Glycerolysis-intertesterification of Palm Stearin and Nyamplung Oil Mixture in High Shear Continuous Stirred Tank Reactor

Bonifasius Rionaldo, Rini Yanti, Chusnul Hidayat*

Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
*Correspondence Author: Chusnul Hidayat, Email: chusnulhi@ugm.ac.id

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ABSTRACT

Glycerolysis-interesterification can be used for the synthesis of products containing high total Mono- and Diacylglycerol (MDAG). Therefore, this study aimed to evaluate the synthesis of products rich in MDAG content using glycerolysis-interesterification method in High Shear Continuous Stirred Tank Reactor (HS-CSTR). The impact of varying material flow rates (6, 10, 14, 18, and 22 mL/min) and processing time on the concentration of MDAG, physical properties of the resulting product, and consistency of product quality throughout the process were assessed. Furthermore, glycerolysis-interesterification reaction was performed at a temperature of 120 °C, with a glycerol and oil mixture mole ratio of 1:5 (mol/mol), 3% NaOH, and a stirring speed of 2000 rpm. Oil mixture consisted of Palm Stearin (PS) and Nyamplung oil (*Calophyllum inophyllum*) (MC) with a PS:MC mole ratio of 80:20 (mol/mol). Subsequently, the acylglycerol concentration and physical properties of the product were analyzed. The results showed that the material flow rate had a significant effect on MDAG and the physical properties of the product. The highest MDAG was obtained at a flow rate of 6 mL/min with content of 58.56±0.91%, and Slip Melting Point (SMP) of 41.44±0.08 °C and 42.9±0.03 °C. The hardness, emulsion capacity, and stability values were 10.88±0.22 N, 85.2±6.93%, and 88.7±5.00%, respectively. The acylglycerol concentration and physical properties of the product did not significantly fluctuate throughout the process, indicating that the process had achieved a steady state condition.

Keywords: Glycerolysis-interesterification; High shear continuous stirred tank reactor; Mono- and diacylglycerol; Nyamplung oil; Palm stearin

INTRODUCTION

Structured lipids can be subjected to chemical or enzymatic reactions to modify their fatty acid composition and the distribution of positions. An example of this lipid that has attracted attention is a product enriched with Diacylglycerol (DAG) and Monoacylglycerol (MAG) (Wang et al., 2022). Triacylglycerol (TAG), DAG, and MAG are acylglycerols found in lipids (Kim and Akoh, 2015). The combination of MAG and DAG, commonly known as MDAG, has gained widespread use as an emulsifier in the food industry, as well as a wetting and lubricating agent in cosmetics and pharmaceuticals (Mamuaja, 2017). The production of MDAG involves interesterification and glycerolysis processes of abundant oil or fat (Wangi et al., 2022; Subroto et al., 2020). One of the raw materials used in the production is Palm Stearin (PS), which is the solid phase of palm oil, and has a high Melting Point (MP) of 44-56 °C (Norazlina et al., 2021). This poses a challenge in the production

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of food fats such as shortening and margarine, resulting in low product plasticity. Furthermore, Nyamplung oil (*Calophyllum inophyllum*) (MC), which has a low MP and contains fatty acids similar to PS (Hasibuan and Buana, 2013), can be used as a mixture to improve its MP.

MDAG is also synthesized through a glycerolysis reaction between TAG and glycerol (Hobuss et al., 2020; Satriana et al., 2016). According to Subroto et al. (2019), the reaction yields a high amount of MDAG since every mole of TAG produces 3 moles of MAG, and in the absence of excess glycerol, the reaction shifts towards DAG formation. Glycerolysis reaction can be accelerated through various means, such as adding solvents or performing the reaction at high temperatures. However, the use of solvents is cost ineffective, and performing the reaction at 210-230 °C may lead to undesirable side effects (Mamuaja, 2017).

Generally, MDAG production is carried out in a batch system, and Arum et al. (2019) reported that the procedure decreases significantly after exceeding the optimum reaction time. The production can also be conducted in a continuous system (Bôas et al., 2021), and the use of a reactor system is easily controlled, and very fast. Meanwhile, continuous reactor can be equipped with high-shear mixing to improve mass transfer in reactor at a lower temperature. High-shear mixing can reduce the size of material droplets (Tamminen and Koiranen, 2015) to expand the contact surface area, and cause the occurrence of a reaction.

In this study, MDAG was synthesized by reacting a mixture of raw materials PS and MC in an HS-CSTR. Reactor was equipped with a high-speed agitator. Furthermore, the use of HS-CSTR was expected to increase MDAG synthesis, and product quality parameters were maintained during the production process. The factors evaluated included material flow rate and processing time on the characteristics of TAG, MAG, DAG concentration, Slip Melting Point (SMP), MP, hardness, stability, and emulsion capacity.

MATERIAL AND METHOD

Material

PS, MC and Glycerol were obtained from PT Smart (Tbk, Indonesia), Cilacap, and Merck KGaA (Darmstadt, Germany). Meanwhile, NaOH, NaCl, citric acid, phosphoric acid, and bleaching earth were obtained from Sigma Aldrich (St. Louis, MO, USA).

Method

The experimental design was completely randomized, and the factor was the flow rate at 6, 10, 14, 18, and 22 mL/minute. The fixed variables were the reaction temperature of 120 °C, oil to glycerol molar ratio of 1:5, mixture of PS and MC at 80:20, and the addition of 3% (w/w) chemical catalyst NaOH based on the mass of oil.

