palm oil to capric acid of 1:1, 1:2, 1:3 and 1:5. Lipase 1, 3-specific from *Rhizomucor miehei*; lipozyme RM IM was used in this study. Cloud point value was determined using PORIM p4.3 method (1995). Results showed that by increasing the mole ratio of palm oil to capric acid resulted in the increase of cloud point value before and after acidolysis significantly ($p < 0.05$). The cloud point value was decreased after acidolysis for each mole ratio.

Key words: Palm Oil • Cloud Point • Enzymatic Acidolysis

INTRODUCTION

Oil palm (*Elaeis guineensis*) is a plant which produces two different types of oils which are palm oil and kernel palm oil [1]. The composition of fatty acid in palm oil shows that it is inclusive of the type of saturated fat where the highest fatty acid is palmitic acid which is 46% followed by oleic acid, 38%. Meanwhile, for the kernel palm oil, it is included in the saturated fat type because the content of lauric acid is the highest which is at 51% followed myristic acid, 18%.

Both of these oils are important for commercial purpose and are not only used in a variety of food but in non-food industries as well. Nonetheless, the crude oil obtained from oil palm need to be purified through a subsequent process before it can be used. The early stage of processing included the extraction of the crude oil from its fruit and its purification to eliminate unwanted components and existing impurities [1].

To diversify the functions and usage of fat and oil, modification is done especially to improve the physicochemical characteristics and stability. The frequently used modification methods are fractionation, hydrogenation and transesterification which include acidolysis, alcoholysis, glycerolysis and interesterification. Palm oil is formed mainly from three groups, triacylglycerol (TAG) which is 8% trisaturated TAG mainly PPP, 45% disaturated TAG mainly POP and

PLinP and 40% monosaturated TAG mainly POO and PLinO [2].

In studies involving palm oil especially, that of acidolysis is one of the important transesterification reactions involving the reaction between TAG and fatty acid (FA). This reaction will result in changes of fatty acid arrangement inside the TAG molecule [2]. Acidolysis is also referred to as the changes between acyl and acid groups (fatty acid) and ester. Acidolysis is an effective method to consolidate specific fatty acid inside the triacylglycerol to achieve the wanted functions. Structured lipid is also produced through acidolysis to increase or change the physical and/or chemical TAG characteristics. Structured lipid is produced through the TAG modification to change the fatty acid composition and/or position on the glycerol backbone through the chemical or enzymatic methods [3].

Structured lipid is TAG that consists of short chain and/or medium chain fatty acid and long chain fatty acid on an equal glycerol molecule [4]. This type of structured lipid cannot be found naturally and cannot be produced through chemical reaction. It can only be synthesized through enzymatic method using specific lipase *sn*-1,3 [5]. Medium chain fatty acid on TAG contains caprylic acid (C_8) and capric acid (C_{10}), with caproic acid (C_6) and lauric acid (C_{12}) as the minor components [6]. Compared to long chain TAG, the medium chain TAG is more prone to be turned into body fat during metabolism because it is not re-estered to TAG [7]. For structured lipid, the medium

chain fatty acid stands at *sn*-1,3 and fatty acid needs a long chain at *sn*-2 standing on the glycerol backbone. This unique combination gives a fast power impact and other health benefits [8].

The study on the crystallization of palm oil at a low temperature is important to determine the stability of the palm oil at cold temperature and prevent the palm oil from turning cloudy at different temperatures. Normally, olein palm oil crystallizes at the temperature between 5 and 28°C [9]. Even though the crystallization of palm oil at low temperature does not damage the quality, yet the cloud can damage consumer acceptance and it need to be dealt with.

In this study, palm oil is chosen to conduct an acidolysis reaction. By using specific lipase1,3, capric acid can be included at palm oil *sn*-1,3 standing while fatty acid at *sn*-2 standing is retained for a more effective absorption during metabolism [8]. Therefore, the objective of this study is to reduce the value of palm oil cloud point through the enzymatic acidolysis reaction.

MATERIALS AND METHODS

Materials: The brand of the palm oil that is used as the study's substrate is Vesawit and it is bought from Warta Supermarket, Bandar Baru Bangi, Selangor. Fatty acid that is used is capric acid (C₁₀) which is bought from Southern Acids (M) Bhd. The enzyme that is used is specific lipase 1,3 from *Rhizomucor miehei*, produced inside *Aspergillus oryzae* which is bought from Science Technics Sdn. Bhd., Subang Jaya, Selangor.

