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Chemical synthesis mono- and diacylglycerol from palm stearin-olein blend using continuous high shear stirred tank reactor

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KEYWORDS

Continuous high shear stirred tank reactor

Diacylglycerol

Monoacylglycerol

Palm stearin-Palm olein blend

ABSTRACT

This research aimed to evaluate the effect of flow rate and processing time on the synthesis of high mono- and diacylglycerol (MDAG) from palm stearin-olein blend using high shear continuous stirred tank reactor (HS-CSTR). Glycerolysis-interesterification was performed at 120 ºC and flow rates of 6, 10, 14, 18, and 22 mL/min. Glycerol:oil ratio, stearin:olein ratio, NaOH concentration, and agitation rate were 1:5 (mol/mol), 60:40 (w/w), 3%, and 2000 rpm, respectively. The result showed that flow rate significantly affected MDAG concentration and the product's physical characteristics. The highest MDAG was obtained at a flow rate of 6 mL/min. MDAG concentration, slip melting point (SMP), melting point (MP), hardness, emulsion capacity, and stability were 60.36 ± 1.61%, 42.3 ± 0.01 ºC, 43.3 ± 0.06 ºC, 6.04 ± 0.32 N, 87.6 ± 1.75 % and 91.8 ± 2.99 % respectively. An increase in residence time, which means flow rate decreased, increased MDAG, SMP, MP, hardness, emulsion capacity, and stability of the product. Processing time did not significantly affect MDAG concentration and the product's physical properties. It means that acylglycerol concentrations and physical properties of the product did not fluctuate significantly during the process. Thus, it confirmed that the continuous process was stable and reached a steady state throughout the process.

Introduction

Mono- and diacylglycerols (MDAG) can be produced by chemical and enzymatic glycerolysis reactions (Subroto et al., 2018). The enzymatic reaction has disadvantages because it has a long reaction time and high production costs (Naik et al., 2014). Chemical glycerolysis is faster than enzymatic reaction. However, the chemical reaction in stirred tank reactor requires high temperature and energy consumption, and it is not suitable for producing heat-sensitive polyunsaturated fatty acids (Zhang et al., 2017).

A problem in glycerolysis is that glycerol cannot dissolve in an oil phase. High temperature and solvent addition were applied to improve the reaction. However, the high temperatures caused a side effect reaction, resulting in a dark color and an off-flavor (Putri et al., 2020). In addition, the use of solvents is more expensive and requires a separation process.

An alternative to improve the reaction is the use of a high mixing rate. High shear mixing (HSM) can reduce the particle size and increase the surface area of the particle so that the intermolecular contact becomes better. This alternative can overcome the use of high reaction temperatures without using a solvent. HSM has been widely used in energy-intensive processes such as homogenization, dispersion, emulsification, milling, dissolving, food manufacturing, and chemical reaction processes (Zhang et al., 2012).

Besides, the glycerolysis process for producing MDAG based on vegetable fats, such as palm stearin (PS) and palm olein (PO), can be performed in a batch or continuous system. Batch processes have a disadvantage, such as requiring larger reactor volume, hence higher capital investment (Cole and Johnson, 2018). Continuous systems can increase production at lower costs because they are easy to control, have high productivity, and have a faster process (Florit et al., 2018). However, a factor, which should be considered is reaction time. A reversible reaction

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occurs when the glycerolysis time is much longer. It inhibits the conversion of triacylglycerol (TAG) to monoacylglycerol (MAG) and diacylglycerol (DAG) (Arum et al., 2019). In a continuous system, reaction time correlates with the residence time of the reactants in the reactor and subsequently the flow rate. The suitable residence time can prevent the occurrence of a reversible reaction that causes a decrease in MDAG production. Sufficient residence time is required to ensure the interaction between molecules reactants to achieve higher ester production (Boukhalkhal et al., 2019).

In a continuous process, the characteristic of the product must be consistent and not fluctuate after reaching a steady state (Lindeque and Woodley, 2019). Besides, residence time controls the reaction. Therefore, this study aimed to evaluate the effect of flow rate and processing time on the conversion of palm oil into MAG and DAG using high shear continuous stirred tank reactor (HS-CSTR). The characteristics of the interesterification product including the composition of MAG, DAG, TAG, slip melting point (SMP), melting point (MP), hardness, emulsion capacity, and stability were evaluated.

Research Methods

Materials

Palm stearin and palm olein were obtained from PT Smart Tbk, Indonesia. Glycerol 85%, NaOH, acetic acid, n-Hexane, ethyl ether, and methanol were obtained from Merck KGaA (Darmstadt, Germany). NaCl, citric acid, and brilliant blue were obtained from Sigma Aldrich (St. Louis, USA).