The Effect of Flow Rate on the Concentration of Acylglycerol and Free Fatty Acids

Oil phase was a mixture of PS and MC (PS-MC) with a ratio of 80:20 (w/w), and glycerol was mixed with 3% NaOH based on oil phase. The reaction started by mixing oil and glycerol phase at a ratio of 5:1 (mol/mol) in HS-CSTR. Meanwhile, HS-CSTR was operated at flow rates of 6, 10, 14, 18, and 22 mL/minute and a reactor volume of 210 mL. Based on the flow rate and reactor volume, the residence time was 35, 21, 15, 11.67, and 9.55 minutes, respectively. The temperature and the stirring speed for mixing the ingredients were set at 120 °C and 2000 rpm. Subsequently, the reaction was stopped by adding 20% citric acid solution until the pH was 7, and the product was washed with 5% NaCl at a ratio of 1:1 (v/v). The analyzed product included the concentration of acylglycerol and free fatty acid (FFA).

The Effect of Processing Time on the Concentration of Acylglycerol and Free Fatty Acids

The effect of processing time was analyzed according to the flow rate at sampling periods of 0, 10, 20, 30, 40, 50, and 60 minutes. The product was analyzed for the concentration of acylglycerol and FFA.

The Effect of Flow Rate on Product Physical Characteristics

The flow rate on the physical characteristics of the product was measured by determining SMP, MP, hardness, as well as stability, and emulsion capacity.

The Effect of Processing Time on Product Physical Characteristics

The effect of processing time on the physical properties was measured according to the processing time. The product was analyzed for SMP, MP, hardness, as well as stability, and emulsion capacity.

Product Analysis Using the Thin-Layer Chromatography (TLC) Method

The composition of acylglycerols and FFA was analyzed using a TLC plate with a size of 20x20 cm (Wangi et al., 2022), and the distance between the spots was 1.5 cm. The starting and ending spots of the top and bottom parts of the plate were 1 cm each. Furthermore, the TLC plate was heated at ±105 °C for

±30 minutes, and 1 µL of liquid product sample was dropped onto the plate. The TLC plate was then placed in a chamber containing a developing solution with a composition of hexane, ethyl ether, and acetic acid at a ratio of 80:20:2 (v/v/v). The chamber was saturated with the developing solution for ±1 hour before use, and the TLC plate was removed and dried in an acid cabinet for ±16 hours. Subsequently, it was dipped into a 0.02% Coomassie blue solution dissolved in acetic acid, methanol, and distilled water at a ratio of 1:3:6 (v/v/v) and left for ±1 minute. The plate was then dried at room temperature in the acid cabinet for ±5 hours. The sample was analyzed using the Camag Automatic TLC Scanner III "dummy" S/N (1.14.16) with Camag WinCATS planar chromatography software. In addition, scanning was performed at λ = 629 nm, where the peak area of monoacyl, diacyl, TAG, and FFA on the chromatogram corresponded to the concentration of each acylglycerol and FFA in the product (Jin et al., 2017).

MP and SMP Analysis

The capillary tube was immersed in the sample at a temperature of 85°C for 15 minutes with a depth of 10 mm, then placed in a refrigerator for 16 hours. Similarly, the tube was attached to the thermocouple by partially dipping it into 400 mL water in a beaker placed on a hot plate and stirred at a speed of 400 rpm. The water was set at 8-10°C, and the temperature was adjusted in the range of 0.5-1ºC/minute. The sample was recorded as SMP and MP after moving upward in the capillary tube and became fully transparent (AOCS 2003).

Hardness Analysis

The product hardness was analyzed using a Universal Testing Machine (UTM) from Zwick/Z0.5 with a 12.7 probe. Furthermore, the sample was placed in a plastic container with a height of approximately 2 cm and a penetration depth of 4 mm (Kadivar et al., 2016).

Emulsion Capacity and Stability Analysis

Emulsion capacity analysis was conducted according to the study of Subroto et al. (2020; 2029), where 3 mL of the sample was mixed with 37.5 mL of cooking oil and 75 mL of distilled water. The solution was homogenized at 10,000 rpm for 30 seconds using an Ultra Turrax, then 37.5 mL of cooking oil was added, and homogenized at 10,000 rpm for 90 seconds. Mixture was placed in a centrifuge tube and centrifuged at 2500xg for 10 minutes. Meanwhile, the solution formed a cloudy white layer that represented the emulsion, and the capacity can be calculated using Equation 1.

$$\text{Emulsion Capacity (\%)} = \frac{\text{Emulsion volume formed}}{\text{Total volume}} \text{ x } 100 \text{ (\%)} \quad (1)$$

The sample from the emulsion capacity measurement was heated in a water bath without shaking at a temperature of 80°C for 30 minutes and was calculated using Equation 2. The sample was then cooled to room temperature, and centrifuged at 2500xg for 10 minutes.

$$\text{Emulsion Stability (\%)} = \frac{\text{Emulsion volume heating}}{\text{Emulsion volume formed}} \text{ x } 100 \text{ (\%)} \quad (2)$$

Statistic analysis

The experiment was conducted using a Completely Randomized Design (CRD), while the analysis was performed by utilizing ANOVA with a 95% confidence level. Further testing was carried out using Duncan's Multiple Range Test (DMRT) when the ANOVA analysis with a 95% confidence level showed significant differences in treatments.

