

OPTIMISATION OF MULTI-STAGE SILICA-ASSISTED LIQUID-LIQUID EXTRACTION OF PALM STEARIN-BASED EMULSIFIERS AND THEIR PREDICTIVE APPLICATIONS IN FOOD PRODUCTS

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ABSTRACT

Fractionation can be used to enhance the use of palm stearin-based emulsifiers (MDAG) to produce fractions rich in monoacylglycerol (MAG). The emulsifying properties of MAG with a high purity are known to be better than those of its mixture. MAG is an excellent emulsifier in bread, cakes, dairy products, mayonnaise and vegetable oil-based products. Multi-stage silica-assisted liquid-liquid extraction, which is simpler than molecular distillation, has potential as an alternative technology to replace the molecular distillation currently used for MDAG fractionation. This method integrates separation mechanisms based on solubility differences and surface adsorption. Multi-stage silica-assisted liquid-liquid extraction was systematically studied using response surface methodology (RSM). Three parameters including temperature (30°C–50°C), extraction time (15–45 min) and MDAG concentration (30%–50%) were optimised to produce a MAG-rich fraction. Optimal extraction conditions were obtained at a temperature of $39.0 \pm 1.0^\circ\text{C}$, extraction time of 30.0 ± 1.0 min and MDAG concentration of $38.0 \pm 1.0\%$. The optimum extraction parameters are closely related to the melting point and solubility of the dominant compounds in MDAG. Predicted applications in a number of appropriate food products are presented and discussed based on the fatty acid composition and thermal characteristics of each fraction.

Keywords: mono-diacylglycerols, multi-staged silica-assisted liquid-liquid extraction, optimisation.

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INTRODUCTION

Mono-diacylglycerol (MDAG) is an emulsifier that is currently widely used in the food industry (Sagalowicz et al., 2006). Currently, production of mono- and diacylglycerol is mostly achieved by transesterification (glycerolysis) of fats or oils (triacylglycerols [TAG]) with glycerols (Zhong et al., 2014). MDAG based on palm stearin has not been widely developed. Palm stearin contains high amounts of saturated (palmitic) and unsaturated (oleic) fatty acids. The MDAG produced will contain monoacylglycerol (MAG) and diacylglycerol (DAG) of saturated and unsaturated fatty acid composition similar to the feedstock (Nadjamoeddin et al., 2024a). The MDAG produced will have different

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characteristics from the MDAG that is already on the market, which generally contains only saturated fatty acids in stearic and palmitic acid composition.

MDAG is generally a mixture of MAG, DAG and TAG. MAGs have properties that are different from their mixture in MDAG. The emulsifying properties of MAG with a high purity are known to be better than those of its mixture. MAG can disperse in water above the Krafft temperature to form a mesomorphic liquid crystalline phase. This is very useful in food applications where emulsifiers can be added to the aqueous phase to interact with carbohydrates or proteins (Faergemand & Krog, 2003). MAG is commonly used as an emulsifier in bread, cakes, dairy products, mayonnaise and vegetable oil-based products. Meanwhile, DAG also have wide applications especially in the food industry. DAG can be used as a crystallisation modifier emulsifier, fat substitute in meat products and as a cooking oil (Lee et al., 2020). Significant value can be added to MDAG products by fractionating them to produce MAG-rich fractions, DAG-rich fractions and MAG-DAG-rich fractions as new types of emulsifiers. These new emulsifiers are expected to have unique emulsification, stability and performance characteristics. They do not need to be combined with other types of emulsifiers to achieve better performance (McClements & Jafari, 2018).

Several technologies have been developed to separate MDAG into MAG and DAG. Fractionation and purification of MAGs products from palm-based MDAG have been reported extensively using various techniques. Some techniques that have been developed for the fractionation and purification of MAG were using high-pressure molecular distillation (Faergemand & Krog, 2003; Fregolente et al., 2010; Yeoh et al., 2014), supercritical fluid fractionation (King, 2002), supercritical CO₂ (Sahle-Demessie, 1997) and short path distillation with yield up to 90%–95% (Fregolente et al., 2010; Yeoh et al., 2014). However, some of these techniques, such as chromatography and supercritical fluid extraction, are expensive and difficult to scale up. Molecular distillation was the common technique used to separate MAG from DAG and TAG (Feltes et al., 2013; Hobuss et al., 2020). This process is very energy consuming and leads to significant degradation of labile unsaturated fatty acids.

Currently, MDAG fractionation studies using simple solvent extraction are limited. A simulation design performed by Sánchez et al. (2018) predicted that extraction with a dilute ethanol solution (65% ethanol + 35% water) to purify MAGs is expected to produce MAG with 90% purity and 70% yield. The MDAG fractionation technique using multi-stage silica-assisted liquid-liquid extraction has been

introduced in previous studies (Nadjamoeddin et al., 2024a). Using silica as a solid support increases the separation efficiency of MAG and DAG. Silica gel has hydroxyl groups on its surface, enabling it to attract MAG and separate it from TAG and DAG. Silica gel has previously been used as a support agent for the purification of MAG from cooking oil using column chromatography with silica gel as the stationary phase (González-Fernández et al., 2017). This method can produce MAG with a purity above 90% (González-Fernández et al., 2017). Other studies have demonstrated the impact of solid media, such as silica gel, on the MAG purification process via flash chromatography (Compton et al., 2013).

This study aims to optimise the parameters of multiple-staged silica-assisted liquid-liquid extraction using response surface methodology (RSM) with a Box-Behnken design (BBD) as the experimental design. The optimised parameters can provide useful information for industry in applying multiple-staged silica-assisted liquid-liquid extraction techniques as a simple alternative technology for MDAG purification. The use of RSM decreases the number of experiments involved to produce statistically significant data. The effect of extraction temperature, extraction time, MDAGs concentration and their interactive effects on the extract yield was studied and discussed. The extract yield obtained at various conditions and the optimum condition were characterised by gas chromatography (GC) compared with the raw MDAG (palm stearin based MDAG), reported and discussed. The scheme of multiple stage silica assisted liquid-liquid extractor is proposed in this article to provide an overview of the extraction process on a larger scale. The extracted fractions were then characterised and predicted for use in appropriate food products.

MATERIALS AND METHODS

Synthesis and Fractionation of MDAGs

Refined bleached deodorised palm stearin (RBDPS) as raw materials was obtained from PT Smart Tbk., Jakarta, Indonesia. Laboratory-scale synthesis of MDAGs according to the method from Triana et al. (2014). Acylglycerols profile and fatty acids components of MDAG and its fraction are determined using GC-flame ionisation detector (GC-FID) (Hewlett Packard 6890 with DB5 HT column, Agilent Technologies, CA, USA). The thermal characteristics of the MDAG and its fraction were determined using differential scanning calorimetry (DSC) (Shimadzu DSC-60, TA-60WS, Shimadzu Corp., Kyoto, Japan).

The extraction of MDAG was carried out in a glass bottle with a lid. In each experiment, approximately 20.0 g of MDAG were heated at 60°C–70°C. After all the MDAG had melted, a selected volume of solvent was added and stirred until homogeneous. Then 10.0 g of silica was added and stirred until homogeneous. The extraction flask was then placed in a water bath heated to the extraction temperature. Extraction was allowed to proceed for 15, 30 and 45 min. The silica and solution were then separated using a vacuum filter. The silica was re-extracted with another solvent at the specified temperature and time. The order of solvents used was chosen based on a previous study (Nadjamoeddin et al., 2024b). The solvent used in Stage 1 was hexane, the solvent used in Stage 2 was hexane:ethanol (8:2), the solvent used in Stage 3 was hexane:ethanol (4:6) and the final step to ensure that all MDAG were removed from the silica was ethanol. A schematic diagram of the experimental setup is shown in *Figure 1*.

