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Enzymatic preparation and facile purification of medium-chain, and medium- and long-chain fatty acid diacylglycerols

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ABSTRACT

High purity diacylglycerols (DAG) rich in medium-chain fatty acid diacylglycerols (MCD) and medium- and long-chain fatty acid diacylglycerols (MLCD) were prepared via the enzymatic esterification of monoacylglycerols (MAG) with caprylic acid followed by molecular distillation (MD), solvent fraction and low-temperature centrifugation. The content of DAG in the crude product was 44.8±0.1%, under the selected esterification conditions, which were MAGs/caprylic acid mole ratio of 1:3, reaction temperature of 65 □, reaction time of 30 min and enzyme load of 5 wt.%. Subsequently, the one-step MD and solvent fraction in methanol/ethanol increased the DAG content to 61.3±0.8%. Eventually, the product containing 86.6±0.6% of DAG with 39.3±1.3% of MCD and 47.3±0.6% of MLCD was obtained by the methanol crystallization at 0 □ with a water content of 9 wt.% and a 1:3 ratio of glycerides/methanol (v/v) followed by the centrifugation separation at 0 □.

Keywords: medium chain diacylglycerols; medium- and long-chain diacylglycerols; enzymatic esterification; molecular distillation; solvent fraction

1. Introduction

With the increasing concerns of high fat intake risks such as obesity, cardiovascular disease and high blood pressure, novel healthy food lipid and oil are becoming more and more popular among consumers who have increasing demands for healthy food (Hassel et al, 1993; Koh et al, 2010; Naughton et al, 2016). A variety of novel food lipid and oil has been investigated and among them products with both medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) are of great research interests (Kasai et al, 2003; St-Onge et al, 2003; Kim & Akoh 2005; Lu et al, 2017). Studies revealed that MCFAs, which are a mixture of fatty acids (FAs) with 6~12 carbon chains, exhibit several beneficial physiological functions, such as reducing fat accumulation improving glucose tolerance and insulin sensitivity (Nagao et al, 2010; Zhao et al, 2013; Li et al, 2016). LCFAs are FAs with more than 14 carbon atoms. Long-chain fatty acid triglycerides (LCT) with LCFAs are the major components of dietary lipids, which supply energy and provide essential FAs (Murillo et al, 2015; Wang et al, 2016).

Medium- and long-chain fatty acid triacylglycerols (MLCT), which have the combined advantages of medium-chain fatty acid triglycerides (MCT) and LCT, have been heavily investigated for their nutritive properties and have been commercialized (Socha et al, 2007; Koh et al, 2010; St-Onge et al, 2014). Previous studies state that MLCT are suitable for cooking/frying purposes, due to their capabilities of reducing body fat, body weight, and total serum cholesterol (Kasai et al, 2003; Koh et al, 2010). Furthermore, previous studies have confirmed that MLCT could significantly reduce low-density-lipoprotein cholesterol without affecting circulating high-density-lipoprotein cholesterol (St-Onge et al, 2003; Lu et al, 2017). Similarly, DAG rich in MCFAs and LCFAs are supposed to exhibit their combined benefits and medium- and long-chain fatty acid diacylglycerols (MLCD) have good potential applications (Nagao et al, 2010; Zhao et al, 2013). Moreover, some advantages diacylglycerols (DAG) has over triglycerides (TAG) add to the significance of study on MLCD. Studies have revealed that the stability of thermal oxidation (around 170 □) and autoxidation of DAG-rich cooking oil are better than conventional TAG oil

(Voll et al, 2013). Furthermore, relevant studies report that nutritive properties of DAG do not change during the test period of deep-frying (Shimizu et al, 2013). In spite the functional and healthy properties of MLCD, to our knowledge, the preparation of MLCD remains unexplored. In this work, we aimed to investigate the preparation of the high-purity MCD- and MLCD-rich DAG, through the application of Novozyme 435 and the development of a facile purification approach.