Effect of flow rate on acylglycerols concentrations and their physical properties

The oil phase was a mixture of PO and PS with a ratio of 40:60 (w/w) and NaOH 3%. The ratio of glycerol to the oil phase was 5:1 (mol/mol). The reaction was started by continuously flowing both glycerol (mixed with NaOH) and oil from the vessel into a high-shear continuous stirred tank reactor (HS-CSTR). The HS-CSTR was operated at 6, 10, 14, 18, and 22 mL/min based on the best reaction time (15 min) on the batch system (Bramasta et al., 2019). These flow rates corresponded to total residence times of 35, 21, 15, 11.67, and 9.55 min, based on the reactor volume of about 210 mL. The temperature was set at 120°C, and mixing agitation was set at 2000 rpm. After the glycerolysis reaction, the reaction was stopped by adding 20% citric acid solution dropwise into the product until pH 7. Then, the product was washed with 5% NaCl salt solution at a ratio of 1: 1 (v / v). Samples were analyzed, including MDAG, MAG, DAG, free fatty acid (FFA), TAG proportion, slip melting point (SMP), melting point (MP), and hardness.

Effect of processing time on acylglycerols concentrations and their physical properties

The glycerol and oil phase (5:1 mol/mol) continuously flowed into the reactor. The flow rate was 6 mL/min, corresponding to a residence time of 35 min. Sampling was carried out every 10 min for 60 min. Furthermore, the glycerolysis reaction was stopped by adding a 20% citric acid solution dropwise into the product until pH 7. Then the product was washed with 5% NaCl salt solution (1:1 v/v). Samples were analyzed, including MDAG, MAG, DAG, FFA, TAG concentration, SMP, MP, hardness, capacity, and emulsion stability.

Analysis properties of acylglycerols and free fatty acid concentrations

The thin layer chromatography (TLC) plate was activated by heating at ~105 ºC for ~30 min. One µL sample was then put on the TLC plate, which was placed above the hotplate until the sample dried. The TLC plate was developed in a TLC chamber containing a mixture of hexane: ethyl ether: acetic acid = 80: 20: 2 (v / v / v) as a developing solution. Chamber was saturated for approximately 1 hour prior to use. After that, the TLC plate was dried in the fume hood chamber and then left to stand for approximately 16 hours. TLC plates were stained using 0.02% coomassie brilliant blue in a solution of acetic acid: methanol: water = 1: 3: 6 (v / v / v) by immersing in the dye solution for ~5 min and drying at room temperature for approximately 5 hours. Then the sample composition was analyzed using Camag Automatic TLC Scanner III "dummy" S / N (1.14.16) with Camag WinCATS, the planar chromatography software at λ = 850 nm. The chromatogram had peak areas corresponding to mono-, di-, triacylglycerol, and free fatty acids concentration (Arum et al., 2019).

Analysis of slip melting point and melting point on the product

The melting point analysis was carried out according to the AOAC 920.157 method. A capillary tube (D = 1 mm) were immersed in samples that had been completely prepared at a temperature of 85 ºC for 15 min until they were

filled with samples as high as ~10 mm, then stored for 16 hours in the refrigerator. After that, the capillary tube is glued to the thermometer. The tip was parallel to the thermometer's tip and immersed in a beaker glass containing about 400 mL of water, and heated while stirring at 400 rpm. The heating temperature rise was controlled at 0.5-1 ºC / min. When the sample starts to slide upward, it is SMP, whereas MP is the temperature when it is completely clear (transparent).

Analysis of product hardness

The hardness was determined using Universal Testing Machines (UTM) (ZwickiLine Z0.5 to Z5.0, Germany) for the texture analyzer. Samples with a thickness of 2 cm were stored at 5 °C for 24 hours, and hardness measurements were performed at 25 °C. Hardness was determined at a speed of 1 mm/sec for 4 seconds with a depth of 4 mm (Biswas et al., 2017).

Analysis of the product emulsion capacity and stability

The sample (3 mL) was mixed with 37.5 mL of cooking oil and 75 mL of distilled water, then homogenized for 30 seconds at 10,000 rpm using Ultra Turax (Ika ultra-turrax® t 50 digital, Germany). Then, 37.5 mL of cooking oil was added and then homogenized again at the same speed for 90 seconds. The mixture was put into a centrifuge tube and centrifuged at 2500 g for 10 min. An emulsion is a cloudy white layer, and emulsion capacity can be measured using Equations (1).

$$Ec\ (\%) = \frac{V_{formed}}{V_{total}}\ x\ 100\% \quad \text{.....(1)}$$

where E_c is the percent emulsion capacity, V_{formed} is the volume of the emulsion formed, and V_{total} is the total volume sample.