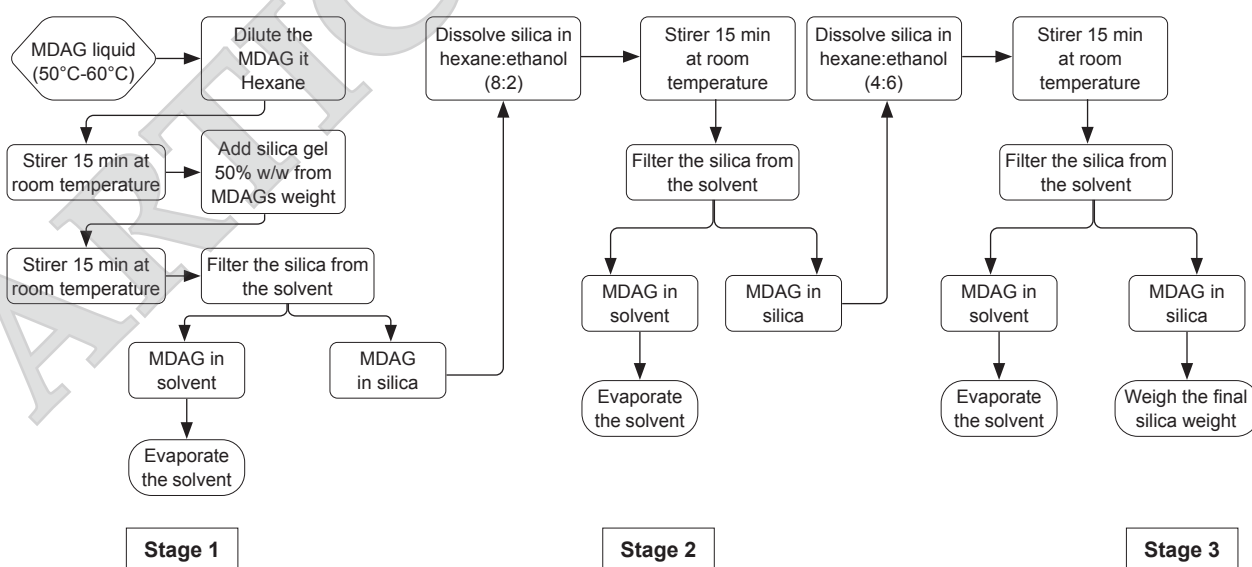
Characterisation of MDAG and Fractionation Results

Fatty acid composition of fatty acid methyl esters (FAME) was prepared as described by the American Oil Chemists' Society (AOCS) method Ce 2-66 (AOCS, 1996b). The AOCS method Ce 1e-91 (AOCS, 1996a) was followed to identify and quantify FAME by GC. GC (Shimadzu 2100, Shimadzu Corp., Kyoto, Japan) equipped with a C-18 Rtx-5 capillary chromatography column (30 m, inner diameter 0.25 mm and film thickness 0.25 µm) was used. A flame ionisation detector (FID) was used to detect the FAME. GC was

performed isothermally at 198°C, with a split ratio of 1:40, an injector temperature of 240°C and a detector temperature of 280°C.

The determination of MDAG glyceride composition by GC follows AOCS Official Method Cd 11b-91 (AOCS, 2003a) with modifications. A sample of 0.025 was placed in a test tube, then 10 µL of THF and 50 µL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide were added. Subsequently, the reaction tubes were sealed and the mixture was vortexed at 2,400 rpm for 90 s. The tubes were then placed in a dark room for 10 min. Next, 2 mL of heptane is added, stirred (vortex) again at a speed of 2,000 rpm, for 30 s. The outside of the tube is coated with parafilm. The sample is stored for 30 min before being injected. GC-FID (Shimadzu 2100) analysis conditions: Analysis was conducted using a 30 m DB-5HT column. The initial column temperature was 50°C which was gradually increased to 180°C at a rate of 15°C/min, then increased at a rate of 7°C/min to 230°C and finally to 380°C. The detector temperature and injector temperature were 380°C and 390°C respectively. The flow velocity of the carrier gas was 0.7 mL N₂/min, airflow velocity 450 mL/min and injection volume 1 µL. The calculation was carried out using the percentage method of area composition.

Thermal property characterisation was carried out using DSC (Shimadzu DSC-60) based on AOCS Official Method Cj 1-94 (AOCS, 2003b) with modifications. A total of 5 mg of the sample was placed in a hermetically sealed aluminium container. An empty hermetically sealed aluminium container was used as a comparison. DSC (Shimadzu DSC-60) analysis conditions: Thermal analysis of the



Source: Nadjamoeddin et al. (2024a).

Figure 1. Flowchart of multiple-stage silica-assisted liquid-liquid extraction.

sample was carried out at temperatures ranging from -10°C – 80°C . The exothermic (crystallisation) curve was obtained by heating the test sample at 80°C for 5 min, followed by cooling to -10°C at a cooling rate of $5^{\circ}\text{C}/\text{min}$. The endothermic (melting) curve of the test sample was obtained by heating the sample at -10°C for 5 min, then heating it to 80°C at a heating rate of $5^{\circ}\text{C}/\text{min}$. Based on this analysis method, the crystallisation and melting curves of the sample are obtained to determine the crystallisation temperature (T_c), melting temperature (T_m) and enthalpy value (ΔH).

The determination of solid fat content (SFC) from samples was carried out using the integration method on the area of the DSC curve/unit of time. This method uses the proportion of remaining melting energy compared to the total melting energy, which directly represents the solid fraction at temperature (Márquez et al., 2013). Then multiplied by 100% to obtain the percentage. The SFC value was determined by the following Equation (1):

$$\text{SFC (\%)} = \frac{\int_T^{T_f} E (dT)}{\int_{T_0}^{T_f} E (dT)} \quad (1)$$

where, T_0 is the initial melting temperature (onset of the melting peak on the DSC curve) ($^{\circ}\text{C}$), T is the desired temperature for determining the SFC ($^{\circ}\text{C}$), T_f is the final melting temperature (end of melting peak) ($^{\circ}\text{C}$). $E (dT)$ is the DSC signal related to heat flow rate (e.g., mW/mg) as a function of temperature.

Design of Experiment, Data Analysis and Model Fitting

This study used a BBD to systematically investigate the effect of three variables and their interactions on MAG extract yield using Minitab 14. The investigated range for temperature (X_1), extraction time (X_2) and MDAGs concentration (ratio of sample MDAG and solvent used) (X_3) were 30°C – 40°C , 15–45 min and 30–50 wt%, respectively.

Determination of X_1 was conducted above room temperature and below the boiling point of hexane. Determination of X_2 referred to the previous study (Hasibuan et al., 2021) and the preliminary study conducted by the research team. Determination of the X_3 referred to the MDAG solubility in several solvent compositions of hexane:ethanol, in which the maximum solubility obtained was around 26% at room temperature (based on a previous study). The solubility may increase with the increase in temperature used during extraction. Moreover, efficiency and applicability in industry also became the factors being considered for selecting the extraction capacity, time and temperature used.

Numerical optimisation was performed on the parameters of X_1 , X_2 and X_3 . The optimisation results based on the RSM model were then verified experimentally in the laboratory for five repetitions.

RESULTS AND DISCUSSION

Synthesis and Fractionation of MDAGs

The composition of MAG and DAG in MDAGs is $51.11 \pm 3.46\%$ and $34.86 \pm 0.10\%$, respectively. This result is consistent with a previous study which showed that MAG content of $46.68 \pm 0.26\%$ and DAG content of $32.57 \pm 0.82\%$ were observed in MDAG (Faridah et al., 2020). Palmitic acid (56.01%) and oleic acid (30.82%) are the dominant components in MDAG.

The characteristics of MDAG such as acylglycerol composition, fatty acid type and composition have a great influence on the fractionation process and the optimisation of the fractionation. The solubility of MDAG in the fractionation process depends on the composition of MAG and DAG, especially the number of available free hydroxyl groups and the hydrophobic interactions caused by the carbon chains in the fatty acids. In addition, the composition and type of dominant fatty acid indirectly influence the melting point of the compound. Melting point strongly influences the solubility of a compound in a particular solvent (Blanco & Blanco, 2017; Ran et al., 2001).

Silica gel was added to the fractionation to increase the effectiveness of adsorption and separation of compounds with hydroxyl groups from the mixture. It was expected that the MAG would adsorb onto the silica, leaving the DAG and TAG in solution. This is because silica gel has a greater number of OH groups on its surface, which can interact with compounds that have different OH groups. The illustration of silica gel's interaction with MAG and DAG is shown in Figure 2. Previous studies have shown that the effectiveness of silica gel in absorbing monoglycerides is very efficient and reliable (Mazzieri et al., 2008).

The multiple-stage silica-assisted liquid-liquid extraction is a combination of two mechanisms that is adsorption of MDAG by silica and solubilisation of MDAG by the polarity value of the appropriate solvent. Both mechanisms work simultaneously and are effective to separate MAG and DAG in MDAG. Many studies have shown that separation mechanisms which combine adsorption and partitioning are effective in macromolecular separations such as column chromatography (Coskun, 2016).

