Statistical Mixture Design for Modelling and Optimization of Feed Mixture in the Chemical Interesterification to produce Cocoa Butter Alternatives

Jenny Rizikiana 1,2,3, Nathania 1, Oktalia Putri Pratama 1, and Dianika Lestari 1,3

1 Department of Food Engineering, Institut Teknologi Bandung, Bandung 40132 Indonesia
2 Department of Bioenergy Engineering and Chemurgy, Institut Teknologi Bandung, Bandung 40132 Indonesia
3 Department of Chemical Engineering, Institut Teknologi Bandung, Bandung 40132 Indonesia

*Corresponding author: dianika@che.itb.ac.id

Abstract. This study aimed to find the optimum feed mixture consist of palm stearin (PS), palm olein (PO), and coconut oil (CO), for chemical interesterification to produce cocoa butter alternatives (CBA) with the most similar melting profile with cocoa butter. The feed mixture was chemically interesterified using sodium ethylate as the catalyst at a temperature of 75°C for 8 hours. Based on the melting profile analysis of the products, the pure coconut oil (CO-100) and pure palm stearin (PS-100) gave the most similar melting profile of cocoa butter alternatives. The CO-100 melting profile gave the least value of Sum of Square Error compared to Cocoa Butter Substitute while the PS-100 melting profile gave a similar result compared to Cocoa Butter Equivalent (CBE) and Cocoa Butter Replacer (CBR). However, PS-100 product does not meet the other specifications, such as Iodine value and slip melting point. Therefore, it can be concluded that pure coconut oil was the best feed for chemical interesterification to produce cocoa butteralternatives (CBA).

Keywords: Chemical interesterification, Melting profile, Mixture Design, Optimization

1. Introduction
Cocoa butter is a unique fat as it is the only type of fat consumed directly and is widely produced in the market. Cocoa butter has a melting temperature of around 35 °C or close to the human body temperature [1]. Chocolate fat (cocoa butter) is fat with unique physicochemical characteristics because its 80% triacylglycerol composition is dominated by three symmetric, saturated-monounsaturated-saturated triglycerides (TAGs), namely palmitate-oleate-stearate (POS, 36-42%), stearate-oleate-stearate (SOS, 23-29%) and palmitate-oleate-palmitate (POP, 13-19%) [1]. Aside from the declining production, the high price of cocoa butter, which reached Rp 45,000 per 100 grams, constrains chocolate fat for the food industry. Various attempts have been made to find alternative cocoa butter use, one of them by developing specialty fats from other oils with lower prices to produce alternative cocoa butter. Cocoa butter alternative can be made using various vegetable oil sources through methods such as mixing fat, hydrogenation reaction, and interesterification reaction. In this research, coconut and palm oil were used due to the abundant availability in Indonesia. In industry, chemical interesterification is widely used because it requires a relatively faster time than enzymatic interesterification [2]. The results of interesterification were dependent on the composition of the feed mixture and each fat mixture's interesterification time with different characteristics of triglyceride content and melting characteristics of the fats. Slip melting point (SMP) is the temperature when fat is observed to melt entirely or visually change its shape and color due to solid fat content has reached below 10% [3]. Solid fat content control is the key to fat character modification. Solid fat content responsible for controlling the hardness of the
fat in various temperatures. Therefore, the effect of the feed mixture's composition on the content of solid fat produced on the interesterification reaction product needs to be investigated.

2. Materials and Method

2.1. Materials
Palm stearin, palm olein, and coconut oil were the raw materials for chemical interesterification. Palm stearin was obtained from PT. Astra Argo Lestari Tbk. Palm olein and coconut oil were purchased from Lion Super Indo. The raw materials were stored at room temperature. Sodium ethoxide was purchased from Sigma Aldrich.

2.2. Blend Preparation
Fat blends formulated with palm stearin, palm olein, and coconut oil were mixed at different ratios. Based on mixture design- second-degree lattice, simplex lattice design, fat blends were prepared as indicated in Table 1.