The emulsion stability analysis was then performed by transferring the emulsion into Erlenmeyer then incubated in a water bath (without shaking) at 80 ºC for 30 min; after that, the sample was cooled at room temperature until the temperature reached room temperature; the last step sample was re-centrifuged at a rate of 2500 g for 10 min. Emulsion stability can be calculated using Equations (2).

$$Es\ (\%) = \frac{V_{heated}}{V_{total}}\ x\ 100\% \quad \text{.....(2)}$$

where Es is the percent of emulsion stability, V_{heated} is the volume after heating, and V_{total} is the total emulsion volume before heating (Subroto et al., 2018).

Determination of the best flow rate

The best flow rate was determined by calculating the effectiveness index (De Garmo et al., 1984). Each analysis parameter had a score (0 - 1) based on the level of importance, and the weight was calculated. Then the value of the effectiveness of DeGarmo for each treatment on each parameter of the analysis was determined.

The effectiveness value (NE) can be calculated using Equation 3.

$$NE = \frac{(N_p - N_{tj})}{(N_{tb} - N_{tj})} \quad \text{..... (3)}$$

where NE is the effectiveness value, N_{tj} is the worst value, N_p is the treatment value, and N_{tb} is the best value. Then the result value (NH) is determined by multiplying the effectiveness value (NE) with the score. Next, the sum of NH of all parameters was calculated, and the highest NH is the best treatment.

Statistical analysis

The experiment followed a Completely Randomized Design (CRD) design. Data represented the mean of duplicate analysis and were analyzed using ANOVA (analysis of variance). Duncan's test ($p<0.05$) was carried out when a significant difference was detected. Data were analyzed using the software SPSS version 2.2.

Results and Discussion

Characteristic of olein, stearin, and olein-stearin blend

The characteristics of olein, stearin, and olein-stearin blend as raw material for glycerolysis-interesterification are shown in Table 1. Palm stearin had a high SMP and MP, although the MAG and DAG content in stearin was relatively low (<17%). The high values of SMP and MP are because palm stearin is dominated by saturated fatty acids (Hasibuan, 2012). Besides, the unsaturated fatty acid is more dominant in olein (Karouw, 2014), resulting in a low melting point. Therefore, blending PS with PO reduced SMP and MP of the product compared to PS. The hardness of the PS-PO blend also decreased compared to the hardness of stearin, and it is suggested due to lower SMP and MP.

Table 1. Characteristics of olein, stearin, and a mixture of olein-stearin

Characteristic	Palm Olein (PO)	Palm Stearin (PS)	PO : PS blend (40:60)
Water content (% w/w)	0.09 ± 0.01	0.03 ± 0.01	0.06 ± 0.030
FFA (% w/w)	0.17 ± 0.01	0.20 ± 0.01	ND
Triacylglycerol (% w/w)	76.12 ± 0.45	84.27 ± 1.06	82.66 ± 5.50
Diacylglycerol (% w/w)	20.51 ± 0.82	14.04 ± 0.50	16.71 ± 4.99
Monoacylglycerol (% w/w)	3.36 ± 0.37	1.69 ± 1.56	0.63 ± 0.50
Slip Melting Point (°C)	13.20 ± 0.85	50.80 ± 0.42	45.50 ± 0.57
Melting Point (°C)	13.90 ± 0.28	51.30 ± 0.59	45.90 ± 0.14
Hardness (N)	-	66.72 ± 3.86	14.34 ± 1.18