Best Flow Rate Analysis

The best material flow rate was determined by the DeGarmo effectiveness value (DeGarmo, 1984), and each parameter was given a value of 0-1 based on its importance. The effectiveness value assessment was carried out for each treatment based on the parameters of MDAG, SMP, MP, and hardness. This was because MDAG, SMP, and MP affected the product quality parameter, namely hardness, and DeGarmo's effectiveness can be calculated using Equation 3.

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

where NE, Np, N_{tj}, and N_{tb} are the effectiveness, treatment, worst, and best values. The effectiveness value (NE) was multiplied by the score to obtain the result value (NH). The NH obtained from all parameters was added up, and the treatment with the highest value was the best.

RESULTS AND DISCUSSION

Characteristics of Raw Materials

The characteristics of the raw materials used can be seen in Table 1. Water and FFA content in MC are relatively higher compared to PS, which causes PS-MC mixture to have relatively higher water and FFA content compared to PS. According to Hasibuan & Buana (2013), MC has a water content of 0.14%.

Table 1. Characteristics of PS, MC, and the proportion of PS:MC

Characterization of raw materials and products	MC	PS	PS:MC (80:20)
Water content (% b/b)	0.28±0.01	0.03±0.01	0.23±0.04
Free fatty acids (% b/b)	11.26±0.01	0.2±0.01	3.44±0.03
Acylglycerol:			
TAG %	82.78±5.67	75.86±1.61	78.80±5.75
DAG %	16.39±5.43	18.54±1.42	14.91±5.87
MAG %	0.50±0.70	5.60±0.1 8	2.86±0.14
SMP (°C)	16.0±0.28	50.8±0.42	42.5±0.14
MP (°C)	16.6±0.14	51.3±0.14	43.1±0.28
Hardness (N)	-	66.72±3.86	23.53±3.14

Description: The data is the mean of 2 repetitions.

The concentration of TAG in PS-MC mixture is 4.43 times greater than the total DAG and MAG. This is because TAG is the main component of fat storage in plant and animal cells (Mamuaja, 2017). SMP and MP values in PS-MC mixture are 1.2 times lower than PS and 2.6 times higher than MC. This is due to mixture of PS with high MP, which is proportional to the hardness value (Devi and Katkhar, 2017). Therefore, the hardness of PS decreases after being mixed with MC along with the decreased value of SMP and MP in PS-MC mixture.

Effect of Flow Rate on the Concentration of Acylglycerol and Free Fatty Acids

Figure 1 shows that the trend of TAG conversion to MDAG decreases with increasing flow rate. Based on statistical analysis, the flow rate has a significant effect on MDAG concentration ($p<0.05$), and the material is correlated with the residence time. Furthermore, the slowest flow rate is 6 mL/min, equivalent to a residence time of 35 minutes. Increasing the flow rate causes a decrease in residence time (Mándity et al., 2015). These results in suboptimal molecular contact in HS-CSTR reactor, leading to a decrease in MDAG concentration. Therefore, a flow rate of 6 mL/min produces the highest MDAG concentration compared to others, as shown in Figure 1.

The concentration of MAG is inversely related to DAG during the process. Based on statistical analysis, DAG, MAG, and FFA at various flow rates are not significantly different ($P<0.05$). This may be due to the dominant mechanism of MAG formation originating from DAG glycerolysis and hydrolysis. It causes an increase in MAG followed by a decrease in DAG and an increase in FFA (Wangi et al., 2022). Meanwhile, DAG can be formed from TAG hydrolysis or glycerolysis.

Effect of Processing Time on the Concentration of Acylglycerol and Free Fatty Acids

The concentration of MDAG during the 60-minute process can be seen in Figure 2. Based on statistical analysis, the concentrations of MAG, DAG, TAG, total MDAG, and FFA do not differ significantly at each sampling time ($p<0.05$). The reason for the efficient mixing of materials in HS-CSTR is attributed to high-shear mixing, which accelerates the mass transfer process. Consequently, the reaction time is reduced, leading to rapid attainment of steady-state conditions, and the product quality remains stable without significant fluctuations. Meanwhile, the average concentration of MDAG in the product is 58.55±0.91%. The result of MDAG synthesis using HS-CSTR for 60 minutes is almost equal to the result of Arum et al.'s (2019) study, where the reaction took place in a batch system for 6 hours and produced MDAG at 61.43%. The use of high-shear mixing in HS-CSTR plays a role in increasing the mixing of materials to produce a product with consistent results throughout the process.