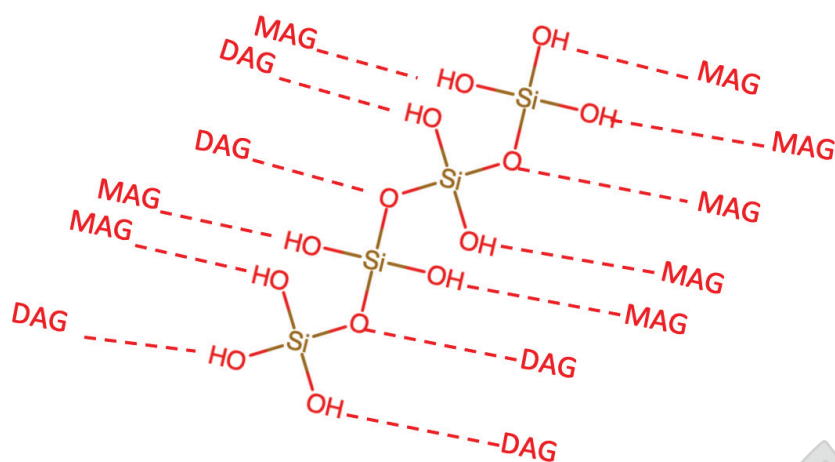


Figure 2. Illustration of silica gel's interaction with MAG and DAG.

The polarity of the solvent has a significant influence on the separation process in multiple-stage silica-assisted liquid-liquid extraction than the involvement of silica. In Stage 1, hexane can dissolve more DAG than MAG, while in Stage 2, hexane:ethanol (8:2) can dissolve more MAG than DAG. DAG is more soluble in a less polar solvent (hexane), while MAG is more soluble in a more polar solvent [hexane:ethanol (8:2)]. A similar separation principle is also used in the AOCS method (Cd 11c-93, reapproved in 2009) in the quantitative separation of monoglycerides, diglycerides and triglycerides using silica gel column chromatography. The method used the binary solvent petroleum ether:diethyl ether (9:1) for the separation of TAG, the binary solvent petroleum ether:diethyl ether (7.5:2.5) for the separation of DAG and the solvent diethyl ether for the separation of MAG. This method demonstrates that MAG and DAG can be efficiently separated with an increase in solvent polarity.

Linear Regression Models and Validation of the Models

The process parameters optimised in this study include temperature, extraction time and MDAG's concentration. Temperature, time and solvent concentration were critical to the success of the high purity lignin fractionation process from lignocellulosic biomass (Cheiwanich et al., 2017). To optimise the extraction of oil from *Calophyllum inophyllum*, Fuad et al. (2016) investigated the effects of extraction time, ultrasonic power, extraction temperature and liquid/solid (L/S) ratio on oil yield. Ogbeide et al. (2018) studied the interaction effect of process parameters agitation time, solvent volume and particle size on oil yield to optimise the extraction process conditions of gmelina seed oil. Oniya et al. (2017) also used the seed/solvent ratio, extraction temperature and extraction time

to optimise the solvent extraction of oil from grains in a sand box. Results from the experimental design using RSM showed a relationship between temperature, extraction time and extraction capacity.

The coefficient of determination (R^2 and R^2 adj) result show that the prediction model of MDAG composition percentage has R^2 values range between 77.62% and 99.68% and R^2 adj values varies between 37.33% and 99.10%. Based on the result of this study, the MAG_3 and DAG_3 gave an R^2 value that is close to 1, but MAG_1 , DAG_1 , MAG_2 and DAG_2 models show lower R^2 values. The low R^2 value indicates that other factors affect the achievement of the response variable, which is also evidenced by the p -values for each independent variable in the insignificant MAG_1 and DAG_1 models.

Numerical Process Optimisation

Anuar et al. (2013) suggested that the numerical optimisation required the targets to be defined for the independent variables and responses in which all targets were combined into one desired function. In this study, the independent variables of (i) temperature (30°C–50°C), (ii) extraction time (15–45 min) and (iii) MDAG's concentration (30–50 wt%) were used.

Response optimisation was performed for the MAG_3 model as the highest goodness-of-fit valued model. Determination of response optimisation is shown in Figure 3a. A calculation using response optimiser shows that in the value of $X_1 = 39.29^\circ\text{C}$, $X_2 = 30.15$ min and $X_3 = 38.10\%$, the expected target of 89.72% MAG purity is achieved. Figure 3b-3c shows the contour plots for the MAG models. Based on the graph, at 40.00% weight, a temperature range of 35°C–45°C and a reaction time of 25–35 min, the production of MAG with a composition above 87.50% is achievable.

TABLE 1. RESPONSE VARIABLE COEFFICIENTS AND VALIDATION OF THE MODELS

Term	Response variable coefficients					
	MAG ₁	DAG ₁	MAG ₂	DAG ₂	MAG ₃	DAG ₃
Constant Linear	179.9	-79.9	-33.33	133.33	-78.3	175.7
X ₁	-2.5	2.5	-0.55*	0.55*	2.62	-2.52
X ₂	-0.558	0.558	2.514	-2.514	2.242*	-2.213
X ₃	-4.16	4.16	4.34	-4.34	4.35*	-4.41
Quadratic						
X ₁ *X ₁	0.0284	-0.0284	0.0066	-0.0066	-0.0384*	0.0384*
X ₂ *X ₂	0.013	-0.013	-0.0379*	0.0379*	-0.0453*	0.0453*
X ₃ *X ₃	0.0344	-0.0344	-0.0483*	0.0483*	-0.0707*	0.0707*
Interaction						
X ₁ *X ₂	0.0218	-0.0218	0.0032	-0.0032	-0.0037	0.0037
X ₁ *X ₃	0.0247	-0.0247	-0.009	0.009	0.0131*	-0.0131
X ₂ *X ₃	0.0178	-0.0178	-0.0076	0.0076	0.01687*	-0.01687
R ²	77.62	77.62	87.97	87.97	99.68	93.75
R ² adj	37.33	37.33	66.33	66.33	99.10	82.51
Lack of fit	20.33	20.33	19.76	19.76	0.70	14.26
Model					*	*

Note: MAG₁ and DAG₁ were the fractionation results in Stage 1 using hexane single solvent. MAG₂ and DAG₂ were the fractionation results in Stage 2 using hexane:ethanol (8:2) binary solvent. MAG₃ and DAG₃ were the fractionation results in Stage 3 using hexane:ethanol (4:6) binary solvent. * - was significant in the confidence range of 95%.