Generally, purification methods of DAG include molecular distillation (MD), column chromatography, supercritical CO_2 extraction and solvent fraction (Sebari et al, 2011; Gunawan et al, 2008; Holser et al, 2002; Akamatsu taku et al, 1994). However, for the purification of MCD- and MLCD-rich DAG in our study, one-step MD is inadequate because of low separation ratio, and it also results in the increase of TAG under higher temperature. Column chromatography suffers from complicated operation and long purification time (Yang et al, 2012). As for supercritical CO_2 extraction, it is still associated with high cost, long extraction time, relatively low extraction yield and high operation pressure. Meanwhile, CO_2 is suspected of damaging the composition of products (Yang et al, 2012). In contrast, solvent fraction is featured with short separation time, simple operation, ease of forming high separation ratio and good product purity (Akamatsu taku et al, 1994). The mechanism is that under low temperature, oils dissolve in the solvent while fats form stable crystals because of their high melting point and poor solubility, allowing for the separation of oils and fats. A mixture of chloroform-methanol-water mixture is frequently applied due to high extraction efficiency (Zbinden et al, 2013). Therefore, the present study also investigated the possibility of using solvent fractionation to purify the DAG containing MCD and MLCD.

Owing to enzyme's significant advantages such as mild reaction conditions, higher catalytic efficiency, and high regioselectivity, the application of lipases in producing DAG has become increasingly popular (Liu et al, 2016; Phuah et al, 2012). On the basis of previous research results, MCD and MLCD (Fig. 1) were prepared through the esterification of high purity monoacylglycerols (MAG) and caprylic acid catalyzed by Novozyme 435, and the reaction conditions of MAG/caprylic acid mole ratio, reaction temperature, reaction time and enzyme load

were investigated, respectively. The crude esterification products were then subjected to a facile and efficient purification process. Molecular distillation, low temperature solvent fraction and high-speed centrifugation were applied successively, affording the MCD- and MLCD-rich DAG. Conditions of different water content, centrifugation temperature and glycerides/methanol (v/v) were screened to improve the purification. It is expected that the MCD- and MLCD-rich DAG will be used as a basis for the subsequent development of low saturated fats, based on DAG-SLNs (solid lipid nanoparticles) Pickering emulsions, a novel nutritional, healthy and high quality lipid food system.

2. Materials and methods

2.1. Materials

High-purity MAG (53% palmitic acid MAG and 42% stearic acid MAG) was provided by Meichen Group Co., Ltd. (Guangzhou, China), Novozyme 435 (immobilized *Candida antarctica* lipase B) was supplied by Novozymes (Copenhagen, Denmark). Methanol (≥ 99.5%), ethanol (≥ 99.5%) and caprylic acid (99%) were purchased from Tianjin Chemical Reagent Co., Ltd (Tianjin, China). Deionized water was used and all other reagents were of analytical grade.

2.2. Production of products containing MCD and MLCD by enzymatic esterification

High-purity MAG (≥ 95%) was weighed in a 250 mL round bottom flask and heated in an oil bath at 85 □ with a magnetic stirrer at 200 rpm on until MAG completely melted. Then a certain amount of caprylic acid (MAG/caprylic acid mole ratio from 1:1 to 1:5) and Novozyme 435 (from 2.5 wt.% to 9.0 wt.%) were added to the flask. Esterification reaction was stirred at 200 rpm under a vacuum condition (0.1 MPa). After reaction, the crude products composed of free fatty acid (FFA), MAG, DAG and TAG were obtained after filtrating Novozyme 435.

2.3. Purification of esterification products by MD, solvent fractionation and centrifugation

The crude product was purified by a MD □ molecular distillation equipment (Foshan Handway Technology Co., Ltd, Foshan, China) to obtain high-purity DAG products. Use

parameters as follows: distillation temperature of 160 □, pressure of 3.6 Pa and wiped film speed of 200 rpm. The MD residue was then further purified using a solvent fraction method. A mixture of methanol (135 mL) and ethanol (15 mL) was added to 50 mL crude products in a 500 mL beaker, which was then placed in a constant temperature tank (15 □) stirring at 80 rpm for 30 min. Solid fat was removed by filtration after crystallization and the filtrate was concentrated under vacuum at 65 □ for 30 min to give the liquid oil. Next, the liquid oil was mixed with methanol in a certain proportion (volume ratio from 1:1 to 1:9) and the mixture was added into a 2 mL centrifuge tube followed by the addition of water (0-17 wt.%). Subsequently, the products containing MCD and MLCD were obtained after high-speed centrifugation (Legend Micro 17R, Guangzhou Bio-Key science & technology Co., LTD) at different temperatures (-5-15 □), water content and glycerides/methanol (v/v) with a speed of 11100 × g for 5 min.

