

CHAPTER V

Physicochemical Properties of Purified Modified Palm Stearin

5.1 Introduction

Palm stearin characteristics differ from those other fractioned palm oil products; palm olein and palm kernel products group. According to Lai (1998) palm stearin is a solid fraction of palm oil with a higher range of melting points (44.5°C – 56.2°C). It consists of high amounts of saturated fatty acid which contribute to that melting profile. Thus to enhance the PS compatibility, blending of palm stearin with unsaturated fatty acids or any other oils are necessary (Fauzi et al., 2013)

Modification of palm stearin is aimed to improve its physical and chemical properties such as melting profile, iodine value, TAG composition and solid fat content. Based on Soares et al. (2009), incorporation of 50% palm olein to palm stearin reduced the level of saturated fatty acids in 20% and increased in 48% of polyunsaturated fatty acids (PUFA) and 50% of monounsaturated fatty acids (MUFA) respectively. The increase in PUFA and MUFA alter the TAG composition and then promote changes in end product melting point.

Besides, Zainal and Yusoff (1999) supported that blends containing a substantial amount of palm stearin in modified products should lower the melting point. They studied the blends of 30:70 and 70:30 palm stearin and palm kernel olein respectively, modified by enzymatic interesterification for 6 hours and found that the modifying process lower the slip melting point from 40.0°C and 47.7°C to 29.9°C and 37.5°C respectively. On the other hand, in 2009, Costales-Rodríguez et al. modified the palm stearin by blending it with soybean oil with 70/30 wt%. The enzymatic interesterification result in 9°C lower in its clear melting point because of the alteration in TAG profile (Costales-Rodríguez et al., 2009).

Besides measures in its melting point and fatty acids content, the free fatty acids (FFA) analysis gave advantages on determined the quality of the finished product. It appears that enzymatically interesterified product had higher FFA content as compared to the chemically interesterified products of the same blend (Mayamol et al., 2007). Thus as in this research minimal amount of FFA was favourable and the optimization process have done by considering this factor.

Modification of palm stearin in this study done by incorporates more mono-unsaturated fatty acid (oleic acid) via acidolysis reaction to increase its healthy benefits. The exchange of oleic acid with existing fatty acid in the triacylglycerol have changed its TAG composition and increased the total oleic acid percentage in the modified palm stearin. These changes successfully promotes the decrement in slip melting point of the modified palm stearin.

5.2 Materials

Lipozyme TL IM was purchased from Novozymes, Germany. Lipase AK-Amano 20 purchased from Sigma-aldrich, Japan. Palm Stearin was obtained from Sime Darby Jomalina, Malaysia. Oleic acid, n-hexane of GC grade, chloroform of liquid chromatography (LC) grade, acetonitrile, acetone, and sodium methoxide were purchased from Merck, Germany.

5.3 Methods

5.3.1 Production and Purification of Modified Palm Stearin using Short Path Distillation

Refer method 4.3.1 in chapter IV.

5.3.2 Determination of TAG Composition for physicochemical characteristics

Refer method 4.3.3 in chapter IV.

5.3.3 Fatty acid composition (FAC) analysis for physicochemical characteristics

Refer method 3.3.5 in chapter III.

Corresponding fatty acids of interest to be observed were palmitic acid (C 16:0), stearic acid (C 18:0), oleic acid (C 18:1) and linoleic acid (C 18:2).

5.3.4 Analysis of Slip Melting Point (SMP)

The analysis was done by using melting point apparatus model SMP30, Stuart®, Baby Scientific. Sample was crystallized overnight in both open end capillary tubes. Then, three tubes accommodated in an illuminated chamber within the aluminium block prior to heating. The heating rate was set between 0.5°C per minute. Once the sample begins to melt, the temperature displayed on the screen for record. The sample was in triplicate.

5.4 Results and Discussion

5.4.1 TAG Composition

Analysis of TAG was focused on four types of TAG, which are triolein (OOO), palmitic-oleic-oleic (POO), palmitic-palmitic-oleic (PPO) and tripalmitin (PPP). Among other various TAG, these four types TAG has the major changes in each sample because the idea was to incorporate more oleic acid to the glycerol. Results in Table 13 show TAG composition among the samples.