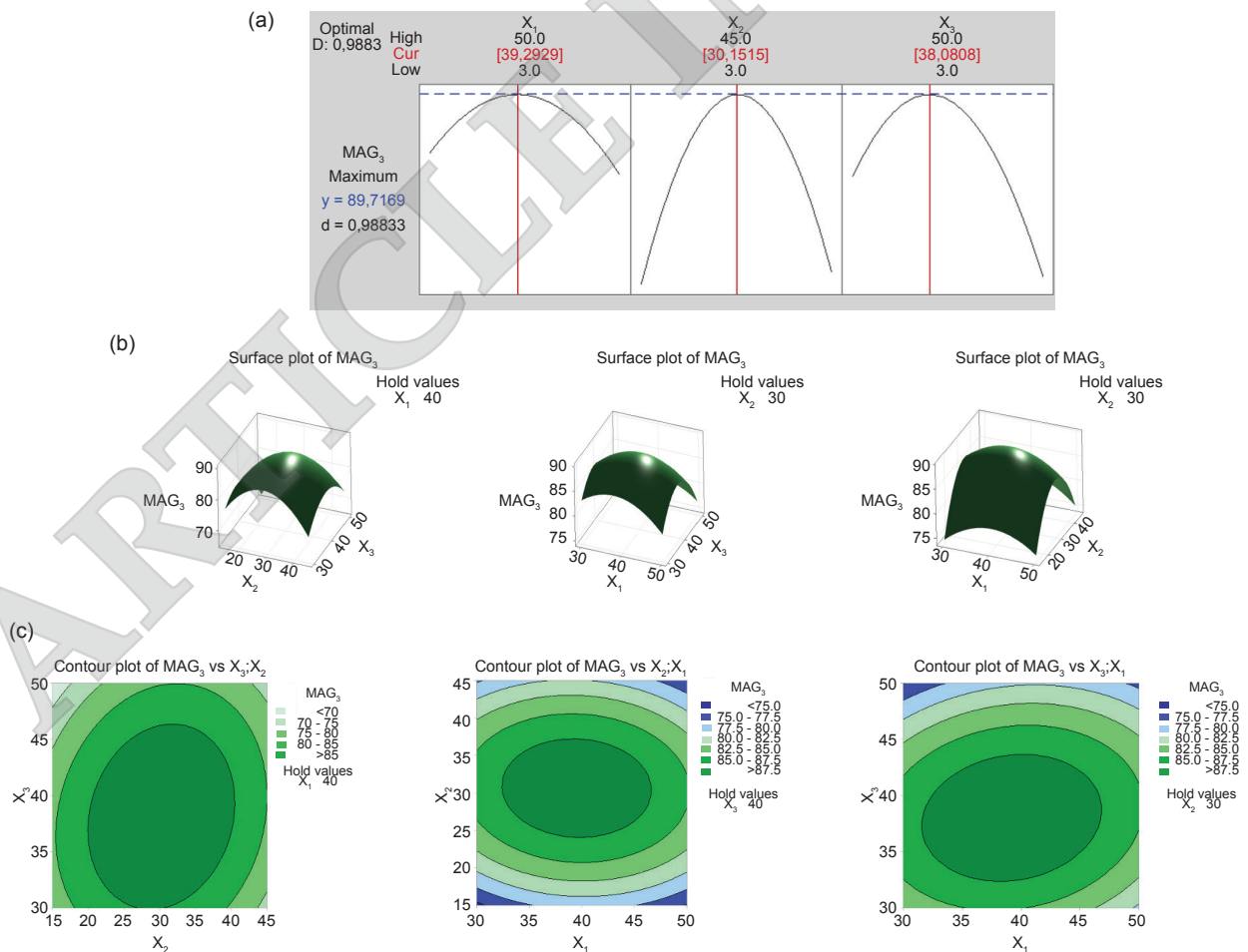


Figure 3. Response optimisation of fractionation MDAG.

The experimental results in *Table 2* show that the extraction at Stage 3 yields a MAG composition of $89.86 \pm 3.91\%$. The chromatogram of MDAG, DAG-rich fraction, MAG-DAG fraction and MAG-rich fraction is shown on *Figure 4*. The verification results do not differ significantly from the predicted values generated by the RSM model, indicating that the model accurately represents the experimental system and reliably predicts the response under the given conditions.

The optimisation process has some drawbacks regarding the yield obtained at each stage (*Table 2*). However, based on the yield data, it is clear that the largest mass fraction of approximately 50 wt% was produced in Stage 1 (hexane), followed by approximately 30 wt% in Stage 2 [hexane:ethanol (8:2)], approximately 12 wt% in Stage 3 [hexane:ethanol (4:6)]. Therefore, further investigation of the optimisation parameters is necessary to achieve the optimal yield in Stage 3.

TABLE 2. THE EXPERIMENTAL EXTRACT RESULTS IN RSM VERIFICATION PROCESS

Fraction	Yield (wt%)	Average composition percentage (%)	
		MAG	DAG
Stage 1: (hexane)	56.12 ± 3.58	43.71 ± 1.56	56.29 ± 1.57
Stage 2: [hexane:ethanol (8:2)]	31.18 ± 3.70	73.15 ± 1.75	26.85 ± 1.74
Stage 3: [hexane:ethanol (4:6)]	12.70 ± 1.94	89.86 ± 3.91	10.14 ± 3.90

Note: MAG - monoacylglycerol; DAG - diacylglycerol

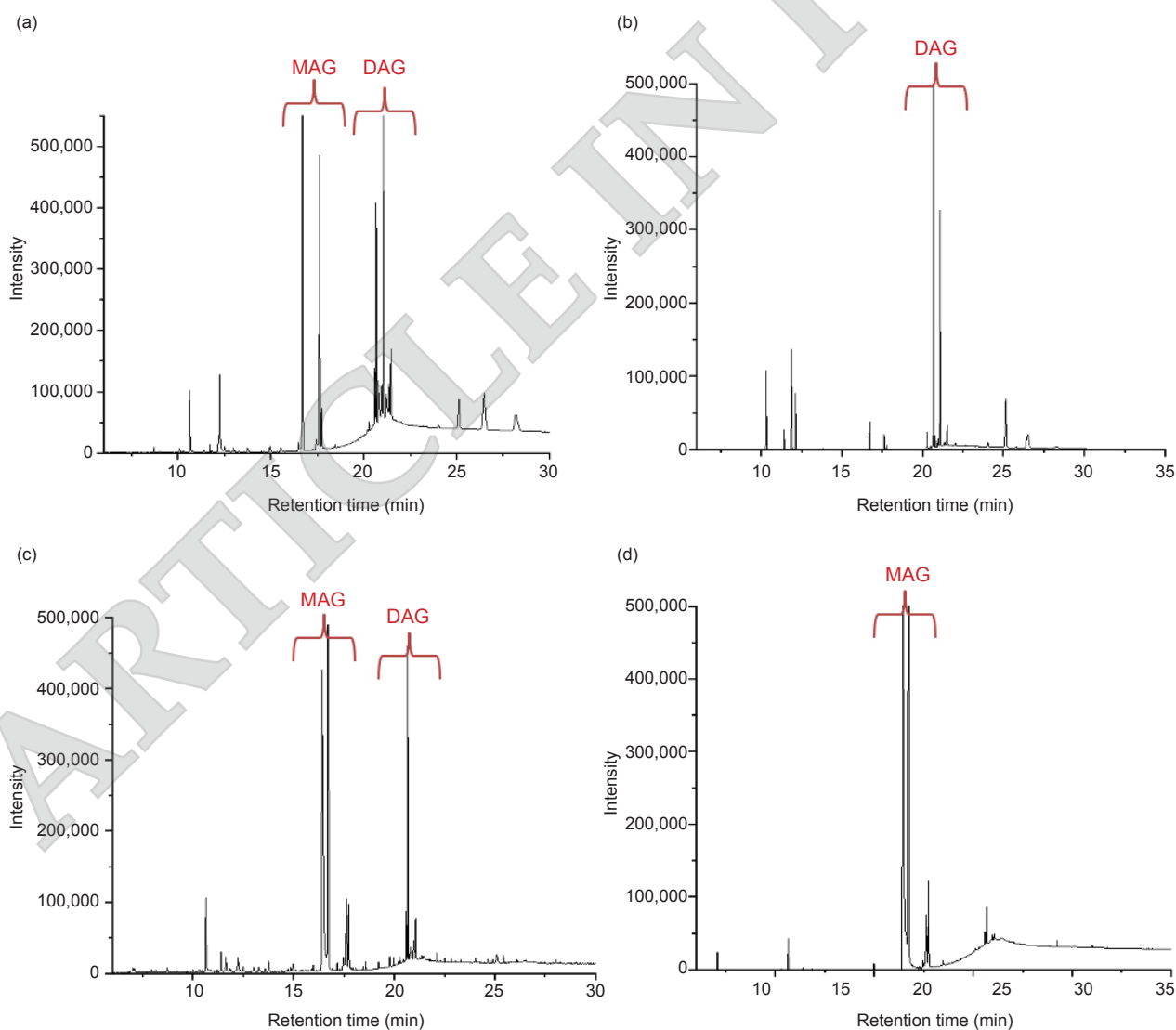


Figure 4. Chromatogram of (a) MDAG, (b) DAG-rich fraction, (c) MAG-DAG fraction and (d) MAG-rich fraction.

Characteristics of MDAG Fractions

Multi-stage silica-assisted liquid-liquid extraction for MDAG produced a DAG-rich fraction, a MAG-DAG fraction and a MAG-rich fraction (Figure 5). The three MDAG fractions obtained from the three extraction stages have been found to have different shapes and textures. Stage 1 of extraction using hexane as a solvent results in a semi-solid, oily fraction that has a soft texture and pale yellow colour. The fraction obtained from Stage 2 of the extraction using hexane:ethanol (80:20) solvent has a semi-solid form with a harder texture and is white in colour. The fraction obtained from Stage 3 of the extraction using a hexane:ethanol (40:60) solvent is a white, powdery solid.