Table 1. Compositional design of the blends.

| Blends | Mass Compositions (w/w %) of 100 grams feed |
|--------|---------------------------------------------|
|        | Palm Stearin (PS) | Palm Olein (PO) | Coconut Oil (CO) |
| 1      | 0               | 100             | 0               |
| 2      | 50              | 0               | 50              |
| 3      | 16.67           | 66.67           | 16.67           |
| 4      | 33.33           | 33.33           | 33.33           |
| 5      | 0               | 0               | 100             |
| 6      | 66.67           | 16.67           | 16.67           |
| 7      | 100             | 0               | 0               |
| 8      | 50              | 50              | 0               |
| 9      | 0               | 50              | 50              |
| 10     | 16.67           | 16.67           | 66.67           |

Figure 1. Simplex design plot.

2.3. Water content
The water content of raw material could be determined by heating the sample. About 1 g of sample was placed in a porcelain cup which was mass known. Then, the sample was dried for 3 hours.
at 105°C. After that, the sample was cooled in a desiccator for 15 minutes, and mass was pondered by analytical balance. Water content could be determined using the equation below.

\[
\text{content} = \frac{W_2-W_1}{W} \times 100\%
\]  

where: \(W_2\): The weight of porcelain and sample before dried (g), \(W_1\): The weight of porcelain and sample after dried (g), \(W\): the sample’s weight (g)

2.4. Batch Chemical Interesterification
The chemical reaction was started by adding 0.4% w/w sodium ethoxide (Sigma Aldrich) as the catalyst into fat blends prepared before, according to table 1. Chemical interesterification was conducted at 75°C using sodium ethoxide as the catalyst. The chemical interesterification was carried out in a laboratory-scale batch reactor with an agitator. The sample was agitated with an average speed of 300-400 rpm for 8 hours. To terminate the reaction, 20 ml 20% (w/w) citric acid solution was added. By water addition, it would non-activate catalyst by changing it into ethanol (Sreenivasan, 1978). Citric acid would react with sodium into sodium salt. Interesterified fat blends was dried again to reduce the water content of a sample.

2.5. Slip Melting Point
The slip melting point of the interesterified blends was determined according to the AOCS Method No. Cc 3-25 and slip melting point digital. A capillary tube filled with a 1 cm high column of the sample was chilled at 4 ± 1°C for 16 hours before being immersed in a beaker with cold water. The water was stirred and heated slowly. The temperature was recorded when the blend in the tube started to rise due to hydrostatic pressure. The temperature at the moment was taken as the SMP.

2.6. Iodine value
The iodine number was determined by the volumetric titration method. This method referred to AOCS (1998). Iodine numbers are expressed as grams of iodine absorbed per 100 gram sample. The samples were melted at 60-70°C and stirred until smooth. A sample of 0.13-0.15 gram was put into the Erlenmeyer and then added with chloroform p.a. 25 ml and 25 ml Wijs solution, then covered and stirred until homogeneous. The mixture was put down in a dark room for an hour. After that, the mixture was added with 20 ml KI 10% and 150 mL aqua dm. The solution was mixed and titrated with 0.1N thiosulfate solution until a purple-purplish color changes precisely disappear. Next, a 10% 2 mL starch indicator is added. The iodine number can be calculated as follows.

\[
\text{Iodine Value (g iod / 100 g sample)} = \frac{12.69 \times N \times (V_2-V_1)}{W}
\]  

Where \(N\) is the normality of thiosulfuric acid, \(V_2\) volume of thiosulfate used in the blank determination (mL), \(V_1\) volume of thiosulfate used in sample determination, \(W\) is the test weight (gram). The following is a workflow diagram for the analysis of fat iodine numbers.

2.7. Experimental Design and Statistical Analysis
Statistical analysis was done using Response Surface Methodology (RSM) and ANOVA. The type of RSM that was used was the Mixture Design method, simplex lattice design. Experimental results for SMP was applied to obtain the regression models, as a function of the proportions of each ingredient (x1 = palm stearin, x2 = palm olein and x3 = coconut oil) present in blends: SMP (°C) = \(\beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{123}x_1x_2x_3\), where SMP was estimated response, \(\beta\) was coefficient estimated by least-squares method, x was a dependent variables. The quality of the models was evaluated by ANOVA and adjusted coefficient of determination \((r^2)\). ANOVA that used was Univariate Multi-Way Analysis of Variance using Microsoft Excel.