TABLE 13: percentage of TAG composition and its values among the samples

Sample (%) [*]	OOO	POO	PPO	PPP
PS	1.84 ± 0.05 ^a	16.15 ± 0.40 ^a	46.88 ± 1.41 ^a	35.14 ± 1.76 ^a
PMPS	7.33 ± 0.05 ^b	23.97 ± 0.28 ^b	45.37 ± 1.00 ^a	26.08 ± 1.43 ^b

^{*ab} shows the tukey test among the sample. Different letters indicates there was a significance difference between sample. $p < 0.0005$ from one-way ANOVA.

^{*}OOO indicates (triolein), POO (palmitic-oleic-oleic), PPO (palmitic-palmitic-oleic), PPP (tripalmitin), PS (Palm Stearin), PMPS (Purified Modified Palm Stearin).

Based on Table 13, Tukey test shows that there was a significance difference ($p < 0.0005$) between PS and PMPS for TAG of OOO, POO and PPP. The PPO and PPP shows a decrement while OOO and POO showed an increment. This resulting from the breakdown of TAG PPO and PPP at the *sn*-1,3 position. This allow more oleic acid can incorporates to the glycerol bond thus making up the OOO and POO have higher percentage in the end product (PMPS). This result supports the idea to incorporate more oleic acid and replace palmitic acid in TAG, whereby more PPO and PPP have been rearranged to become POO and OOO.

As compared with Ibrahim et al. (2007), the study of interesterification of palm stearin with canola oil with 7:3 ratio of lipase AK to TL IM has promoted the changes on the chromatogram peak by the exist of ECN 44 group after enzymatic interesterification. While in this research, by the same approach of reaction, dual lipase, however with different substrate (palm stearin with oleic acid) and enzyme ratio (5:5, lipase AK and TL IM), the changes mainly occur on the increase of amount OOO and POO, and decrease of PPO and PPP triglycerides.

5.4.2 Fatty acid composition (FAC)

Fatty acid content analysis can be used to determine the increment of oleic acid in the, purified modified palm stearin. Table 14 shows several fatty acids observed, which are palmitic, stearic, oleic and linoleic acids. Palmitic and stearic acids are responsible for the solid structure of palm stearin. Both fatty acids were more unfavourable to human

diet if taken in excess. Thus incorporating more oleic acid was believed to enhance the healthy benefits of palm stearin since that oleic acid is monounsaturated fatty acid. Nutritional experts generally recommended limiting the consumption of saturated fatty acid and trans isomer while promoting the consumption of unsaturated fatty acid (Bednarski and Adamczak, 2003).

TABLE 14: FAC of palm stearin and purified modified palm stearin

Fatty acid (%)	Palmitic acid (C16)	Stearic acid (C18)	Oleic acid (C18:1)	Linoleic acid (C18:2)
PS	56.08 ± 0.00	4.80 ± 0.53	30.80 ± 0.08	5.97 ± 0.10
PMPS	37.22 ± 0.13	3.71 ± 0.20	48.55 ± 0.44	3.47 ± 0.43

*all analysis being done in triplicate and represent by its mean.

*PS: Palm Stearin, PMPS: Purified Modified Palm Stearin

Based on the Table 14, PMPS showed major decreased in palmitic acid percentage and major increased in oleic acid percentage. The result was in lined with TAG percentage as the increment in OOO and POO indicates that high incorporation of oleic acid in the final product, PMPS. With a molar ratio of palm stearin to oleic acid at 1:3 respectively, the ratio gave approximately 50% of the amount of each substrate to be blended in the reaction.

Thus, with this ratio, high incorporation of oleic acid had found (48.55%), unsaturated fatty acid, in the PMPS compared to PS. The palmitic acid has been reduced in percentage from 56.08% to 37.22% while stearic acid and linoleic acid do not have major changes with the values obtained was 3.71% and 3.47% respectively in PMPS after acidolysis reaction. This results supported by Soares et al. (2009) where they found the

same pattern of increment in interesterification of palm stearin and palm olein. Soares et al. (2009) found that incorporation of 50% of palm olein to palm stearin reduced the level of saturated fatty acid in 20% and increased the levels of monounsaturated and polyunsaturated fatty acid.