The Stage 1 fraction is a non-polar fraction that has been separated from MDAG-RBDPS. It is dominated by DAG and MAG, and its composition is palmitic acid and oleic acid. The Stage 1 fraction is called the DAG-rich fraction because the chemical composition of DAG is greater than that of MAG. Stage 2 extraction produces an MDAG fraction with a higher composition of MAG than DAG, and a higher composition of palmitic acid than oleic acid. The second fraction has a more solid texture than the previous fraction. This is due to the higher amount of MAG with a high saturated fatty acid composition. This solid texture is due to the compact arrangement of saturated fatty acid chains, which are accompanied by hydrogen bonds formed between the MAG molecules. The fraction obtained from Stage 2 is then referred to as the MAG-DAG fraction. The same phenomenon occurs in the fraction obtained from Stage 3. This fraction is dominated by MAG containing palmitic acid, giving it a denser texture than the previous fraction. This fraction is then called the MAG-rich fraction. The complete characteristics of each MDAG fraction, synthesised MDAG and glycerol monostearate (GMS) as a reference are

shown in Table 3. These characteristics include the glycerol acyl profile, fatty acid composition, melting point, crystallisation point and SFC value obtained from the DSC curve. The DSC curve used to determine the SFC value is presented in Figure 6.

The DAG-rich fraction with almost 60% DAG composition, exhibits the highest yield of 56.29 ± 1.57 wt%. The fatty acid composition in Table 3 shows that the DAG-rich fraction is dominated by palmitic acid and oleic acid in the largest amounts compared to the other two fractions. This composition was not found in Stage 2 or Stage 3. This indicates that oleic acid-rich MAG-DAG was obtained in Stage 1 extraction. The oleic acid-rich MAG-DAG is more concentrated in Stage 1, which is not well known at present and requires further study. The DAG-rich fraction has a melting point of $50.20 \pm 0.32^\circ\text{C}$ and a crystallisation point of $32.72 \pm 0.23^\circ\text{C}$. Based on the SFC data obtained, storage at 10.00°C , storage and distribution at 20.00°C and at a simulated oral cavity temperature of 35.00°C , the DAG-rich fraction will be stable in solid form.

The application of DAG-rich fraction can be an alternative to DAG oil. DAG oil has been recognised as a fat substitute for conventional vegetable oils and fats (Yeoh et al., 2014). DAG can be used as an emulsifier in oil-in-water emulsions (mayonnaise, ice cream), in water-in-oil emulsions (butter, margarine), as a crystallisation modifier, as a fat substitute in meat products (sausages) and as a cooking oil (Lee et al., 2020). DAG show advantages mainly in terms of health-beneficial properties. DAG has been found to have positive effects on human health as an anti-obesity agent by being able to reduce postprandial fatty acid levels and lower total cholesterol and LDL cholesterol in serum, liver and adipose tissue and suppress fat accumulation (Lai, 2011). DAG also shows potential in improving cardiovascular health and preventing systemic inflammatory diseases (Wang et al., 2016).

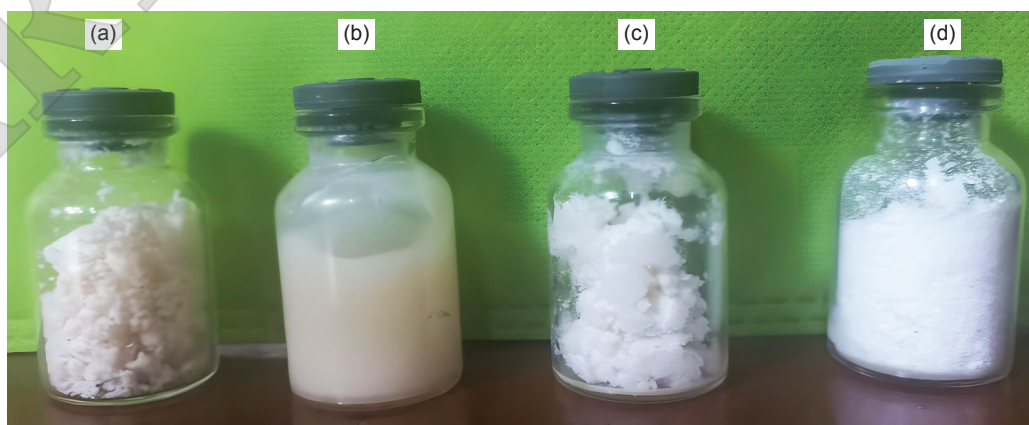


Figure 5. Fractionation results from (a) MDAG-RBDPS, (b) DAG-rich fraction, (c) MAG-DAG fraction and (d) MAG-rich fraction.

TABLE 3. CHEMICAL AND THERMAL CHARACTERISTICS OF MDAG FRACTIONS

No	Compound	MDAG	DAG-rich fraction	MAG-DAG fraction	MAG-rich fraction	GMS (reference)	Unit
1	MAG	51.11 ± 3.46	43.71 ± 1.56	73.15 ± 1.75	89.86 ± 3.91	98.88	%
2	DAG	34.86 ± 0.09	56.29 ± 1.57	26.85 ± 1.74	10.14 ± 3.90	0.65	%
1	C14:0	0.76 ± 0.17	1.06 ± 0.03	1.07 ± 0.03	0.87 ± 0.01	0.83	%
2	C16:0	55.69 ± 0.45	55.45 ± 0.76	66.60 ± 4.39	83.05 ± 3.83	44.63	%
3	C18:0	5.17 ± 0.49	4.87 ± 0.02	5.41 ± 0.07	5.67 ± 0.46	54.54	%
4	C18:1n9c	32.03 ± 1.72	33.90 ± 0.76	25.12 ± 3.95	9.26 ± 3.39	0.00	%
5	C18:2n6c	5.06 ± 0.32	4.71 ± 0.01	1.81 ± 0.49	1.15 ± 0.90	0.00	%
1	MP	50.17 ± 0.23	50.20 ± 0.32	50.83 ± 4.43	57.37 ± 9.12	71.41	°C
2	CP	38.03 ± 0.34	32.72 ± 0.23	38.38 ± 0.58	45.09 ± 9.01	61.07	°C
3	SFC 10°C	89.19 ± 0.16	84.40 ± 0.14	87.41 ± 7.11	87.11 ± 1.42	90.66 ± 0.18	%
4	SFC 20°C	77.24 ± 0.16	67.84 ± 0.02	72.53 ± 8.59	74.38 ± 2.88	78.69 ± 0.14	%
5	SFC 35°C	39.30 ± 0.17	44.98 ± 0.80	49.16 ± 8.01	55.79 ± 5.05	66.24 ± 0.13	%

Note: MDAG - mono-diacylglycerol; DAG - diacylglycerol; MAG - monoacylglycerol; GMS - glycerolmonostearate; MP - melting point; CP - cloud point; SFC - solid fat content.