3. Results and Discussion
3.1. Effect of Variation in Chemical Interesterification Raw Material Composition on Solid Fat Content (SFC)
In cocoa butter, solid fat content is responsible for the characteristics of fat hardness and quickmelting. SFC is also essential for the implications of fat stability at room temperature. High storage temperatures at room conditions above 20 °C determine product stability and resistance to oil exudation, which requires SFC to survive above 10% (Graef, 2012). This standard specification is also needed to produce cocoa butter equivalent and cocoa butter replacer. Cocoa butter equivalent and cocoa butter replacer are alternatives to
cocoa butter, similar to the original cocoa butter seen from the resulting SFC profile. CBS's SFC value does not maintain solid fat content far above 10% at 25 °C. The CBE, CBR, and CBS standard specifications are presented in the following table. The addition of fatty acids in the form of triglyceride alloys to the mixture will change the ratio between saturated and unsaturated fatty acids that affect the content of solid fat in the mix, the polymorphic structure and, melting temperature as described previously in Figure 4. The gentle melting pattern results in melting points (SMP) visually identified appear to be lower due to decreased solid fat content below 10% (Hernqvist, 1990). Under conditions of 10% SFC, colorless fat appears to melt visually completely. This melting profile is undesirable in alternative chocolate fat products. Profile of melting of fat resulting from interesterification of variations 1 to 10 is presented in Figure 2.

### Table 2. Differences of CBE, CBS, and CBR.

| Characteristic | CBE | CBS | CBR |
|---------------|-----|-----|-----|
| Main Fatty Acid | Palmitic, stearic, oleic, linolec, arachidic | Laurac, myristic | Elaidic, stearic, palmitic, linoleic |
| % Solid Fat Content | 10 °C = 90-95 % | 16 °C = 70-72% | 10 °C = 91-93 % |
| 20 °C = 85-92 % | 18°C = 60-68% | 20 °C = 65-70 % |
| 25 °C = 80-88 % | 22 °C = 31-40 % | 25 °C = 40-43 % |
| 30 °C = 65-89 % | 26 °C = 9-18% | 30 °C = 28-30 % |
| 35 °C = 10-25 % | 34 °C = 5-7% | 35 °C = 15-20 % |
| Iodine Value | 30-34 (g iod/ 100 g) | 4-17 (g iod/ 100 g) | 30-34 (g iod/ 100 g) |
| SMP | 33- 42 °C | 33- 42 °C | 33- 40 °C |

Sources: SNI Document and Jurnal *Cocoa Butter and Its Alternatives: A Review*, 2014 dan Delta Wilmar

The profile of solid fat content is specific to the content of fatty acids in oil/fat. The melting profile in Figure 2 was obtained from the results of reprocessing the literature data from the research of Andreia, F et al. (2012) using multiple regression methods to get the coefficient of determination of the SFC value.

\[
SFC(T) = B_1X_1 + B_2X_2 + B_3X_3 + B_{1,2}X_1X_2 + B_{1,3}X_1X_3 + B_{2,3}X_2X_3 + B_{1,2,3}X_1X_2X_3
\]

Prediction of solid fat content percentage at the measurement temperature using slip melting point experimental results of each feed variation. Slip melting point experimental results at the 8th hour presented in Table 3.

### Table 3. Experimental Slip Melting Point Result Feed Mixture Variations.

| Variations | Palm Stearin | Palm Olein | Coconut Oil | Slip Melting Point (°C) |
|------------|--------------|------------|-------------|-------------------------|
| 1          | 0%           | 100%       | 0%          | 10                      |
| 2          | 50%          | 0%         | 50%         | 30                      |
| 3          | 17%          | 67%        | 17%         | 18                      |
| 4          | 33%          | 33%        | 33%         | 40                      |
| 5          | 0%           | 0%         | 100%        | 33                      |
Based on Figure 2, it can be observed that the profile of fat melting has a very gentle melting pattern, gentle melting pattern, and steep melting pattern. However, based on the CBR triglyceride component’s basic specifications, no alternative fat types will be produced from this mixture so that only alternative CBE and CBS fats will be identified from the melting profile variations.