2.4. Analysis for acylglycerols by gas chromatography (GC)

Analysis of the acylglycerol composition was conducted in accordance to Liu et al. (2012) and Wang et al. (2010). The composition of acylglycerols was analyzed by GC-FID (Agilent 7820A, Agilent Technologies Inc., Santa Clara, CA, USA). All product was dissolved in hexane of 2.0 mL and filtrated through a filter membrane of 0.45 μm. A capillary DB-1ht column (15 m × 0.25 mm i.d., 0.1 μm film thickness, Agilent Technologies Inc., USA) was used. Nitrogen was used as carrier gas at a column constant pressure of 20.0 psi. Sample volumes of 0.5 μL were injected with a split ratio of 20:1. The temperature for both detector ports and injector was set at 380 □. The oven temperature program was set as follows: the initial oven temperature was held at 50 □ for 1 min and then raised to 100 □ at 50 □/min; at the second stage, it was raised to 220 □ at 80 □/min； at the last stage, it was raised to 330 ℃ at 50 ℃/min and held 2 min; and finally, it was raised to 380 ℃ at 50 ℃/min and held for 3 min. The composition of acylglycerols was quantified as relative percentages of the total acylglycerols.

2.5. DAG analysis by low and high resolution-MS (LRMS and HRMS)

According to the method of Wan et al. (2016), samples were dissolved in methanol and filtered through a 0.22 μm filter. The mass spectra (MS) was used by a 4000 QTRAP triple quadrupole/linear ion trap mass spectrometer (AB SCIEX, Framingham, MA, USA) equipped with an ESI source, and detected in the positive ion mode within a *m/z* range of 100-2000. The other parameters were as follows: capillary temperature of 250 □, capillary voltage of 40 V, and ions spray voltage of 5500 V.

2.6. Statistical analysis

All of the experiments were performed in triplicate. The analysis of variance (ANOVA) method was used to analyze the data, the mean values were compared using Duncan's new multiple range test at a 95% significance level ($p<0.05$). Origin 8.0 software version and SPSS software version 16.0 were employed to analyze the measured data.

3. Results and discussion

3.1. Effect of enzymatic reaction conditions

3.1.1. Effect of MAG/caprylic acid mole ratio

The effects of different substrate (MAG/caprylic acid) mole ratio on the esterification were investigated under the conditions of reaction temperature 65 □, enzyme load 5 wt.%, and reaction time 30 min. The results are shown in Fig. 2 (A1).

With the decrease of substrate (MAG/caprylic acid) mole ratio from 1:1 to 1:3, the DAG content increased significantly and reached its highest value of 44.8±0.1%. Nonetheless, if the mole ratio was below 1:3, the DAG content began to decrease significantly. Meanwhile, the contents of both MAG and TAG decreased reversely with substrate mole ratio. In addition, the significant increasing trend of the contents of FFA was recorded. The result shows that, along with the decreasing substrate mole ratio, the esterification would be promoted to shift reaction equilibrium to yield more DAG. But the increase of caprylic acid increases the viscosity of substrate which may impede the water removal from the system, leading to the hydrolysis of DAG. Furthermore, the

increased viscosity of reaction system might reduce mass transfer efficiency so as to inhibit the reaction (Arpi, Lubis, & Supardan, 2016). Therefore, taking both the costs and the DAG content into consideration, the mole ratio of 1:3 was selected for further reaction.

3.1.2 Effect of reaction temperature

The effects of different reaction temperature on the esterification were investigated under the conditions of substrate (MAG/caprylic acid) mole ratio 1:3, enzyme load 5 wt.%, and reaction time 30 min. The results are shown in Fig. 2 (A2). The highest content of DAG in crude products was obtained as 44.8±0.1% at 65 □, and over 65 □ the content decreased insignificantly. In contrast, TAG had a continuously increasing trend with the increasing temperature and significantly. Opposite to TAG, MAG content underwent a decreasing trend and increased significantly over 75 □. This could be ascribed to that higher temperatures favor the production of TAG, resulting in the consumption of DAG and MAG (Mu et al., 1998). In addition, along with the increase of reaction temperature, to a certain extent, the thermal stability of the lipase would be affected which in turn will limit the enzyme application to a certain extent (Wang et al., 2016). Therefore, temperature of 65 □ was selected in this work.

3.1.3 Effect of reaction time

The effects of different reaction time on the esterification were investigated under the conditions of substrate (MAG/caprylic acid) mole ratio of 1:3, enzyme load of 5 wt.%, and reaction temperature of 65 □. The results are shown in Fig. 2 (A3).