Note: ND is not defined

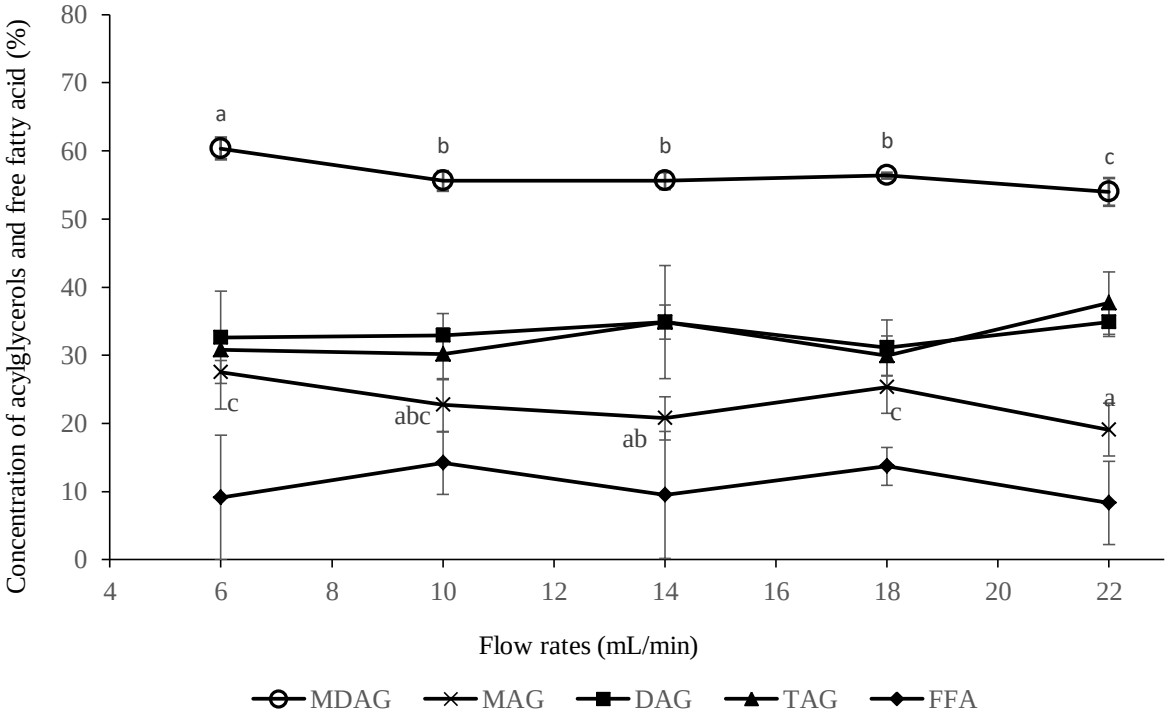

Figure. 1 Effect flow rate on mono-, di-, and triacylglycerols, total mono and diacylglycerols, and free fatty acid of the product. The reaction was performed at 120 ºC, PS:PO ratio of 60:40 (w/w), oil:glycerol ratio of 1:5 (mol/mol), and 3% NaOH. Error bars represent standard deviation from duplo measurements.

Effect of flow rate on acylglycerols and free fatty acid concentrations

The flow rates affected significantly MDAG concentration (Figure 1) and correlated with residence time. The flow rate of 6 mL/min correlated with a residence time of 35 min and resulted in the highest MDAG (60.36 ± 1.61%). The high MDAG is due to the lowest flow rate causing the longest residence time in the reactor. It is suggested that changes in acylglycerols compositions during the glycerolysis-interesterification are caused by the transferring of fatty acids from oil into glycerol. Several bound fatty acids on TAG will release from the glyceride oil backbone, then react with glycerol to produce MAG, DAG, and FFA (Affandi et al., 2017). Thus, an increase in reaction time resulted in more reactant molecules colliding with each other leading to an increase in MAG and DAG contents (Affandi et al., 2017). When the flow rate increases, it will reduce the residence time in the reactor. Therefore, only a few contacts between oil and glycerol molecules occurred so that the MDAG conversion decreased.

The flow rate had a significant effect on MDAG (Figure. 1), but it did not have a significant effect on MAG, DAG, TAG, and FFA. The highest MAG was obtained at the lowest flow

rate because of the longer reaction time. More acyl groups are released from TAG molecules and react with glycerol molecules to produce MAG and DAG. Besides, acyl groups of DAG are also released and react with glycerol to form MAG so that the number of MAG will increase. DAG in excess of glycerol tends to form more MAG (Zhang et al., 2017).

Effect of processing time on acylglycerols and free fatty acid concentrations

The MDAG, MAG, and DAG were not significantly affected by processing time (Figure 2). They tended to be stable from 0 min to 60 min, indicating that the continuous process had reached a steady state throughout the process. It is suggested that the continuous HS-CSTR allows a high mixing intensity, which significantly enhances heat and mass transfers (Boukhalkhal et al., 2019). Besides, HS-CSTR can break down fat globules into micro-emulsions that allow wider contact between molecules and subsequently enhance the reaction. Therefore, the process reaches faster to a steady state. MAG production was almost comparable to DAG production during the process, while FFA value was low. The production of MDAG by chemical glycerolysis-interesterification was relatively high compared to enzymatic glycerolysis-interesterification using CSTR (Arum et al., 2019; Subroto, 2020). Glycerolysis leads to the formation of MAG under an excess of glycerol, while the reaction leads to the formation of DAG when the amount of glycerol is limited (Subroto, 2020).