Effect of Flow Rate on SMP and Product MP

The effect of flow rate on SMP and MP can be seen in Figure 3 with a significant effect on the variables ($p<0.05$). The trend of SMP and MP tends to decrease with increasing flow rate, and the highest values are obtained at 6 mL/min. This is because the variables are affected by the concentration of MAG and DAG in the product (Zhang et al., 2017). MAG and DAG molecules have two and one hydroxyl bonds, while

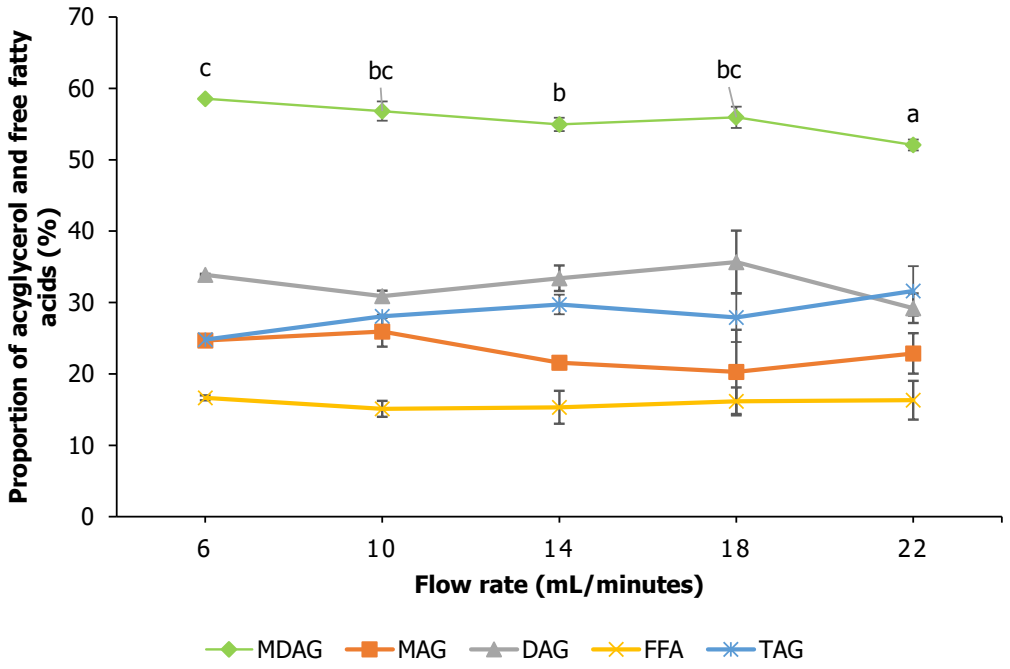

Figure 1. Effect of flow rate on MAG, DAG, and TAG, and total MDAG and free fatty acids. The reaction was carried out at 120 °C, PS:MC 80:20 w/w, oil:glycerol 1:5 mol, and 3% NaOH. Data followed by different lowercase letters on the same line color indicate significantly different data ($p<0.05$). The data is the mean of 2 repetitions

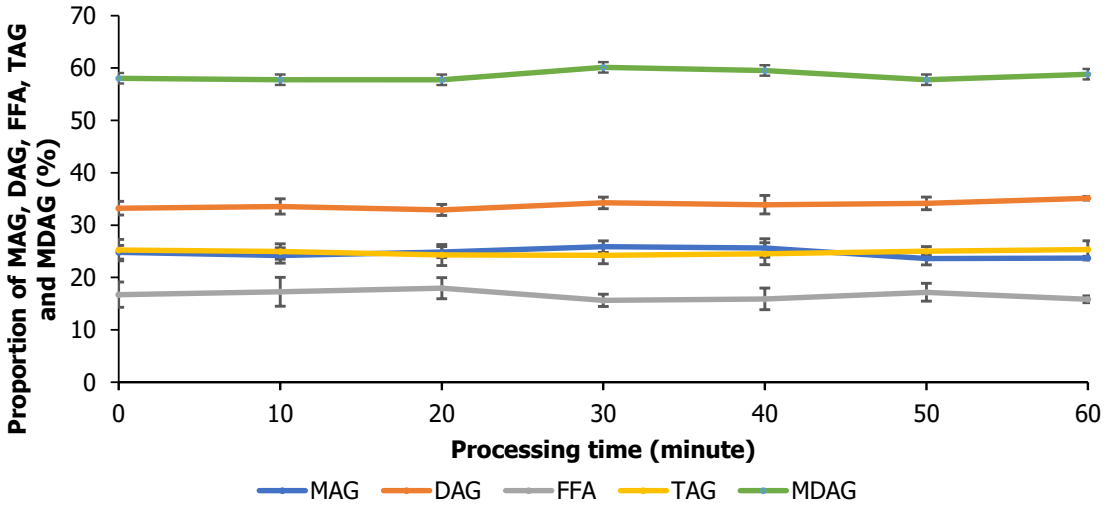

Figure 2. The effect of processing time on MAG, DAG, and TAG, as well as total MDAG and free fatty acids. The reaction was carried out at a flow rate of 6 mL/min for 60 minutes, a temperature of 120 °C, PS:MC 80:20 w/w, oil:glycerol 1:5 mol, and 3% NaOH. The data is the mean of 2 repetitions

TAG molecules have no bond (Wang et al., 2022). The presence of hydroxyl groups in MAG and DAG requires more energy to break the bond, resulting in high SMP and MP values. The trend in MDAG, SMP, and MP products decreases with an increasing flow rate as shown in Figures 1 and 3.