Distinctively opposite trends of the contents of DAG and FFA were recorded with reaction time. In the first 30 min, the content of DAG increased significantly and reached the highest value of 44.7±0.1% whereas FFA content decreased significantly to 41.3±0.0%. After 30 min, the content of DAG began to decrease significantly while FFA content increased to 48.7±0.5% at 45 min but changed insignificantly after that. The content of MAG decreased significantly with enlonged reaction time. As for TAG, its content increased continuously in the first 75 min to 14.6±0.3% and

insignificantly changed after that. Therefore, to ensure the higher yield DAG as well as the followed better purification, the reaction time was selected as 30 min.

3.1.4 Effect of enzyme load

The effects of different enzyme load on the esterification were investigated under the conditions of substrate (MAG/caprylic acid) mole ratio 1:3, reaction time 30 min, and reaction temperature 65 □. The results are displayed in Fig. 2 (A4). Noticeably, a watershed at the 5 wt.% enzyme load could be found.

The content of DAG increased significantly with enzyme load under 5 wt.% and maximized (44.5±0.2%) at 5 wt.%, whereas the DAG content decreased significantly with enzyme load over 5 wt.%. The TAG content showed a similar tendency. Clearly, the contents of FFA and MAG presented trends exactly opposite to that of DAG. This phenomenon might be attributed to the increasing number of active sites the more lipase provided, resulting in improved catalytic efficiency. Thereby, more products, DAG, were formed and more substrates, FFA and MAG, were consumed (Wang et al., 2011). The decreasing content with enzyme load over 5 wt.% might come from the hydrolysis of DAG, and the hydrolysis products were FFA and MAG. Therefore, based on the operability and economy, enzyme load 5 wt.% (substrates mass) was selected in this study.

3.2. Purification of esterification products by centrifugation

3.2.1. Effect of water content

Lipid crystallization as a purification approach is frequently applied in industrial production. In an aqueous environment, most lipids self-assemble into different crystalline, liquid crystalline or sometimes macroscopically disordered phases, whereas in a dehydrated state most lipids form well-ordered crystals (Jiménez, Fabra, & Talens, 2013). Young et al. (2010) reported that methanol as a co-solvent was capable of extracting lipids from biomass sources and they also used centrifugation to separate the solution and the solid lipid layer. In our study, the crude product of liquid oils and solid fats was further purified by further methanol crystallization followed by centrifugation separation.

Accordingly, the effects of different water content on the purification by centrifugation were investigated under the conditions of centrifugation temperature 0 □, glycerides: methanol 1:3 (v/v), centrifugation time 5 min and rotating speed 11100 × g. The effects of different water content on the DAG yield and the purification of DAG (containing MCD and MLCD) are depicted in Fig. 3 (A1).

As described in Fig. 3 (A1), with the increasing of water content under 9 wt.%, DAG content increased significantly to the highest value of 85.6±0.2% while the content of TAG and DAG yield declined significantly to 14.4±0.2% and 33.0±1.7%, respectively. Meanwhile, the contents of MCD and MLCD were 38.6±0.1% and 47.0±0.1%, respectively. MLCD accounted for 55% of the total content of DAG. After the water content increased to 13 wt.%, the total DAG content was 65.8±0.4% with 36.0±0.2% MLCD and 29.8±0.1% MCD. It is obvious that higher water content did not favor the separation of solid fats and liquid oils as well as the purification of DAG products (including MCD and MLCD). This might be because when the water content was above a critical value, the mixture became rubbery, which rendered unfavorable molecular mobility rate. Thus, the FFA would not crystalize in a well-ordered form (Jiménez, Fabra, & Talens, 2013). In summary, water content had a significant effect on purifying DAG and water content of 9 wt.% was selected in this work.

3.2.2. Effect of temperature

Temperature could be used to control crystal growth (Li, Shah, & Caffrey, 2013). López-Martínez et al. (2004) applied solvent fraction at low temperatures. They chilled the liquid oils to allow solid fats to crystallize and the subsequent filtration of the two phases. It is a process of the removal of solid fats by controlled crystallization and filtration. Different products could be selectively crystallized at different temperatures and separated. Accordingly, solvent fraction is quite suitable for the separation of DAG and TAG with differing melting points (Fats, 1994).

The effects of different centrifugation temperature on the DAG yield and the purification of DAG were investigated under the conditions of water content 9 wt.%, glycerides:methanol 1:3 (v/v), centrifugation time 5 min, and rotating speed 11100 × g. The results are shown in Fig. 3 (A2).

