Glycerolysis-interesterification of chicken-palm stearin blend: effect of solvent and chicken stearin to palm stearin ratio

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Abstract. Both glycerolysis and the interesterification reactions take place in one reaction system. The objectives of this research were to obtain chicken stearin and to evaluate the effect of solvent to fat ratios and chicken stearin to palm stearin ratios on total mono-acylglycerol (MAG) and diacylglycerol (DAG) of product by the chemical glycerolysis-interesterification (GIE). GIE was initiated by adding NaOH to the reactant. Structured Lipids were evaluated based on acylglycerol composition and melting behavior. Results show that the yield of chicken stearin was 13%. The highest total MAG and DAG was obtained at the stearin: solvent ratio 1:3 (w/v). An increase in chicken fat stearin to palm stearin caused a slight increase in the total MAG and DAG. However, it didn’t have a significant increase in the total MAG and DAG. The best ratio of palm stearin to chicken stearin was 40:60 because slip melting point and melting point were 36.16 ± 4.9°C and 37.59 ± 5.96°C. Thus, the best condition was obtained at the ratio of stearin to solvent 1:3 (w/v), temperature 50°C, oil to glycerol ratio 1:5, 300 rpm and NaOH 3%. The reaction was performed for 2 h. Total MAG and DAG was 67.87 ± 1.97%.

1. Introduction
Structured lipids (SLs) synthesis can be used to produce lipids for the desired characteristics and/or nutritional benefits [1]. SLs were synthesised by blending of vegetable fats/oils, such as cottonseed oil and palm stearin [2-3]. Vegetable fats/oils blending have disadvantage as most of them have low stearic acid content. As consequence, they had a lower melting profile and a soft texture [4-5].

Some sources of vegetable fats or oils that have high stearic acid content were kokum (Garcinia indica), sal (Shorea robusta), mango (Mangifera indica), mahua (Madhuca longifolia) and sunflowers (Helianthus annuus) obtained from genetically modified organism (GMO) trait [6-9], pumpkin seed [10]. Meanwhile, the stearic acid source from animal fat or oil was chicken [11-12]. Chicken fat has not been widely used in Indonesia, so it can be a potential source of stearic acid.

As an alternative, lauric fats/oils were also used to increase the hardness [13-15]. Moreover, the hydrogenated fat, such as hydrogenated palm oil, sunflower oil and soybean oil can be used to enhance stearic acid content [16-18]. However, the disadvantage of the hydrogenated oils is that they may contain trans-fat.

Properties of SLs can be enhanced through interesterification of fat blend. The interesterification of a fat/oil blend, such as palm olein and stearin, which had low and high melting point, was also able to improve physicochemical properties of SLs [19-20]. However, the hardness of SLs was still low.
On the other hand, high monoacylglycerol (MAG) and diacylglycerol (DAG) contents may increase the melting point (MP) of fats. They also led to a large number of crystallization nuclei formation, accelerating the crystal formation and improve the hardness of product [21-22]. MAG and DAG may be used for developing a new product with certain physical properties, such as melting and crystallization behaviors [23-24].

In this research, the glycerolysis-interesterification reaction (GIE) was used to synthesise SLs that contain a high concentration of MAG and DAG. The objective of this research was to evaluate the effect of solvent to fat ratios and chicken stearin to palm stearin ratios on total MAG and DAG of product by using the GIEchemical. GIE was initiated by adding NaOH to the reactant. SLs were evaluated based on acylglycerol composition and melting behavior.

2. Materials and methods

2.1. Materials

Chicken fat was obtained from PT So Good Food, Boyolali. Palm stearin was obtained from PT Smart Tbk. Molecular sieve was obtained from Sigma-Aldrich (St Louis, USA). NaOH, glycerol, n-hexane, methanol, acetic acid, t-butanol and TLC plate silica gel 60 F254 were obtained from Merck KgaA (Darmstadt, Jerman). Ethyl ether was obtained from Mallinckrodt Chemicals.