A slight slope of melting profile is produced by variations in a composition dominated by a mixture of palm olein such as pure palm olein, PS: PO: CO (16.67: 66.67: 16.67), and PO: CO (50:50). The melting profile produced is very gentle, with 10 °C indicating that the percentage of solid fat is in the range of 20%. Thus from this mixture, it can be seen that the characteristics of the melting characteristics of palm olein significantly affect the characteristics of the fat variety. The dominant saturated fatty acid content makes it difficult to form solid fat structures from polyunsaturated or monounsaturated triglycerides so that the fat content at low temperatures is very low.

The mixture shows different things with more solid fat content due to the mixing process. In this condition, a slightly steeper melting profile is formed, which is included in variations of the composition of PS: PO: CO (50: 0: 50), PS: PO (50:50), PS: PO: CO (16.67: 16, 67: 66.67), PS: PO: CO (66.67: 16, 67: 66.67).
16.67: 16.67), and PS: PO: CO (1: 1: 1). In this composition, the gentle melting pattern is obtained. This better melting pattern results from adding the amount of saturated fatty acids to the mixture. This melting pattern is expected to occur in margarine functional fat (Soekopitojo, 2011) but is undesirable in the production of CBE and CBS fat. This is because the pattern of gentle melting produces less comfortable melting in the mouth (melting behavior).

Furthermore, the steep melting profile from the data analysis results resulted from variations in the composition of 100% palm stearin and 100% coconut oil. This composition found that coconut oil produces SMP between 25-33°C (SFC <10%) and palm stearin spanning 42-45°C. Of these two variations, only 100% coconut oil has a melting profile similar to cocoa butter alternatives. The value of 100% palm stearin SMP is very far above the human body temperature causing this variation to have no potential to produce cocoa butter. Based on the graph, it can be seen that the 100% coconut oil melting curve coincides with the CBS line crew starting at a measurement temperature of 20°C. As such, 100% coconut oil is the only variation with a similar melting profile to CBS-type butter alternatives.

3.2. Optimization Feed Mixture for Solid Fat Content Profile by Response Surface Method

Cocoa butter alternative based on the literature consists of 3 types, namely cocoa butter equivalent (CBE), cocoa butter substitution (CBS), and cocoa butter replacer (CBR). The three alternative types of chocolate have differences from varieties of fatty acids, enabling properties, physicochemical properties, solid fat content, iodine numbers, and melting profiles (Naik & Kumar, 2014). Based on these characteristics, the desired interesterification product has a melting profile similar to alternative cocoa butters and has the appropriate melting temperature of the product and iodine number. Thus, the melting profile is the main factor used to determine the best variation in producing alternative cocoa butter, verified by melting temperature and iodine number. Slip melting point data for each variation is presented in table 3, while iodine number data for experimental results for each variation is shown in table 4.

Table 4. Experimental Iodine Value Result Feed Mixture Variations.

| Variations | Palm Stearin | Palm Olein | Coconut Oil | Iodine Value |
|------------|--------------|------------|-------------|--------------|
| 1          | 0%           | 100%       | 0%          | 56.86        |
| 2          | 50%          | 0%         | 50%         | 9.79         |
| 3          | 17%          | 67%        | 17%         | 59.35        |
| 4          | 33%          | 33%        | 33%         | 34.04        |
| 5          | 0%           | 0%         | 100%        | 7.98         |
| 6          | 67%          | 17%        | 17%         | 38.72        |
| 7          | 100%         | 0%         | 0%          | 33.56        |
| 8          | 50%          | 50%        | 0%          | 48.7         |
| 9          | 0%           | 50%        | 50%         | 42.05        |
| 10         | 17%          | 17%        | 67%         | 20.97        |

The statistical mixture design is used to determine the mixture's optimal composition to be adjusted to the response factor. All variations of the experimental design presented in Table 1 observed the closeness of the response factor's actual value. This optimization technique is called the Response Surface Method. The response factor used to determine the optimal composition is the melting fat profile against the Cocoa Butter (CB) reference fat. Tracing the optimal value of all variations of the mixture is done by minimizing the difference in profile (Sum of Square Error / SSE) the results of various composition experiments on the trend of Melting reference fat. The least value of SSE means that the melting profile of the sample is closer to the profile of CB melting profile. The results of the SSE calculations for each variation of the composition are presented in Figure 3.
Figure 3. Distribution of SSE values to Cocoa butter Profile (CB) in all variations of a mixture of Palm Olein (PO), Palm Stearin (PS), and Coconut Oil (CO).