Effect of Flow Rate on Hardness

The hardness of the products produced at various flow rates can be seen in Figure 4. Statistical analysis shows that flow rate has a significant effect on the hardness value of MDAG product ($p<0.05$), and the value of 6 mL/min has the highest hardness of 10.67±0.22 N compared

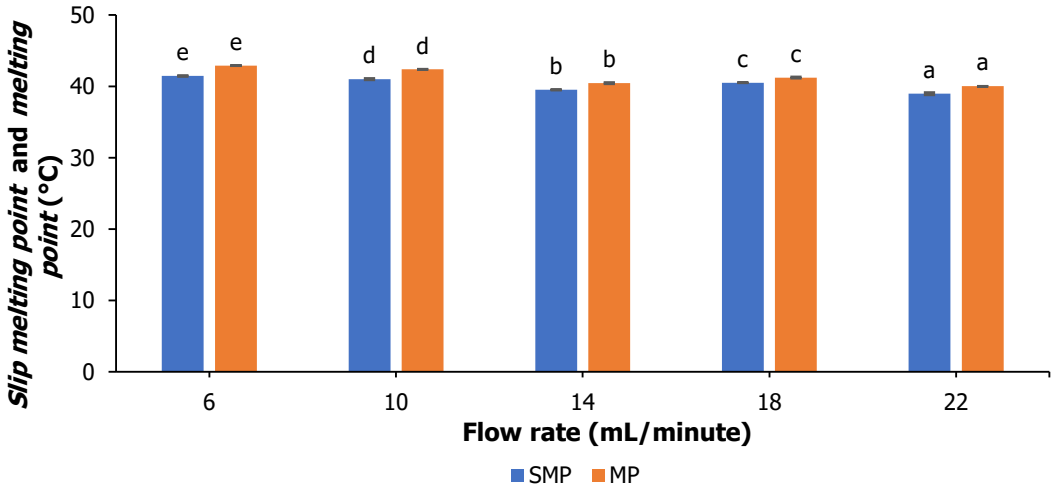

Figure 3. The effect of flow rate on SMP and MP. The reaction was conducted at 120 °C, PS:MC ratio 80:20 w/w, oil:glycerol ratio 1:5 mol, and 3% NaOH. Data followed by different lowercase letters on the same line color indicate significantly different data ($p<0.05$), and the data is the mean of 2 repetitions

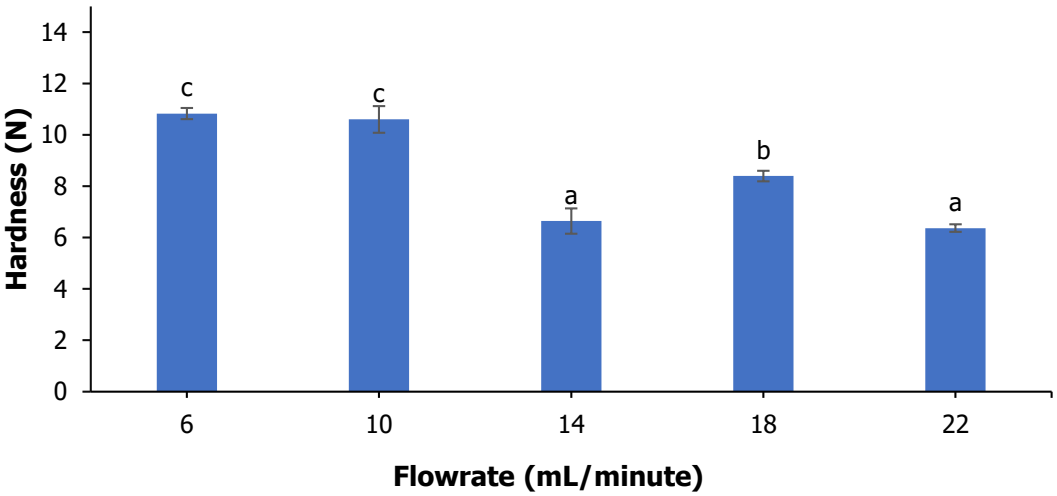

Figure 4. The effect of flow rate on the value of hardness. The reaction was carried out at 120 °C, PS:MC ratio 80:20 w/w, oil:glycerol ratio 1:5 mol, and 3% NaOH. Data followed by different lowercase letters on the same line color indicate significantly different data ($p<0.05$). The data is the mean of 2 repetitions

to others. This is due to the increase in MAG and DAG concentration in the product, which can accelerate the crystal formation process and make the texture harder (Subroto et al., 2020; 2019). Therefore, an increase in flow rate decreases the hardness value caused by a reduction in MDAG concentration, as shown in Figure 1. A low flow rate produces high hardness while increasing the results in a lower hardness value of MDAG product.

Best Flow Rate Determination

The best treatment is determined based on the effectiveness index value using the DeGarmo method (1984) by assigning weights to each parameter based on their importance level, as shown in Table 2.

According to the DeGarmo method, the treatment with the highest total score is selected as the best. Based on the effectiveness values presented in Table 2, the best treatment has the lowest flow rate of 6 mL/minute with a total final effectiveness index of 0.87.