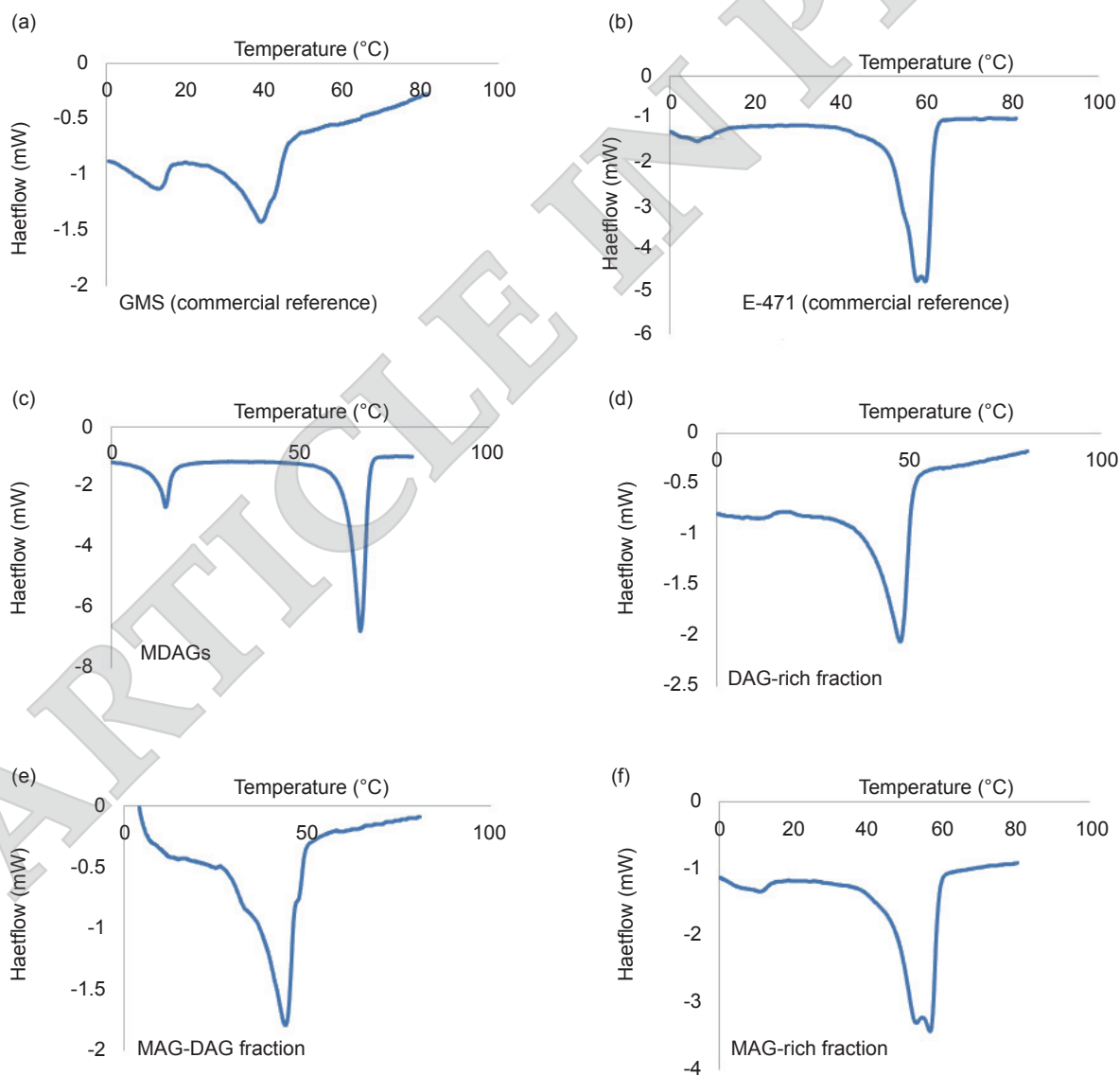


Figure 6. The DSC curve of (a) GMS, (b) E-471, (c) MDAGs, (d) DAG-rich fraction, (e) MAG-DAG fraction and (f) MAG-rich fraction.

Stage 2 extraction produced the MAG-DAG fraction, which contains a greater MAG composition than DAG. *Table 3* shows the chemical composition and thermal characteristics of the MAG-DAG fraction. The MAG-DAG fraction possesses a MAG composition of $73.15 \pm 1.75\%$ and a DAG composition of $26.85 \pm 1.74\%$, with a yield of 31.18 ± 3.70 wt%.

The MAG-DAG fraction obtained from Stage 2 extraction has an almost identical MAG and DAG composition to that of commercial MDAG. It contains >55% MAG, <45% DAG and traces of TAG, glycerol and free fatty acids (Faergemand & Krog, 2003). However, in terms of fatty acid composition, the MAG-DAG fraction is dominated by palmitic and oleic acids. This is unlike the fatty acid composition of commercial MDAG. That is dominated by palmitic and stearic acids (Faergemand & Krog, 2003). The MAG-DAG fraction will have characteristics that are more easily dispersed in water and more suitable for oil-in-water emulsions, given the larger amount of MAG compared to DAG. The MAG-DAG fraction with unsaturated fatty acids is a potent emulsifier with good aeration properties and an alternative to current commercial MDAG (Zhang et al., 2017). The MAG-DAG fraction, which has an unsaturated fatty acid composition, can significantly affect the fat polymorphism patterns of palm oil-based shortening systems when used as an emulsifier. This is achieved by maintaining the stability of the relatively soft β' form, thereby reducing the rate of fat crystallisation and inhibiting clear crystal aggregation. This maintains a stable level of hardness in palm oil-based shortening system samples, preventing an increase in hardness during storage (Zhang et al., 2017).

Stage 3 extraction produced a MAG-rich fraction. The MAG-rich fraction with high purity was the target of the optimisation process. The MAG-rich fraction had MAG and DAG compositions of 89.86 ± 3.91 and 10.14 ± 3.90 , respectively. The MAG composition of this fraction is close to the 90% composition of commercial monoglycerides, which are generally produced by molecular distillation techniques. Thus, in addition to molecular distillation, multi-stage silica-assisted liquid-liquid extraction could be an alternative MDAG fractionation technique to produce commercial monoglycerides. The separation process of multi-stage silica-assisted liquid-liquid extraction is simpler and cheaper than molecular distillation, but can achieve similar purity. This is based on the separation mechanism in the multi-stage silica-assisted liquid-liquid extraction process, which relies on dissolution in a suitable solvent and adsorption on the silica surface, while the separation mechanism in molecular distillation

is more complex. The complexity of molecular distillation is related to the principle of separating compounds based on differences in molecular weight using very low pressure (<0.01 Torr). Therefore, molecular distillation techniques require more sophisticated instruments, greater power and the assistance of trained operators. The multi-stage silica-assisted liquid-liquid extraction technique does not require specialised instruments and can be self-developed by industry. The use of silica and organic solvents in the extraction process can be made more efficient through the reuse of silica and the recovery of solvents through distillation during the evaporation of the extraction results. Reusable silica can be used because the adsorption of MAG and DAG on silica is temporary, so that during the dissolution process, MAG and DAG adsorbed on the silica surface tend to dissolve in the solvent and detach from the silica surface. However, further study is needed to determine how many times silica can be reused. Another challenge in this study is that the 3rd extraction stage, which produces the MAG-rich fraction, has a very low yield of 12.70 ± 1.94 wt%, much lower than the molecular distillation, which yields more than 90% (González-Fernández et al., 2017).

The fatty acid composition was the main difference between the MAG-rich fraction and glycerol monostearate-kosher (GMS-K). The MAG-rich fraction is dominated by palmitic acid ($83.05 \pm 3.83\%$) and contains unsaturated fatty acids (oleic and linoleic), whereas GMS-K is dominated by stearic acid (54.54%) and palmitic acid (44.63%). This difference in fatty acid composition causes the thermal properties of the MAG-rich fraction to differ from those of the GMS-K. The MAG-rich fraction has a lower melting point and crystallisation point than GMS-K. The SFC of the MAG-rich fraction is also lower than that of GMS-K at the same temperature. Different MAG fatty acid compositions, such as MAG-oleic and MAG-stearic, give different emulsion system properties (Fredrick et al., 2013). The addition of MAG-oleate to the emulsion system will reduce the stirring and shaking time and increase the firmness of the whipped cream. The addition of MAG-stearate has the opposite effect. MAG-stearate behaves as a solid at the oil-water interface whereas MAG-oleate is in a liquid state (Fredrick et al., 2013). The effect of MAG-rich fractions on emulsion system properties requires further study, but it has potential as an emulsifier with unique properties due to its fatty acid composition of saturated and unsaturated fatty acids.

RBBDPS-based MDAG fractions can be an alternative emulsifier that is healthier and more natural, has unsaturated fatty acids (oleic and linoleic acids) and does not contain hydrogenated

fatty acids. MDAG made from olive oil has been shown to provide the highest emulsion stability for mayonnaise compared to other emulsifiers (Kantekin-Erdogan et al., 2019). GMS as a monoglyceride, which is commonly used as an emulsifier, is suspected to be a potential hazard because chronic consumption of GMS can increase the safety risk of phthalate ester contaminants (PAEs) in food (Gao et al., 2016).

The MDAGs fractions provide different characteristics from the MDAG emulsifiers currently on the market, both in terms of chemical composition and thermal properties. The difference in characteristics provides the opportunity to produce emulsifiers with different properties (Figure 7). This will add to the repertoire of different types of emulsifiers for very different food products. This is based on the fact that there is no single type of emulsifier that is suitable for all types of food products. This study is limited to the characteristics of the fractions produced from multi-stage silica-assisted liquid-liquid extraction and predictions of their use. Further study is needed to examine the effectiveness of each fraction in its application to food products and explore the advantages of each fraction as an emulsifier in appropriate food products.