2.2. Preparation of chicken fat stearin

Chicken fat was washed and the remaining water was removed. The chicken fat was then heated at 80-90°C for 15 min. Then, the oil liquid was filtered and weighed. Free fatty acids were analysed using a spectrophotometer at a wavelength of 715 nm. Free fatty acids were firstly neutralized using 0.1M NaOH solution. Furthermore, NaOH was removed using 5% NaCl solution at 60°C. Oil bleaching was carried out using 2% mixture of bleaching earth and activated charcoal (50:50) at 60°C for 30 min. The oil was filtered with a vacuum filter to separate the adsorbent. After that, the oil was fractionated at 20°C for 48 h. Furthermore, the oil was filtered with a vacuum filter to obtain a solid (stearin) and liquid (olein) fractions.

2.3. Effect of glycerolysis-interesterification at various palm stearin-chicken fat blend ratios on total of mono- and diacylglycerol

GIE was performed according to Subroto et al. [25]. Glycerol was added to various mixture molar ratios of palm stearin and chicken fat stearin at molar glycerol to fat ratio 1:5. T-butanol was further added to the mixture with ratio of 1:3 (w/v). The reaction was initiated by adding NaOH (3% w/v). It was performed at 50°C for 120 min. Water formation during the reaction was controlled by molecular sieves (12% of the total reactants). The reaction was stopped by addition of citric acid. All samples were run in duplicate.

2.4. Effect of glycerolysis-interesterification at various fat to solvent ratios on total of mono- and diacylglycerol

A similar procedure of glycerolysis-interesterification [25] was performed to study the influence of fat to solvent ratios on total of Mono- and Diacylglycerol. Glycerol was added to a mixture of palm stearin and chicken fat stearin (1:1) at glycerol to fat molar ratio 1:5. In addition, various amount of t-butanol was added to the mixture.

2.5. Slip melting point and melting point of product

Slip melting point and melting point of the product were analysed according to AOAC 920.157.

2.6. Analysis of acylglycerols

Triacylglycerol (TAG) and its derivatives (MAG and DAG) were analysed by Thin Layer Chromatography (TLC) [25]. Silica gel TLC plate G60F254 was activated by heating at 105°C for 1 h.
The sample was then applied to the TLC plate. The samples on plates were developed using a mobile phase containing hexane: ethyl ether: acetic acid (80:20:2 v/v/v). Chamber was saturated with mobile phase for 1 h prior to development. TLC plate was subsequently dried. Staining of the developed plate was then performed by soaking the plate in 0.02 % Coomassie blue R-350 in a mixture of acetic acid: methanol: H\textsubscript{2}O (1:3:6 v/v/v) for 1 min. Quantification was performed using Camag TLC Scanner III with software CamagwinCATS Planar Chromatography.

3. Results and Discussions

3.1. Rendering of chicken fat

Chicken fat, stearin and olein fractions were obtained by rendering process of chicken fat and skin (table 1). The yield of chicken stearin was 13\%. The low stearin fraction may be due to the high content of oleic acid in chicken fat. Stearin was used as a raw material for the synthesis of monoacylglycerol and diacylglycerol.

| Table 1. Rendering of chicken fat |
|----------------------------------|
|                                   | Chicken Fat | Chicken Olein | Chicken Stearin | Non-fat (g) |
| Amount (g)                        | 1010.41     | 820.89        | 134.35          | 55.17       |
| Yield (%)                         | 100.0%      | 81.2%         | 13.3%           | 5.5%        |

3.2. Composition of palm stearin and palm olein

Moisture content of chicken stearin was 16.5 times greater than that of palm stearin (table 2). It is suggested that high moisture content was due to residue water after washing chicken fat and neutralization process during the preparation of chicken stearin. On the other hand, free fatty acid of palm stearin was 2.9 times greater than that of chicken fat. The presence of free fatty acids was caused by the presence of water in the material. It suggested that moisture of fat caused fat hydrolysis; as a consequence, it produced more free fatty acids. Besides, free fatty acids in chicken stearin may be caused by the lack of neutralization of free fatty acids after the rendering process. High moisture content of chicken fat may also cause fat hydrolysis.