Determination of Saponification Value: Saponification value is determined for the palm oil sample using AOAC method [10]. Saponification value is the weight in milligram (mg) KOH which is needed to saponify 1g of fat and oil.

It is also the triacylglycerol molecule average weight index in fat and oil sample. The reason for the determination is to describe molecular weight and the length of fatty acid chains in fat and oil. The saponification value is inversely proportional with the molecular weight and the chain's length.

In determining saponification value, about 2 ± 0.005g palm oil is weighed in a round flask and 25ml of 0.5N KOH solution is added which is about 95% (volume/volume) ethanol. Next, boiling chip is added and connected to the reflux condenser. The solution is heated for 60 minutes and is left to cool before it is added with 1ml phenolphthalein and titrated with 0.5N HCl until the pink colour disappear. The same process is conducted to

determine blank (without oil). Saponification value is counted using the following formula:

$$\text{Saponification value} = \frac{56.1N (V_b - V_s)}{W} \quad (1)$$

Where : V_b = HCl solution volume (ml) that is used for blank (without oil)

V_s = HCl solution volume (ml) that is used for palm oil sample

N = HCl normality

W = Sample weight (g)

56.1 = KOH molecule weight

Determination of Palm Oil Molecule Weight:

$$\text{The average of TAG molecule weight} = \frac{56.1}{\text{SN}} \times \frac{1000\text{mg}}{1\text{g}} \times 3 \quad (2)$$

Where : 56.1 = KOH molecule weight

SN = saponification value in mg/g

Acidolysis of Palm Oil with Capric Acid: Enzymatic acidolysis of palm oil and capric acid at different molarity ratio of 1:1, 1:2, 1:3 and 1:5 (palm oil:capric acid) is conducted using a water steamer shaker. The enzyme used is specific lipase enzyme 1,3 from *Rhizomucor miehei*. The mixture of the palm oil and capric acid is shaken in the water steamer at 200rpm. The temperature used is 60°C with enzyme concentration 10% from (weight/weight) substrate total and the acidolysis is 24 hours [8]. After 24 hours, the enzyme with the sample is separated through vacuumed filtration. Acetone is used to wash the filter paper and the end product is achieved through the release of acetone through rotating evaporator [11].

Physicochemical Analysis: The palm oil physicochemical characteristic before and after enzymatic acidolysis reaction with capric acid for each different molarity ratio (1:1, 1:2, 1:3 and 1:5) is determined based on the following analysis. Replication is conducted for each analysis and each sample is carried out in duplicates.

Cloud Point: The cloud point analysis conducted is based on PORIM method p4.3 [12]. Cloud point is the temperature where under certain circumstances, the cloud formation start to be induced inside the sample because of the early stage of crystallization. This analysis can be

used for all vegetable oils including palm oil and olein palm oil.

The sample has to be dry before the test is begun. About 60-75g dissolved oil is filtered using Whatman No.1 filter paper. The oil that has been dissolved is heated at 130°C for 5 minutes right before the test is conducted. About 45ml of heated oil is then poured into the sample bottle. Next, the bottle and its content are cooled in a water bath and are stirred so that the temperature is even. When the temperature reaches approximately 10°C above the cloud point level, stirring is done fast by a rotating motion to prevent the occurrence of supercooling and fat crystal solidification on the wall or at the bottom of the bottle. Thermometer is prevented from being released from the sample because this will cause air bubbles to enter and will interfere with the testing. The position of the test bottle is maintained as such so that the level on the sample and the water bath is about the same. After that, the bottle is taken out from the steamer and is constantly checked. Cloud point is the temperature where thermometer substance soaked in the oil is no longer visible when it is looked at through the bottle and sample. The analysis is repeated using water bath with temperature set at 5°C under the cloud point to get the first determination. Cloud point attained from the first determination is recorded in the Centigrade degree, stated in one decimal point. The result from the repeated determination by the same operator must not be more than 0.6°C.

Statistic Analysis: The data obtained is analyzed using *Statistical Analysis System (SAS)* software version 9. The method used is analysis of variance (ANOVA) and analysis procedure *Duncan Multiple Range* to determine the significant differences for mean at confidence level 95% (P: 0.05).