Effect of flow rate on slip melting point and the melting point of products

The flow rates significantly affected SMP and MP of products (Figure 3). The highest SMP and MP values were obtained at 6 mL/min, namely 42.3 ± 0.01 ºC and 43.3 ± 0.06 ºC, respectively. These corresponded to a high concentration of MDAG at low flow rates (Figure 1). MP is related to the chemical composition and concentration of the formed MAG and DAG. An increase in MAG and DAG concentrations caused an increase in the melting point.

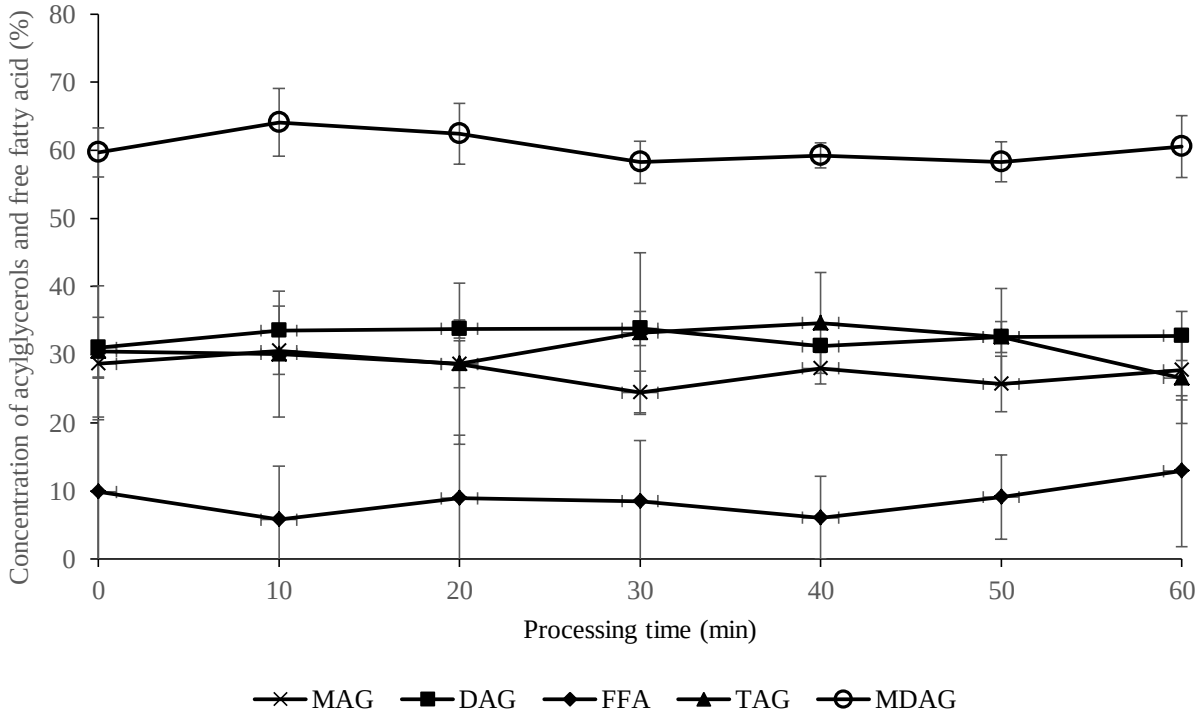

Figure 2. Effect of processing time on mono-, di-, and triacylglycerols, total mono and diacylglycerols, and free fatty acid of the product. The reaction was performed at 120 ºC, PS:PO 60:40 (w/w), oil:glycerol 1:5 (mol/mol), and 3% NaOH. The first coming out of the product was counted as 0 min. Error bars represent standard deviation from duplo measurements.