5.4.3 Slip melting Point

There was a decrement in melting point for PMPS (46.13°C) in comparison to unmodified palm stearin (50.60°C) based on Table 15. Lower in melting point resulted from a rearrangement of fatty acids upon acidolysis reaction. The PMPS sample, however still in the range of melting point as refer to Lai (1998), indicates that slip melting point of palm stearin ranging from 44°C to 56°C. In this temperature range, it shows that PMPS samples still have the solid structure as palm stearin, however, it was rich in monounsaturated fatty acid (oleic acid). This can be concluded by incorporating more oleic acid to lower the melting point of palm stearin and altering the TAG composition was a success.

TABLE 15: Slip melting point of palm stearin and purified modified palm stearin

Sample	SMP (°C)
PS	50.60±0.3
PMPS	46.13±0.1

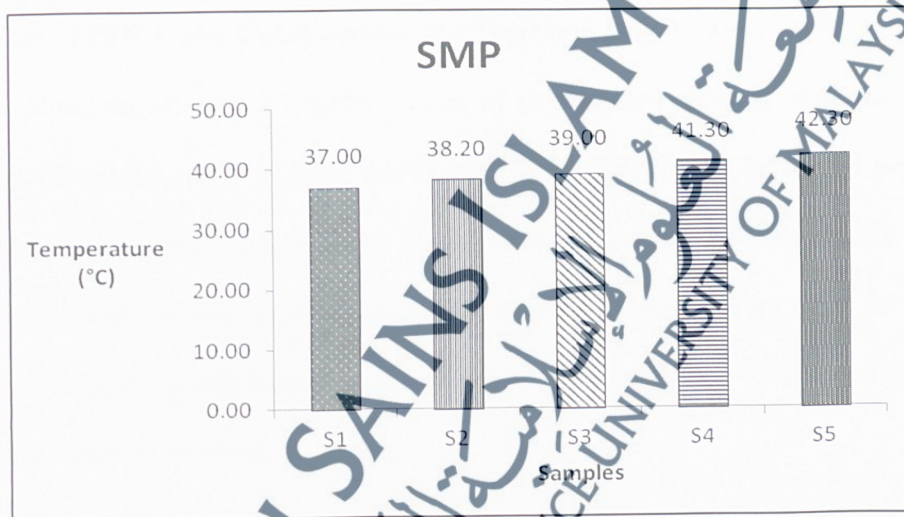
*all analysis being done in triplicate and represent by its mean.

*PS: Palm Stearin, PMPS: Purified Modified Palm Stearin

The amount of SMP reduced in modified stearin sample was low (approximately 4°C) may due to combinations of 5 batch upscale samples by using the reused enzyme. Over

by batch of reaction as shown in Figure 17, there was an increment in slip melting point. The first acidolysis upscale process resulting the highest reduction in its melting point, which is about 37°C. This indicates that the first reaction by using fresh enzyme can lower the melting point of palm stearin up to 10°C and higher. The melting point, however, continues gradually increase until the 5th batch. This may due to the enzyme capability and enzyme activity has reduced by time when reused it over by batch.

FIGURE 17: Slip melting point among reusable enzymes batch samples



*S1,S2,S3,S4,S5: denotes as the batch sample of acidolysis reaction by using the same enzyme which collected after the reaction

From the result in Figure 17, this indicates, that the enzymes ability to cleave the ester bond has been cut even in the presence of new blends of palm stearin and oleic acid. This is in line with Seager and Slabaugh (2010) as they stated that substrate concentration significantly influence the reaction rate but at certain concentration, however, the rate levels remain constant because the enzyme may saturated with the substrate and cannot

work any faster in that condition. This shows that the breaking down of TAG bond in the glycerol decrease thus the incorporation of oleic acid become lower.

5.5 Conclusion

Acidolysis reaction successfully incorporated oleic acid into the ester bond in palm stearin resulting more POO and OOO triglycerides present as well as the oleic acid percentage in PMPS. The higher amount of unsaturated fatty acid may lower the melting point resulting the changes on melting point of palm stearin from 50.60°C to 46.13°C. This support by the result of FAC which PMPS obtained higher oleic acid percentage compared to palm stearin. The batch of reused enzyme has decreased in the enzyme activity. This can be seen in the higher amount of SMP obtained by times of repeated reactions. Thus, the combination of both enzymes did not enhance the reusability of both enzyme to catalyst the reactions.