Effect of Processing Time on Product SMP and MP

The effect of processing time on the product SMP and MP can be seen in Figure 6. Based on the statistical analysis, the values of the variables during

Table 2. Best flow rate based on DeGarmo effectiveness rating

Flow rate (mL/minute)	Effectiveness parameters				
	MDAG	SMP	MP	Hardness	Total value
6	0.39	0.26	0.22	0.01	0.87
10	0.29	0.10	0.13	0.00	0.51
14	0.17	0.16	0.18	0.12	0.64
18	0.23	0.04	0.04	0.06	0.38
22	0.00	0.00	0.00	0.13	0.13

the 60-minute process do not show significant differences ($p<0.05$). Therefore, SMP and MP values are consistent and do not significantly fluctuate during the synthesis of MDAG using HS-CSTR. The mean of SMP and MP at a flow rate of 6 mL/min is 41.44±0.08 °C and 42.9±0.03 °C, respectively. Furthermore, the use of HS-CSTR produces a homogeneous product because the ingredients are stirred at a high speed during the reaction for proper mixing of the ingredients. HSM can accelerate the reaction and increase the contact between oil and glycerol by forming a microemulsion, then increasing the interfacial contact area (Gole & Gogate, 2014). According to Subroto et al. (2020), there is a relationship between the concentration of TAG, MAG, and DAG with SMP and MP. Products with high MDAG concentration and low TAG concentration should have high SMP and MP values.

The Effect of Processing Time on Hardness Value

The effect of processing time on the product hardness can be seen in Figure 6. Based on statistical tests, the hardness values do not significantly differ during the 60 minutes ($p<0.05$). This indicates that the hardness is consistent and does not significantly fluctuate during the process. SMP can characterize the strength of the fat crystal network. The increase in SMP and MP can increase product hardness (Subroto et al., 2020). Meanwhile, SMP and MP are affected by the composition of acylglycerols, and a high SMP value in the product indicates a strong bound fat crystal network, which results in increased hardness. The fat crystal network is directly proportional to the matrix, making it more resistant to temperature and enabling the product to maintain its shape. However, this study indicates that SMP, MP, and acylglycerol composition do not significantly differ during the process.

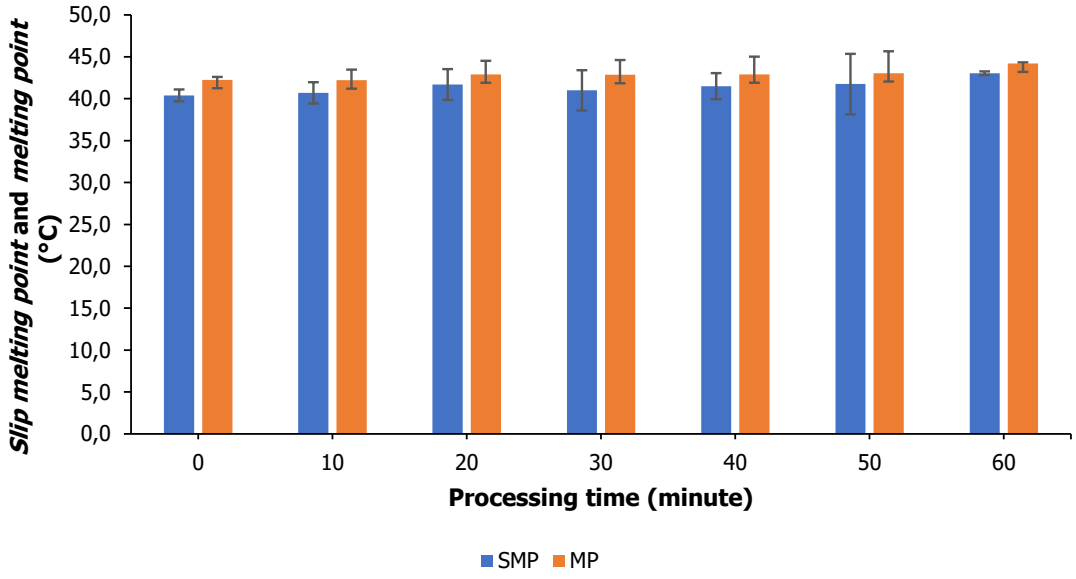

Figure 5. The effect of processing time on SMP and MP. The reaction was carried out at a flow rate of 6 mL/min for 60 minutes, a temperature of 120 °C, a PS:MC ratio of 80:20 w/w, an oil:glycerol ratio of 1:5 mol, and a 3% NaOH. The data is the mean of 2 repetitions

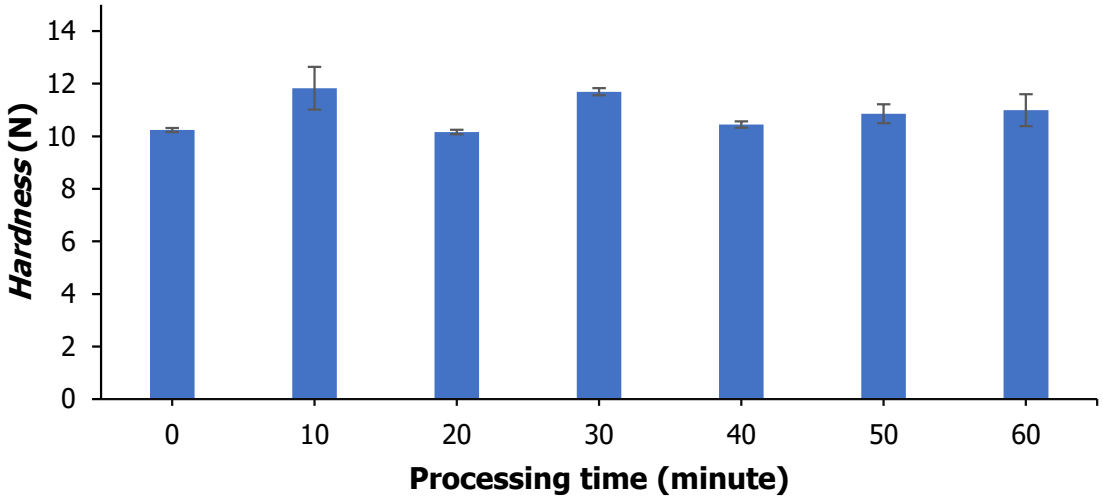

Figure 6. The effect of processing time on hardness. The reaction was carried out at a flow rate of 6 mL/min for 60 minutes, a temperature of 120 °C, PS:MC 80:20 w/w, oil:glycerol 1:5 mol, and 3% NaOH. The data is the mean of 2 repetitions