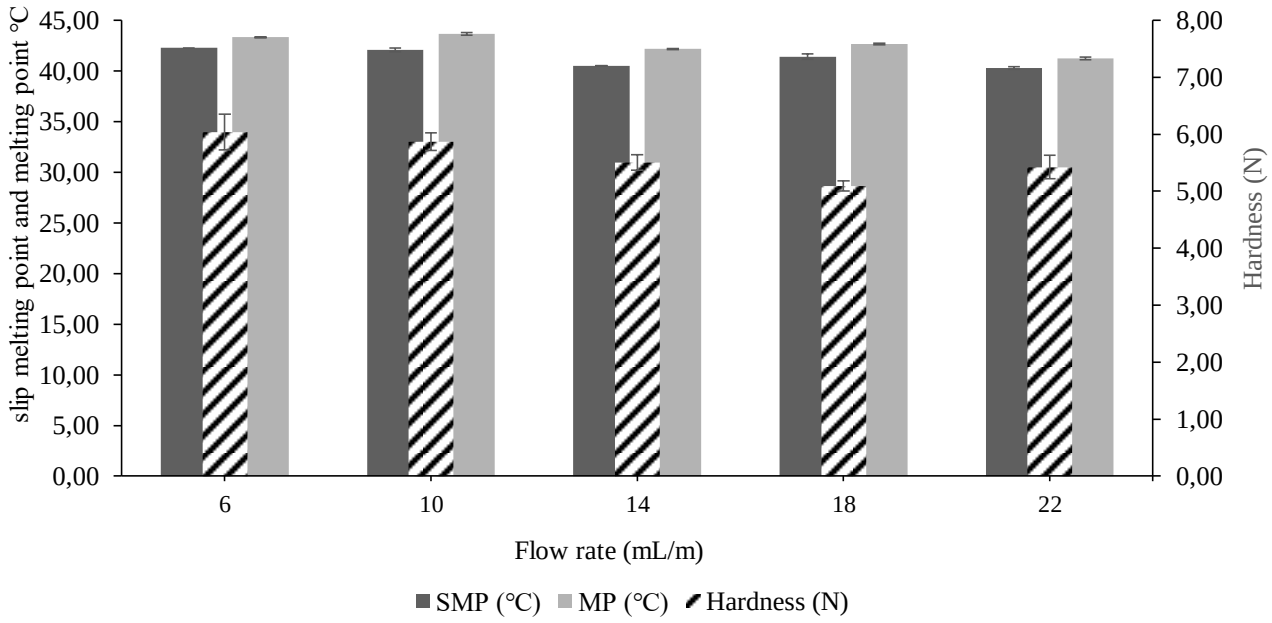

Figure 3. Effect of flow rate on slip melting point, melting point, and hardness of the product. The reaction was performed at 120 ºC, PS:PO 60:40 (w/w), oil:glycerol 1:5 (mol/mol), and 3% NaOH. Error bars represent standard deviation from duplo measurements.

Besides, the difference in melting point is caused by the amount of hydrogen in the fatty acid backbone and hydrophobic interactions along the hydrocarbon chain (Subroto, 2020). The presence of a hydrogen group requires more energy to break the hydrogen interaction, causing high melting points of MAG and DAG. Therefore, fats containing high MAG and DAG have higher SMP and MP than those of lower concentrations of MAG and DAG. This was possibly due to the melting points of MAG and DAG are higher than their triacylglycerol form (Zhang et al., 2017; Saberi et al., 2011).

Effect of flow rate on hardness product

The flow rates significantly affected the hardness of MDAG products (Figure 3). At 6 mL/min, which was the lowest flow rate, the hardness of the product was the highest compared to other flow rates. An increase in the flow rates resulted in a decrease in the hardness value of the product. These are also related to the obtained MDAG (Figure 1). Increasing the content of MAG and DAG in the product caused an increase in the melting point (Figure 3), which also affects the product's hardness. It is suggested that an increase in MAG and DAG in fat accelerates the crystal formation and causes a harder texture (Saberi et al., 2011). Besides, the synthesized MAG and DAG from the stearin-olein blend, which is rich in unsaturated fatty acids, increase crystal stability and produce more stable hardness (Zhang et al., 2017).

Effect of processing time on slip melting point and the melting point of product

The processing time did not significantly affect SMP and MP of the product (Figure 4). It means that the continuous process of HS-CSTR was stable and reached a steady state throughout the process. It is suggested that high stirring reduces the droplet size and results in higher surface contact between molecules. Therefore, it improved the glycerolysis-interesterification reaction rate. As a result, the reaction reached a steady state within a residence time of 35 min in the reactor. It means that the forming of MDAG does not significantly fluctuate (Figure 2). Besides, MP of acylglycerols is MAG > DAG > TAG (Subroto et al., 2018). So, MAG and DAG have more vigorous surface activity than triacylglycerol (Rumondang et al., 2016). Since total MAG and DAG (MDAG) were not significantly different, and MDAG correlated to SMP and MP of the product, then the processing time did not significantly affect SMP and MP of the product.