The DAG content and DAG yield reached 85.3±0.6% and 39.3±0.8%, respectively when centrifugation temperature was 0 □, whereas the contents of MCD and MLCD were 41.8±0.4% and 43.5±0.6%, respectively, and MLCD accounted for 51% of the total DAG content (Fig. 3 (A2)). With the increasing centrifugation temperature, the contents of DAG changed insignificantly whereas the content of MCD and MLCD changed significantly. This indicates that although centrifugation temperature has little effects on the content of DAG, low temperature contributes to MLCD purification. López-Martínez et al. (2004) mentioned that partially crystallized materials were removed from edible oils by filtration to avoid the clouding of liquid oils at refrigeration temperature, and liquid oil can be obtained after filtering the mixture. As the result, the centrifugation temperature 0 □ was selected in the next work.

3.2.3. Effect of ratio of glycerides/methanol

The effects of different ratio of glycerides/methanol (v/v) for DAG yield and purifying DAG (containing MCD and MLCD) were investigated under the conditions of water content 9 wt.%, centrifugation temperature 0 □, centrifugation time 5 min, and rotating speed 11100 × g. The results are shown in Fig. 3 (A3).

The content of DAG was 86.6±0.6% with 31.6±0.6% DAG yield when the ratio of glycerides/methanol was 1:3 (v/v). The contents of MCD and MLCD were 39.3±1.3% and 47.3±0.6%, respectively, and MLCD accounted for 54.6% of the total DAG. With the increasing glycerides/methanol ratio, the content of MLCD increased significantly but the MCD content decreased significantly to 30.8±0.1%. When the ratio of glycerides/methanol was below 1:3, the content of DAG was decreased insignificantly but the DAG yield was increased significantly as the content of methanol increased (Fig. 3 (A3)). This might show that, under the ratio of glycerides/methanol 1:3 (v/v), there was a meta-stable solution which favored the crystal growth of

FFA. The lower glycerides/methanol ratio did not favor crystal growth, thereby decreased the solid fats in the liquid oils (López-Martínez, Campra-Madrid, & Guil-Guerrero, 2004). Thus, the glycerides/methanol ratio of 1:3 (v/v) was selected in this work.

3.2.4 Analysis of products by GC

According to the method of 2.4, GC spectra of the crude products and high purity DAG (containing MCD and MLCD) are shown in Fig. 4 (A1).

According to Fig. 4 (A1), besides MCD and MLCD, there were also impurities including FFA, MAG and trace TAG in the crude products. DAG achieved 44.8±0.1% and MLCD accounted for 47.3% of the total DAG (Table 1). Fig. 4 (A2) demonstrates that after purification, the purity DAG reached 86.6±0.6%, which contained 39.3±1.3% MCD and 47.3±0.6% MLCD (Table 1), and MLCD accounted for 54.6% of the total DAG. Through the comparison of crude product and high-purity DAG, it is obvious that the content of DAG had a significant improvement from 44.8±0.1% to 86.6±0.6% with the combination of MD, constant stirring at 15 □ and high-speed centrifugation at 0 □. Particularly, the content of MLCD more than doubles from 21.5±0.3% to 47.3±0.6%, which also had a significant improvement. The above results indicate that MCD- and MLCD-rich DAG of high purity was achieved and evidenced the high efficiency of the purification approach investigated.

3.2.5 Determination of MCD and MLCDs by LRMS and HRMS

ESI-MS was used for the identification of the products and the result of *LRMS* is shown in Fig. 4 (B) and the analysis is listed in Table 2. The ions of the MCD and two MLCDs were recorded as follows: $[M+H]^+$ at *m/z* 345.6 (MCD, $C_{19}H_{36}O_5$, 344.6), $[M+H]^+$ at *m/z* 457.7 (MLCD, $C_{27}H_{52}O_5$, 456.7), and $[M+H]^+$ at *m/z* 485.6 (MLCD, $C_{29}H_{56}O_5$, 484.6) and they were all in good agreement with theoretical calculation of their molecular weights (Fig. 4 (B) and Table 2). The above results correspond to the analysis results of Fig. 3.

In addition to LRMS, the product was further characterized by HRMS. The HRMS spectrums of MCD and two types of MLCDs are shown in Fig. 4. The ions of the MCD and two MLCDs were

recorded as follows: $[M+Na]^+$ at *m/z* 367.2450 (MCD, $C_{19}H_{36}O_5Na$, calculated for 344.2460), $[M+Na]^+$ at *m/z* 479.3693 (MLCD, $C_{27}H_{52}O_5Na$, calculated for 479.3712), and $[M+Na]^+$ at *m/z* 507.3997 (MLCD, $C_{29}H_{56}O_5Na$, calculated for 507.4025). Results again indicate the good agreement between the HRMS of MCD and MLCDs and the theoretical calculation of their molecular weights. Those spectrums further evidenced the excellent efficiency of the investigated purification process, and the MCD- and MLCD-rich DAG of high purity was obtained.