Effect of Parameter Extraction

The extraction temperature is closely related to the crystallisation process that first occurred in MAGs. This is evidenced by the optimum temperature obtained, which is $39.0 \pm 1.0^\circ\text{C}$. This is similar to the temperature of the first

crystallisation of MAGs in hexane solvent reported by Chetpattananondh and Tongurai (2008).

The process that can occur during the extraction using silica as a solid support is that at a temperature of $\pm 40^\circ\text{C}$, the melting process of MDAG occurs, causing MAG, DAG and TAG compounds to be in the liquid phase and able to interact with the hydroxyl group found on silica. The number of hydroxyl groups dispersed on the surface of the silica allows the MAG, which has two free hydroxyl groups, to adsorb more than the DAG, which has only one free hydroxyl group. This is shown in Stage 1 where the extraction results have a greater amount of DAG than MAG. In Stage 2 and Stage 3 the MAG can be much higher than the DAG. However, the DAG content is still present in the extraction results of Stage 2 and Stage 3. This is an indication of the presence of DAG which can be bound to the silica surface due to the presence of one free hydroxyl group on DAG. The amount of MAG that can be absorbed by silica is influenced by the extraction time. The longer the extraction time, the greater the opportunity for the MAG to interact with the hydroxyl groups on the silica. However, this interaction is limited by the amount of MAG and the surface area of the silica; once the optimum amount of MAG has been absorbed, additional time will not increase the amount absorbed.

MDAG's concentration is closely related to the amount of MDAG that can be dissolved in the solvent used. The solubility of MDAG depends on the temperature used, so the optimal concentration of MDAG is determined by the optimal temperature used in the extraction process.

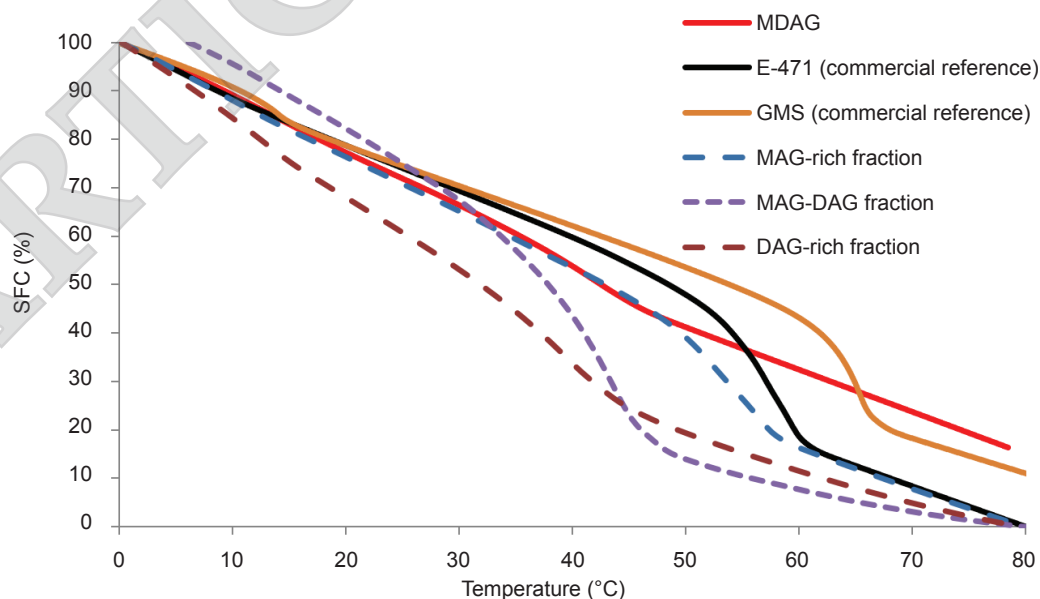


Figure 7. Differences in thermal characteristics possessed by MDAG fractions compared to commercial products.

CONCLUSION

Palm-based MDAG is a palm-derived product that has been extensively developed. Fractionating MDAG into MAG and DAG is challenging and limits product development. The response method was used to find the optimal extraction process parameters to produce MAG with a purity of 89.72%. This was verified experimentally and gave $89.86 \pm 3.91\%$. A significant model based on the results of response surface analysis was obtained for MAG_3 and DAG_3 . Response optimisation was carried out for the MAG_3 model and gave temperature response values $X_1 = 39.29^\circ\text{C}$, $X_2 = 30.15$ min and $X_3 = 38.10\%$. The optimisation results based on the model were verified externally in the laboratory, with the optimal parameters being temperature $39.0 \pm 1.0^\circ\text{C}$, extraction time 30.0 ± 1.0 min and MDAGs' concentration 38.0 ± 1.0 wt%.

This study provides new insights into extracting MAG and DAG from palm-based MDAG. The MDAG fractions differ significantly in chemical composition and thermal properties, offering distinct characteristics from existing MDAG emulsifiers. The key difference is the composition of unsaturated fatty acids, which are generally absent in commercial MDAG. This variation in properties creates an opportunity to develop novel emulsifiers for diverse food products. However, the low yield for the MAG fraction with the highest degree of purity is still a drawback. Further study is needed to explore other variables that can be developed to find the optimum conditions for separating MAG and DAG from MDAG and achieving higher purity of MAG and DAG.

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REFERENCES

- American Oil Chemists' Society (AOCS). (1996a). Official Method Ce 1e-91. Identification and quantification of fatty acid methyl esters by gas chromatography. In *Official methods and recommended practices of the AOCS*. AOCS.
- American Oil Chemists' Society (AOCS). (1996b). Official Method Ce 2-66. Preparation of fatty acid methyl esters (FAME). In *Official methods and recommended practices of the AOCS*. AOCS.
- American Oil Chemists' Society (AOCS). (2003a). Official Method Cd 11b-91. Determination of mono-, di-, and triacylglycerols by gas chromatography. In *Official methods and recommended practices of the AOCS*. AOCS.
- American Oil Chemists' Society (AOCS). (2003b). Official Method Cj 1-94. Thermal analysis by differential scanning calorimetry (DSC). In *Official methods and recommended practices of the AOCS*. AOCS.
- Anuar, N., Mohd Adnan, A. F., Saat, N., Aziz, N., & Taha, R. M. (2013). Optimization of extraction parameters by using response surface methodology, purification, and identification of anthocyanin pigments in *Melastoma malabathricum* fruit. *The Scientific World Journal*, 2013. <https://doi.org/10.1155/2013/810547>
- Blanco, A., & Blanco, G. (2017). Lipids. In *Medical biochemistry* (pp. 99–119). Elsevier. <https://doi.org/10.1016/B978-0-12-803550-4.00005-7>
- Cheiwpanich, S., Laosiripojana, N., & Champreda, V. (2017). Optimization of organosolv based fractionation process for separation of high purity lignin from bagasse. *Materials Science Forum*, 883, 92–96. <https://doi.org/10.4028/www.scientific.net/MSF.883.92>
- Chetpattananondh, P., & Tongurai, C. (2008). Synthesis of high purity monoglycerides from crude glycerol and palm stearin. *Songklanakarin Journal of Science and Technology*, 30(4), 515–521.
- Compton, D. L., Laszlo, J. A., & Evans, K. O. (2013). Influence of solid supports on acyl migration in 2-monoacylglycerols: Purification of 2-MAG via flash chromatography. *Journal of the American Oil Chemists' Society*, 90(9), 1397–1403. <https://doi.org/10.1007/s11746-013-2274-4>
- Coskun, O. (2016). Separation techniques: Chromatography. *Northern Clinics of Istanbul*, 3(2), 156–160. <https://doi.org/10.14744/nci.2016.32757>
- Faergemand, M., & Krog, N. (2003). Using emulsifiers to improve food texture. In B. M. McKenna (Ed.), *Texture in food: Volume 1: Semi-solid foods* (pp. 216–250). Woodhead Publishing Ltd. <https://doi.org/10.1533/9781855737082.2.216>
- Faridah, D. N., Sumitra, N. R., Hariyadi, P., Triana, R. N., Laksana, A. J., & Andarwulan, N. (2020). Laboratory-scale synthesis of mono-diacylglycerol from palm oil stearin using glycerolysis. In *Proceedings of the 2nd SEAFEST*