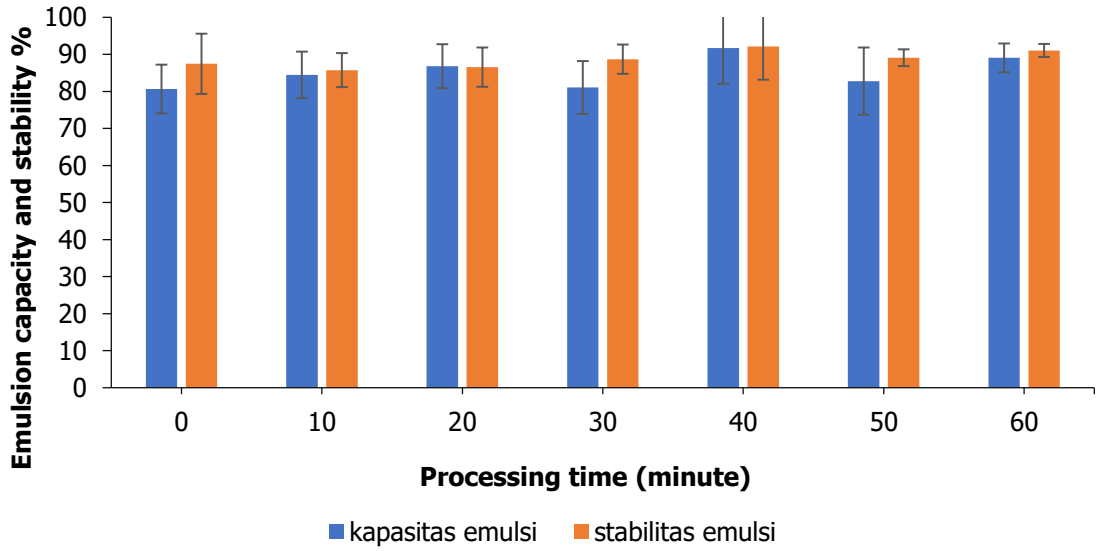

Figure 7. The effect of processing time on emulsion capacity and stability. The reaction was carried out at a flow rate of 6 mL/min for 60 minutes, a temperature of 120 °C, a PS:MC ratio of 80:20 w/w, an oil:glycerol ratio of 1:5 mol, and a 3% NaOH. The data is the mean of 2 repetitions

The Effect of Processing Time on Emulsion Stability and Capacity

The emulsion capacity and stability during the process can be seen in Figure 7. Based on statistical analysis, the values of the variables do not differ significantly during the 60-minute process ($p<0.05$). This indicates that the capacity and stability at average values of 85.2±6.93% and 88.7±5.00% are consistent and not significantly fluctuating. These results are almost comparable to MDAG product from a mixture of PS and palm olein with chemical glycerolysis, which has an emulsion capacity of around 93.63% (Subroto et al., 2020). Furthermore, MAG and DAG have hydroxyl groups, which can act as emulsifiers. The hydroxyl groups on monoglycerides or diglycerides can interact with water through hydrophilic bonds, while the non-polar groups at the end of the fatty acid chain can interact with fats through hydrophobic interactions (Mamuaja, 2017).

CONCLUSION

Based on the results of the study conducted, it can be concluded that the optimal flow rate for continuous synthesis of MDAG using HS-CSTR is 6 mL/minute. The resulting MDAG has a composition of 58.55±0.91%, with physical properties including SMP, MP, and hardness of 41.44±0.08 °C, 42.9±0.03 °C, and 10.67±0.22 N, respectively. The emulsion capacity and stability of the product were also found to be satisfactory at 85.2±6.93% and 88.7±5.00%. Meanwhile, the physical properties of MDAG, SMP, MP, hardness, and emulsion capacity and stability did not significantly differ during the 60-minute process. This indicates that glycerolysis-interesterification reaction in continuous synthesis of MDAG using HS-CSTR occurs consistently, and the quality of the resulting product does not fluctuate. The use of HS-CSTR in the synthesis of MDAG is a more efficient method than the batch process and can produce a product with a nearly equivalent MDAG composition. However, further studies are needed to investigate the use of lower flow rates in glycerolysis reaction to synthesize MDAG as a structured lipid through an HS-CSTR.

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CONFLICT OF INTEREST

The study is not related to any party.

REFERENCES

Arum, A. P., Hidayat, C. & Supriyanto. (2019). Synthesis of Emulsifier from Refined Bleached Deodorized Palm Stearin by Chemical Glycerolysis in Stirred Tank Reactor. *UGM Annual Scientific Conference Life Sciences, KnE Life Sciences,* 130–148. http://doi.org/10.18502/kls.v4i11.3859

Bôas, R. N. V., Lima, R., Silva, M. V. C., Freitas, L., Aguiar, L. G., & de Castro, H. F. (2021). Continuous production of monoacylglycerol via glycerolysis of babassu oil by immobilized Burkholderia cepacia lipase in a packed bed reactor. *Bioprocess and Biosystems Engineering*, *44*, 2205–2215.