RESULTS AND DISCUSSION

Saponification Value and Molecule Weight: Saponification value is determined to obtain palm oil molecule weight that is used in this study. In this study, saponification value obtained is 176.52 ± 0.03 mg/g (Table 1). So the estimation of palm oil molecule weight is 953.43 ± 0.17 (Table 1).

According to a study [13], saponification value for palm oil is in the range of 190.1-201.7 mg/g with the average value of 195.7 mg/g. Meanwhile according to another study [14], saponification value for palm oil is in the range of 196-202 mg/g. Saponification value obtained in the study is much lower compared to stated ranges.

Table 1: Saponification value and palm oil molecule weight

	Average
Saponification value (mg/g)	176.52 ± 0.03
Molecule Weight	953.43 ± 0.17

Table 2: Sample cloud point value at different molarity ratio before and after acidolysis

Sample (palm oil: capric acid ratio)	Cloud Point (°C)	
	Before acidolysis	After acidolysis
1:1	+8.1 ^a	+7.9 ^a
1:2	+18.4 ^b	+9.1 ^b
1:3	+21.9 ^c	+11.2 ^c
1:5	+26.2 ^d	+21.3 ^d

Note: ^{a-d}The same alphabet on the same row shows there is no significant difference ($p > 0.05$).

This shows that fatty acid chain for palm oil used as the research sample is much shorter. This is due to the reason that the longer a certain fat or oil carbon chain is, the higher the saponification value and vice versa [15].

At the same time, the differences in the saponification value is most probable owing to the reason that the palm oil used in the research is not genuine but processed. Therefore, the authenticity has been somewhat compromised. Apart from that, geographical factor also influences the oil palm produced where different plantation area gives different produces.

Based on the saponification value obtained, it can be decided that the molecule weight of the palm oil used in the study is 953.43 ± 0.17 . This molecule weight is used for the determination of the ratio between the palm oil and capric acid for the acidolysis reaction according to a different molarity ratio.

Cloud Point Value: Cloud point is the physical parameter which is frequently used. It is applied in palm oil sample for the purpose of determining the palm oil resistance towards crystallization apart from getting estimations on the stability of the palm oil at a low temperature [9]. On top of that, the cloud point is also closely related to certain samples' unsaturation, the more unsaturated a sample is, the lower the cloud point. According to research [9], the cloud point value for olein palm oil is +11.5°C.

According to Table 3.2, it is found that there is a significant difference ($p < 0.05$) for the four molarity ratio for the samples before and after the acidolysis reaction is carried out. The study result found, the higher the palm oil molarity ratio to the capric acid is, the higher the cloud

point value. This shows that the increase of capric acid in the palm oil sample does not give any decreasing effect on the cloud point value. The reason may be because of the chemical composition of the capric acid itself because capric acid is a saturated fatty acid and is in solid form at room temperature. Therefore, the addition of capric acid in the palm oil sample causes the unsaturation of the palm oil to decrease and duly increasing the cloud point value compared to palm oil without the addition of capric acid.

Nonetheless, based on this study it is found that the cloud point value could be reduced through the acidolysis reaction. This is because the cloud point value before acidolysis is much higher compared to after acidolysis is done to all samples. The minimum reduction is for the sample with 1:1 ratio which is 0.2°C while 1:3 ratio gives the highest reduction which is 10.7°C.

CONCLUSION

Saponification value gained in this study is 176.52 ± 0.03 mg/g. Therefore the estimation of palm oil molecule weight is 953.43 ± 0.17 . Cloud point value shows a significant difference ($p < 0.05$) for the comparison among all four molarity ratio for the samples before and after acidolysis reaction is done. The cloud point value before acidolysis for ratio 1:1, 1:2, 1:3 and 1:5 are 8.1, 18.4, 21.9 and 26.2°C respectively while after acidolysis are 7.9, 9.1, 11.2 and 21.3°C respectively. The result of the study shows cloud point value experiencing a decrease after enzymatic acidolysis is done.

The study of enzymatic acidolysis reaction using the consolidation of palm oil and capric acid like this particular study should be done more to determine the effectiveness of this method in reducing the cloud point value of palm oil so that it is stable at low temperature. For future research, it is suggested that the usage of unsaturated fatty acid such as linoleic acid, linolenic or oleic is applied to replace saturated fat acid which is capric acid that is used in this study. Acidolysis reaction of palm oil with chosen unsaturated fatty acid sample can also be conducted at different molarity ratio.

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