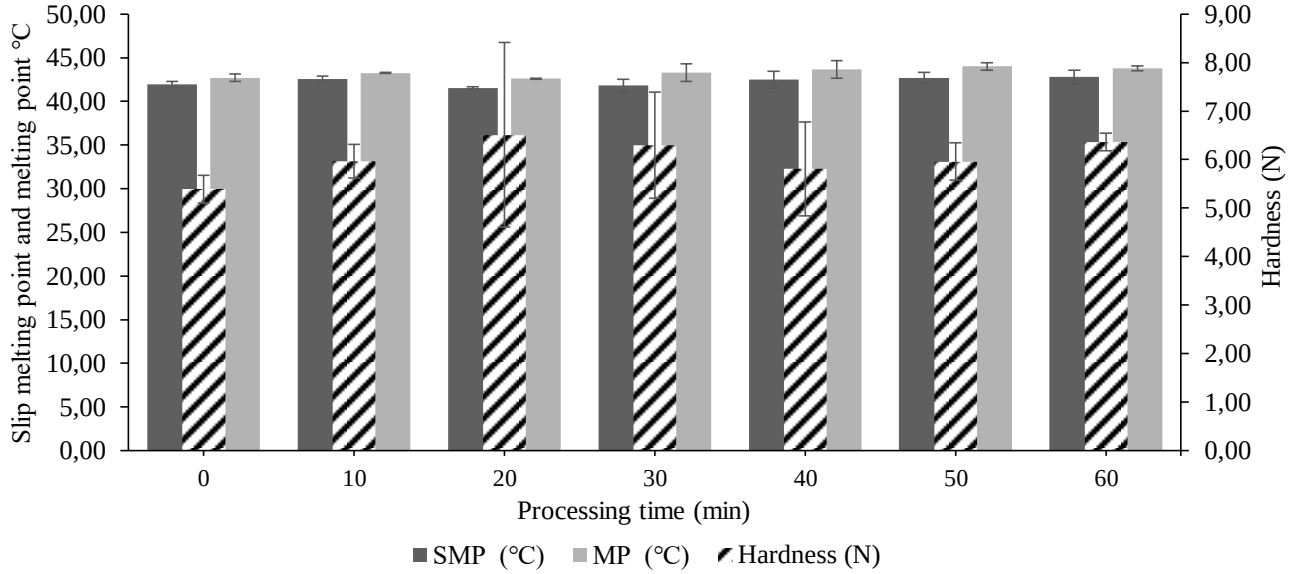

Figure 4. Effect of processing time on slip melting point (SMP), melting point (MP), and hardness of the product. The reaction was performed at 120 ºC, PS:PO 60:40 (w/w), oil:glycerol 1:5 (mol/mol), and 3% NaOH. The first coming out of the product was counted as 0 min. Error bars represent standard deviation from duplo measurements.

Effect of processing time on hardness product

The processing time did not significantly affect the hardness product (Figure 4). It shows that the continuous process was stable and reached a steady state during a processing time of 60 min. The average hardness value of the resulting product was 6.04 ± 0.32 N. It is even higher than the hardness of the enzymatic glycerolysis product at the same proportion of fat, which had a hardness of 5.63 N (Subroto et al., 2018). It is suggested that HS-CSTR creates more the forming of micro-emulsions and causes the reactants to be more homogeneous. The forming of micro-emulsions increased interface surface area. Therefore, it causes the reaction between fat and glycerol to become more intense to produce high MDAG fat. High MDAG causes the product's melting point to increase so that the product becomes harder. The product will have a more rigid texture because the saturated fatty acid composition is more dominant in fat. On the other hand, if unsaturated fatty acids are dominant, the texture will be mushy (Saberi et al., 2011).

Effect of processing time on capacity and stability of emulsion product

The processing time did not significantly affect the capacity and stability emulsion of the product (Figure 5). It shows that the continuous process was stable and reached a steady state during a processing time of 60 min. The average emulsion capacity and stability were relatively high, namely 87.6 ± 1.75 and 91.8 ± 2.99 %, respectively. It is similar to the enzymatic synthesis of MAG and DAG from the PS-PO blend (Subroto et al., 2018). MAG and DAG have a free hydroxyl group (OH), which is a hydrophilic group, and a fatty acid ester group, which is lipophilic. Therefore it can be used as an emulsifier (Rarokar et al., 2017). Glycerolysis-interesterification of the PS–PO blend increases the emulsion capacity of the product. The higher the MAG and DAG content in the fat, the more polar hydrophilic groups so that the capacity and stability also increase. MAG and DAG can also increase polymorphic transition, susceptibility to fat blooming, emulsion stability, and oxidation (Subroto, 2020).