Figure 3 shows that the acquisition of a minimum SSE value will be obtained from 100% palm stearin variation. This statement is verified from the optimizer’s response which shows that an SSE value with a target response close to zero will be obtained from this variation. A low SSE value indicates that pure palm stearin produces a melting profile similar to cocoa butter. However, from observing the melting profile in Figure 2 (a), the 100% palm stearin curve line is still too right, or the temperature drop value is still too high compared to cocoa butter. To explore the optimum potential variation for cocoa butter, the SSE value calculation was performed against alternative profiles of CBE, CBR, and CBS cocoa butter types whose plot contour distribution values are presented in Figure 4.

Figure 4. Distribution of SSE values to Equivalent Cocoa butter Profile (a) Exchanging Cocoa butter Profile (b) Substitution Chocolate Fat Profile (c) for all variations of Palm Olein (PO), Palm Stearin (PS), and Coconut Oil (CO) mixtures.
Based on the analysis results of Figure 4, it was observed that the equivalent cocoa butter profile was approximated by variations of pure palm stearin as the cocoa butter profile (CB). In contrast, the exchanging cocoa butter profile (CBR) and substitution fat profile (CBS) was approximated by variations in pure coconut oil feed. The optimization of the SSE values on the reference profile showed that pure palm stearin and pure coconut oil are the optimum variations resulting from the surface response (RSM). However, coconut oil variations l is superior in advanced selection aspects other than near-zero SSE values. Based on the basic specifications of commercial cocoa butter alternatives regarding SMP values in the range of 33-36°C, it is obtained that coconut oil (100) is following the characteristics of the target SMP value with a melting point value of 33°C. Then, based on observations of the product iodine number, it was found that coconut oil (100) produced an iodine number that was within the range of alternative specifications for lauric fat (4-17 g iodine / 100g fat), which was 7.98 g iodine / 100 g fat. Thus, it can be concluded that variation of virgin coconut oil has a melting profile similar to cocoa butter substitute, the melting temperature of the product close to the target, and the iodine number that meets the standard. Table 2 is the melting profile of the results of interesterification of virgin coconut oil and CBS melting profile literature.

Table 5. Comparison of SFC CBS with CO Optimization Fat (100).

| Temperature | SFC CBS | SFC Coconut Oil (100) | % SFC Differences CBS dan Coconut Oil |
|-------------|--------|-----------------------|--------------------------------------|
| 10 °C       | 84 %   | 77.15 %               | 6.85%                                |
| 20 °C       | 42 %   | 42.85 %               | 8.5%                                 |
| 25 °C       | 10 %   | 12.27 %               | 2.27%                                |
| 30 °C       | 4 %    | 2.76 %                | 1.24%                                |
| 35 °C       | 2 %    | 0 %                   | 2%                                   |

Sources: SNI Document and Jurnal Cocoa Butter and Its Alternatives: A Review, 2014 dan DeltaWilmar

Based on Table 5, it can be concluded that the value of important solid fat content at a temperature of 25 °C is very close to the CBS percentage value of literature with a reasonably steep decrease when reaching the target melting temperature of 35°C so that the fat from the optimization results sufficiently meets the characteristics of the commercial CBS melting profile.

4. Conclusion
Fat melting profile from the observation of solid fat content in the mix showed that from 10 variations in the composition of the feed, only one profile was obtained that matched the characteristics of cocoa butter alternative that variation of pure coconut oil-CO (100) with a melting profile that resembled cocoa butter substitute. The optimization of statistical analysis using the RSM-simplex latticedesign factor with the melting cocoa butter (CB) response profile and alternative cocoa butter CBE,CBR, and CBS produce pure palm stearin and pure coconut oil as the best variation with minimum Sum Square of Error value with the target profile. However, pure palm stearin does not meet the advanced specifications so that it can be categorized as a cocoa butter alternative so that pure coconut oil is a variation that meets the commercial criteria for alternative cocoa butter in terms of slipmelting point, iodine number, and melting profile as an alternative chocolate substitution product.
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