DeGarmo, E. P., Sullivan, W. G. & Canada, C. R. (1984). Engineering Economy. 7th edition *Mc Millan Publ. Co*. New York.

Devi, A. & Khatkar, B. S. (2017). Thermo-Physical Properties of Fats and Oils. *International Journal of Engineering and Technical Research (IJETR)*, *7*(2), 45–50.

Gole, V. L. & Gogate, P. R. (2014). Intensification of glycerolysis reaction of higher free fatty acid containing sustainable feedstock using microwave irradiation. *Fuel Processing Technology*, *118*, 110–116.

Hasibuan, S. & Buana, U. M. (2013). Karakteristik fisikokimia dan antibakteri hasil purifikasi minyak biji nyamplung (*Calophyllum inophyllum L.*). *Agritech*, *33*(3), 311–319. https://doi.org/10.22146/agritech.9553

Hobuss, C. B., Da Silva, F. A., Dos Santos, M. A. Z., De Pereira, C. M. P., Schulz, G. A. S. & Bianchini, D. (2020). Synthesis and characterization of monoacylglycerols through glycerolysis of ethyl esters derived from linseed oil by green processes. *RSC Advances*, *10*(4), 2327–2336. https://doi.org/10.1039/c9ra07834g

Jin, Y., Yuan, Y., Gao, L., Sun, R., Chen, L., Li, D. & Zheng, Y. (2017). Characterization and functional analysis of a type 2 diacylglycerol acyltransferase (DGAT2) gene from oil palm (*Elaeis guineensis Jacq.*) mesocarp in *Saccharomyces cerevisiae* and transgenic *Arabidopsis thaliana*. *Frontiers in Plant Science*, *8*, 1–10. https://doi.org/10.3389/fpls.2017.01791

Kadivar, S., Clercq, N., Dewettinck, K., & Mokbul, M. (2016). Influence of enzymatically produced sunflower oil based cocoa butter equivalents on the phase behavior of cocoa butter and quality of dark chocolate. *LWT-Food Science and Technology*, *66*, 48–55.

Kim, B. H. & Akoh, C. C. (2015). Recent research trends on the enzymatic synthesis of structured lipids. *Journal of Food Science*, *80*(8), 1713–1724.

Mamuaja, C. F. (2017). *Lipida*. *Unsrat Press*, *1*(1), 1–119.

Mándity, I. M., Otvos, S. B., & Fullop, F. (2015). Strategic application of residence-time control in continuous-flow reactors. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. *Chemistry Open*, *4*, 212–223.

Norazlina, M. R., Jahurul, M. H. A, Hasmadi, M., Mansoor, A. H., Norliza, J., Patricia, M., Ramlah, G. M. R., Noorakmar, A. W., Lee, J. S., Fan, H. Y. (2021). Trends in blending vegetable fats and oils for cocoa butter alternative application: A review. *Trends in Food Science & Technology*, *116*, 102–114.

Satriana, Arpi, N., Lubis, Y. M., Adisalamun, Supardan, M. D., & Mustapha, W. A. W. (2016). Diacylglycerol-enriched oil production using chemical glycerolysis. *European Journal of Lipid Science and Technology*, *118*(12), 1880–1890. https://doi.org/10.1002/ejlt.201500489

Subroto, E., Wisamputri, M. F., Supriyanto, Utami, T., & Hidayat, C. (2020). Enzymatic and chemical synthesis of high mono and diacylglycerol from palm stearin and

olein blend at different types of reactor stirrers. *Journal of the Saudi Society of Agricultural Sciences*, *19*(1), 31–36. http://doi.org/10.1016/j.jssas.2018.05.003

Subroto, E., Supriyanto, Utami, T., & Hidayat, C. (2019). Enzymatic glycerolysis–interesterification of palm stearin–olein blend for synthesis structured lipid containing high mono- and diacylglycerol. *Food Science Biotechnology*, *28*(2), 511–517. https://doi.org/10.1007/s10068-018-0462-6

Tamminen, J. & Koiranen, T. (2015). Mixing performance comparison of milliscale continuous high-shear mixers. *The Canadian Journal of Chemical Engineering*, *93*, 2245–2252. https://doi.org/10.1002/cjce.22327

Wangi, I. P., Supriyanto, S., Sulistyo, H., & Hidayat, C. (2022). Sodium silicate catalyst for synthesis monoacylglycerol and diacylglycerol-rich structured lipids: product characteristic and glycerolysis–interesterification kinetics. *Bulletin of Chemical Reaction Engineering & Catalysis*, *17*(2), 250–262. https://doi.org/10.9767/bcrec.17.2.13306.250-262

Zhang, Z., Ma, X., Huang, H., Li, G., & Wang, Y. (2017). Enzymatic production of highly unsaturated monoacyglycerols and diacylglycerols and their emulsifying effects on the storage stability of a palm oil based shortening system. *Journal of the American Oil and Chemists Society*, *94*(9), 1175–1188. https://doi.org/10.1007/s11746- 017-3023-x