Effectiveness index

The effectiveness index is presented in Table 2. Based on the effectiveness index, the best treatment of all tested parameters was obtained at a 6 mL/min flow rate. The total final effectiveness index was 0.54. MDAG parameter has the highest weighing score compared with other parameters because the target was to obtain the highest MDAG. The other parameters that determine the best flow rate are SMP, MP, and hardness, which are the product's physical properties.

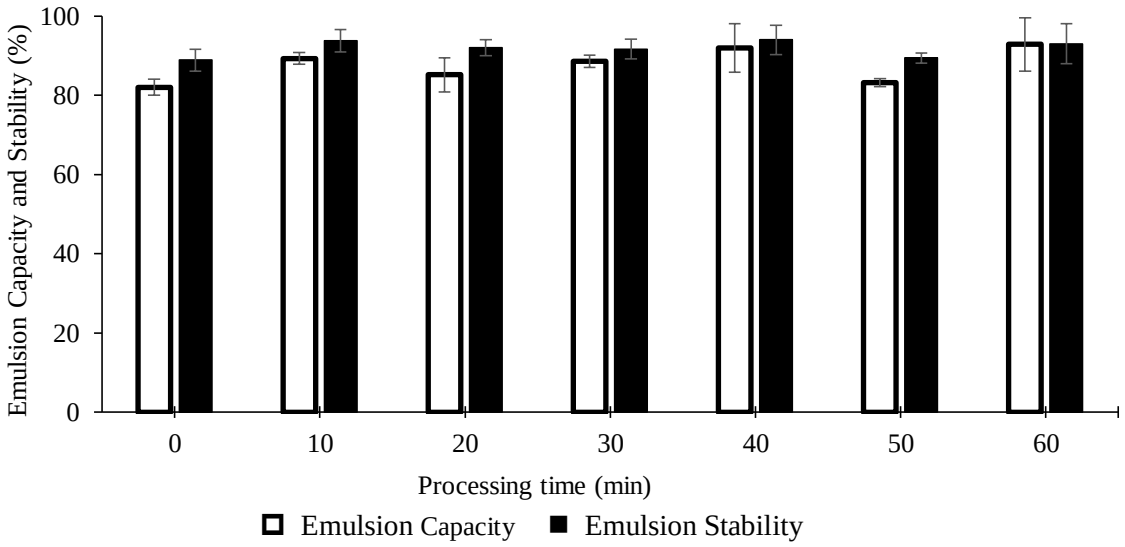

Figure 5. Effect of processing time on emulsion capacity and stability of the product. The reaction was performed at 120 ºC, PS:PO 60:40 (w/w), oil:glycerol 1:5 (mol/mol), and 3% NaOH. The first coming out of the product was counted as 0 min. Error bars represent standard deviation from duplo measurements.

Table 2. The best flow rate is based on the ranking effectiveness index (De Garmo et al., 1982)

Flow rate (mL/min)	Effectiveness Parameter				
	MDAG	**SMP**	**MP**	***Hardness***	**Total Value**
6	0.38	0.00	0.00	0.17	0.54
10	0.10	0.02	0.12	0.11	0.35
14	0.10	0.05	0.12	0.07	0.34
18	0.14	0.11	0.07	0.00	0.31
22	0.00	0.25	0.21	0.06	0.52

Note: MDAG: mono- and diacylglycerol, SMP: slip melting point, MP: melting point

Conclusion

The flow rate had a significant effect on MDAG but it did not have a significant effect on MAG, DAG, TAG, and FFA. The flow rates correlated with a residence time, in which 6 mL/min correlated with a residence time of 35 min. It resulted in the highest MDAG, namely 60.36 ± 1.61%. The flow rate had also a significant effect on SMP, MP, and hardness of products. The highest SMP, MP, and hardness values were obtained at 6 mL/min, namely 42.3 ± 0.01 ºC, 43.3± 0.06 ºC, and 6.04 ± 0.32 N respectively. The average emulsion capacity and stability were relatively high, namely 87.6 ± 1.75 and 91.8 ± 2.99 %, respectively. On the other hand, processing time did not have a significant effect on MDAG concentration, SMP, MP, hardness, emulsion capacity, and product stability during the continuous process. This indicates that the continuous process reached a steady state within 60 min. Therefore, the physicochemical properties of the product did not fluctuate significantly. Based on the effectiveness index, the best treatment of all tested parameters was obtained at a 6 mL/min flow rate. The total final effectiveness index was 0.54. Thus, the continuous glycerolysis-interesterification process of the PS-PO blend using HS-CSTR reached a steady state within 60 min and the product quality did not fluctuate significantly.

Declarations

Conflict of interests The authors declare no competing interests.

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