MAG of both palm and chicken stearin were not detected. This result was similar to Kang et al. [26] and Bankovi et al. [27]. DAG of palm stearin was 6.4 times higher than that of chicken stearin. However, DAG of chicken stearin was greater than the value obtained by Kang et al. [26] and Bankovi et al. [27]. Conversion of TAG to DAG was lower than the value obtained by of Bankovi et al. [27].

| Table 2. Composition of palm stearin and chicken stearin |
|---------------------------------------------------------|
| Moisture content (%) (w/w)                | Palm Stearin | Chicken Stearin |
|                                          | 0.04         | 0.66            |
| Free Fatty Acid (%) (w/w)                | 2.26         | 0.77            |
| MAG (%)                                  | nd\textsuperscript{a} | nd\textsuperscript{a} |
| DAG (%)                                  | 14.51        | 2.25            |
| TAG (%)                                  | 84.69        | 95.95           |

\textsuperscript{a}nd: not detected

3.3. Effect of fat to solvent ration on total of mono- and diacylglycerol

Figure 1 shows that the total MAG and DAG increased with an increase in the stearin-solvent ratio from 1:1 to 1:3 (w/v). A further increase in the stearin-solvent ratio to 1:4 and 1:5 resulted in a decrease in total MAG and DAG. It is suggested that a decrease in total MAD and DAG was due to a decrease in
the concentration of reactants (stearin) because of an increase in the amount of solvent. As a result, GIE reaction was lower. In addition, FFA decreased, while TAG increased with an increase in the stearin-solvent ratio from 1:3 to 1:4 and 1:5. The highest total MAG and DAG was obtained at the stearin:solvent ratio 1:3 (w/v). This is consistent with research conducted by Zhong et al. [28]. Thus, the stearin:solvent ratio of 1:3 (b/v) was used for the further experiment.

![Graph showing the effect of fat to solvent ratio on total of mono- and diacylglycerol](image1)

**Figure 1.** Effect of fat to solvent ration (1:01, 1:02, 1:03, 1:04 and 1:05 (w/v)) on total of mono- and diacylglycerol.

3.4. Effect of palm stearin to chicken fat stearin ration on total of mono- and diacylglycerol

Figure 2 shows the effect of palm stearin to chicken fat stearin ration on the total MAG and DAG.

![Graph showing the effect of palm stearin to chicken fat stearin ratio on total of mono- and diacylglycerol](image2)

**Figure 2.** Effect of palm stearin to chicken fat stearin ration (70/30, 60/40, 50/50, 40/60 and 30/70 (w/w)) on total of mono- and diacylglycerol.

Higher ratios of chicken fat stearin to palm stearin caused an increase in the total MAG and DAG. However, the increases of total MAG and DAG were considered to be small. The total MAG and DAG content was ranging from 80.03 ± 1.43% to 82.70 ± 6.3%. It is suggested that the source of the fatty...
acids and the position of fatty acid on TAG does not have a significant effect on chemical GIE. Either distributed randomly or specific, fatty acid position did not change the reaction equilibrium. Thus in general, it can be concluded that the ratio of palm stearin to chicken fat stearin did not have a significant effect on the total MAG and DAG.

3.5. Effect of palm stearin to chicken fat stearin ration on slip melting point and melting point of product

Figure 3 shows the effect of the ratio of palm stearin to chicken stearin on the slip melting point and melting point of the product. The slip melting point and melting point of the product decrease with an increase in the chicken stearin. It is suggested that this was due to the lower melting point of chicken stearin compared to that of palm stearin. The best ratio of palm stearin to chicken stearin was 40:60 because slip melting point and melting point were 36.16 ± 4.9°C to 37.59 ± 5.96°C and 37.59 ± 5.96°C. It is near similar to the characteristics of cocoa butter.

Note: The reaction was performed at 50°C, fat to solvent ratio 1:3 (w/v), fat to glycerol 1:5, NaOH 3% and 300 rpm for 2 h.

Figure 3. Effect of palm stearin to chicken fat stearin ration (70/30, 60/40, 50/50, 40/60 and 30/70 (w/w)) on slip melting point (■) and melting point (□) of product.

4. Conclusion

The yield of chicken stearin was 13%. The highest total MAG and DAG was obtained at the stearin: solvent ratio 1:3 (w/v). An increase in chicken fat stearin to palm stearin caused a slight increase in the total MAG and DAG. However, it did not have a significant increase in the total MAG and DAG. The best ratio of palm stearin to chicken stearin was 40:60 because slip melting point and melting point were 36.16 ± 4.9°C to 37.59 ± 5.96°C and 37.59 ± 5.96°C. The best condition was obtained at the ratio of stearin to solvent 1:3 (w/v), temperature 50°C, oil to glycerol ratio 1:5 and NaOH 3%, 300 rpm for 2 h. Total MAG and DAG was 67.87 ± 1.97%.

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