- International Seminar* (pp. 112–117). SciTePress. <https://doi.org/10.5220/0009977701120117>
- Feltes, M. M. C., de Oliveira, D., Block, J. M., & Ninow, J. L. (2013). The production, benefits, and applications of monoacylglycerols and diacylglycerols of nutritional interest. *Food and Bioprocess Technology*, 6(1), 17–35. <https://doi.org/10.1007/s11947-012-0836-3>
- Fredrick, E., Heyman, B., Moens, K., Fischer, S., Verwijlen, T., Moldenaers, P., Van der Meeren, P., & Dewettinck, K. (2013). Monoacylglycerols in dairy recombined cream: II. The effect on partial coalescence and whipping properties. *Food Research International*, 51(2), 936–945. <https://doi.org/10.1016/j.foodres.2013.02.006>
- Fregolente, P. B. L., Pinto, G. M. F., Wolf-Maciel, M. R., & Filho, R. M. I. (2010). Monoglyceride and diglyceride production through lipase-catalyzed glycerolysis and molecular distillation. *Applied Biochemistry and Biotechnology*, 160(7), 1879–1887. <https://doi.org/10.1007/s12010-009-8822-6>
- Fuad, F. M., & Don, M. M. (2016). Ultrasonic-assisted extraction of oil from calophyllum inophyllum seeds: Optimization of process parameters. *Jurnal Teknologi*, 78(10), 199–206. <https://doi.org/10.11113/jt.v78.4946>
- Gao, H. T., Xu, R., Cao, W. X., Zhou, X., Yan, Y. H. M., Lu, L., Xu, Q., & Shen, Y. (2016). Food emulsifier glycerin monostearate increases internal exposure levels of six priority controlled phthalate esters and exacerbates their male reproductive toxicities in rats. *PLoS ONE*, 11(8), 1–14. <https://doi.org/10.1371/journal.pone.0161253>
- González-Fernández, M. J., Ramos-Bueno, R. P., Rodríguez-García, I., & Guil-Guerrero, J. L. (2017). Purification process for MUFA- and PUFA-based monoacylglycerols from edible oils. *Biochimie*, 139, 107–114. <https://doi.org/10.1016/j.biochi.2017.06.002>
- Hasibuan, H. A., Sitanggang, A. B., Andarwulan, N., & Hariyadi, P. (2021). Solvent fractionation of hard palm stearin to increase the concentration of tripalmitoylglycerol and dipalmitoyl-stearoylglycerol as substrates for synthesis of human milk fat substitute. *International Journal of Food Science and Technology*, 56, 4549–4558. <https://doi.org/10.1111/ijfs.15206>
- 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>
- Kantekin-Erdogan, M. N., Ketenoglu, O., & Tekin, A. (2019). Effect of monoglyceride content on emulsion stability and rheology of mayonnaise. *Journal of Food Science and Technology*, 56(1), 443–450. <https://doi.org/10.1007/s13197-018-3506-2>
- King, J. W. (2002). Supercritical fluid technology for lipid extraction, fractionation, and reactions. In T. M. Kuo & H. Gardner (Eds.), *Lipid biotechnology* (pp.663–688). CRC Press. <https://doi.org/10.1201/9780203908198-36>
- Lai, O. M. (2011). Diacylglycerol oils: Nutritional aspects and applications in foods. In G. Talbot (Ed.), *Reducing saturated fats in foods* (pp. 158–178). Woodhead Publishing Limited. <https://doi.org/10.1533/9780857092472.2.158>
- Lee, W. J., Zhang, Z., Lai, O. M., Tan, C. P., & Wang, Y. (2020). Diacylglycerol in food industry: Synthesis methods, functionalities, health benefits, potential risks and drawbacks. *Trends in Food Science and Technology*, 97, 114–125. <https://doi.org/10.1016/j.tifs.2019.12.032>
- Márquez, A. L., Pérez, M. P., & Wagner, J. R. (2013). Solid fat content estimation by differential scanning calorimetry: Prior treatment and proposed correction. *Journal of the American Oil Chemists' Society*, 90(4), 467–473. <https://doi.org/10.1007/s11746-012-2190-z>
- Mazzieri, V. A., Vera, C. R., & Yori, J. C. (2008). Adsorptive properties of silica gel for biodiesel refining. *Energy and Fuels*, 22(6), 4281–4284. <https://doi.org/10.1021/ef800479z>
- McClements, D. J., & Jafari, S. M. (2018). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science*, 251, 55–79. <https://doi.org/10.1016/j.cis.2017.12.001>
- Nadjamoeddin, G. L., Faridah, D. N., Andarwulan, N., Hariyadi, P., & Khotib, M. (2024a). Multiple-stage silica-assisted liquid-liquid extraction: A promising extraction method for fractionation mono-diacylglycerols (MDAGs) based palm stearin. *Food Chemistry Advances*, 4, Article 100724. <https://doi.org/10.1016/j.focha.2024.100724>

- Nadjamoeddin, G. L., Faridah, D. N., Andarwulan, N., Hariyadi, P., & Khotib, M. (2024b). Solubility evaluation of palm-based mono-diacylglycerols (MDAGs) in food grade solvent (hexane, ethanol, acetone, water) using QSPR model approach. *Journal of Molecular Liquids*, 400, Article 124531. <https://doi.org/10.1016/j.molliq.2024.124531>
- Ogbeide, S. E., Aniekwe, E. U., & Nwanno, C. E. (2018). Optimization of the extraction process of gmelina seed oil using response surface methodology. *Chemistry Research Journal*, 3(5), 94–102.
- Oniya, O. O., Oyelade, J. O., Ogunkunle, O., & Idowu, D. O. (2017). Optimization of solvent extraction of oil from sandbox kernels (*Hura crepitans* L.) by a response surface method. *Energy and Policy Research*, 4(1), 36–43. <https://doi.org/10.1080/23815639.2017.1324332>
- Ran, Y., Jain, N., & Yalkowsky, S. H. (2001). Prediction of aqueous solubility of organic compounds by the general solubility equation (GSE). *Journal of Chemical Information and Computer Sciences*, 41(5), 1208–1217. <https://doi.org/10.1021/ci010287z>
- Sagalowicz, L., Leser, M. E., Watzke, H. J., & Michel, M. (2006). Monoglyceride self-assembly structures as delivery vehicles. *Trends in Food Science and Technology*, 17(5), 204–214. <https://doi.org/10.1016/j.tifs.2005.12.012>
- Sahle-Demessie, E. (1997). Fractionation of glycerides using supercritical carbon dioxide. *Industrial and Engineering Chemistry Research*, 36(11), 4906–4913. <https://doi.org/10.1021/ie9703853>
- Sánchez, E., Pardo-Castaño, C., & Bolaños, G. (2018). Purification of monoglycerides from palm stearin by liquid-liquid extraction with aqueous ethanol. *Journal of the American Oil Chemists' Society*, 95(2), 217–228. <https://doi.org/10.1002/aocs.12018>
- Triana, R. N., Andarwulan, N., Affandi, A. R., Wincy, & Kemenady, E. (2014). Aplikasi mono-diasilgliserol dari fully hydrogenated palm kernel oil sebagai emulsifier untuk margarin [The application of mono-diacylglycerol from fully hydrogenated palm kernel oil as emulsifier for margarine]. *Mutu Pangan*, 1(2), 137–144.
- Wang, Y. F., Lee, G. L., Huang, Y. H., & Kuo, C. C. (2016). *Sn*-1,2-diacylglycerols protect against lethal endotoxemia by controlling systemic inflammation. *Immunobiology*, 221(11), 1309–1318. <https://doi.org/10.1016/j.imbio.2016.05.007>
- Yeoh, C. M., Phuah, E. T., Tang, T. K., Siew, W. L., Abdullah, L. C., & Choong, T. S. Y. (2014). Molecular distillation and characterization of diacylglycerol-enriched palm olein. *European Journal of Lipid Science and Technology*, 116(12), 1654–1663. <https://doi.org/10.1002/ejlt.201300502>
- Zhang, Z., Ma, X., Huang, H., Li, G., & Wang, Y. (2017). Enzymatic production of highly unsaturated monoacylglycerols and diacylglycerols and their emulsifying effects on the storage stability of a palm oil based shortening system. *Journal of the American Oil Chemists' Society*, 94(9), 1175–1188. <https://doi.org/10.1007/s11746-017-3023-x>
- Zhong, N., Cheong, L. Z., & Xu, X. (2014). Strategies to obtain high content of monoacylglycerols. *European Journal of Lipid Science and Technology*, 116(2), 97–107. <https://doi.org/10.1002/ejlt.201300336>