4. Conclusions

In this study, the preparation and purification of MCD- and MLCD-rich DAG of high purity was developed. Under the selected parameters: reaction temperature 65 □, substrate (MAG/caprylic acid) mole ratio 1:3, reaction time 30 min and enzyme load 5 wt.%, the crude products with 44.8±0.1% of DAG was obtained. Subsequently, the product with 56.3±1.2% DAG content was achieved after one-step MD. DAG content was further improved to 61.3±0.8% by constant stirring with methanol/ethanol (9:1, v/v) at 15 □ for 30 min. Eventually, the high-speed centrifugation of last step's products at 0 □ with a water content of 9 wt.% and a 1:3 ratio of glycerides/methanol (v/v) afforded DAG with a high purity of 86.6±0.6%. The qualification by both LRMS and HRMS showed good agreements to the theoretical calculation of the molecular weights of MCD and MLCD. The obtained MCD- and MLCD-rich DAG of high purity demonstrates the efficiency of both of the preparation and purification approaches proposed herein. Furthermore, the low cost and easy operation of the solvent fraction and low temperature centrifugation suggest the potential industrial values of our process. Future studies may focus on the functional and nutritional properties of MCD- and MLCD-rich DAG to expand the potential usage in food industries.

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Tables

Table 1. Content of MCD and MLCD by GC spectra

	Crude product	DAG of high purity
MCD (%)	23.3±0.1	39.3±1.3
MLCD (%)	21.5±0.3	47.3±0.6

Table 2. Identification of the target products by MS

	Ion type	[M]	$[M+H]^+$
1,2/1,3-octanoyl-glyceride	DOCG	344.6	345.6
1,2/1,3-octanoyl-palmitory-glyceride	DOPG	456.7	457.7
1,2/1,3-octanoyl-stearoyl-glyceride	DOStG	484.6	485.6

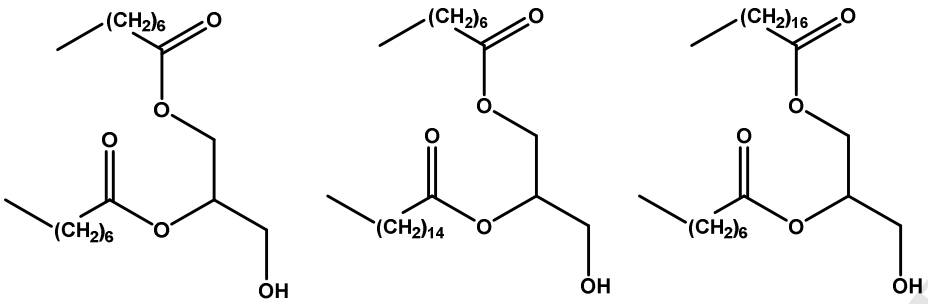
Figure captions

Figure 1. Structures of the medium chain fatty acid diacylglycerols (MCD) and medium- and long-chain fatty acid diacylglycerols (MLCD).

Figure 2. Effect of monoacylglycerols (MAG)/caprylic acid mole ratio (A1), reaction temperature (A2), reaction time (A3) and enzyme load (A4) on esterification reaction. Values are means ±SDs (n = 3); Values of the same series, with the different letter are significantly ($p < 0.05$) different.

Figure 3. Effect of water content (A1), temperature (A2) and glycerides/methanol ratio (A3) on the purification of diacylglycerols by methanol crystallization at different temperature by centrifugation. Values are means ±SDs (n = 3); Values of the same series, with the different letter are significantly ($p < 0.05$) different.

Figure 4. Chromatograms and mass spectra of crude and purified products. A1: compositions of diacylglycerols (DAG) of crude products (CA: caprylic acid; PA: palmitic acid; SA: stearic acid; MAG, monoacylglycerols; MCD, medium chain fatty acid diacylglycerols; MLCD, medium- and long-chain fatty acid diacylglycerols; TAG, triglycerides); A2: Compositions of glycerides of high purity DAG (TCG, caprylic triglycerides); B: low-resolution mass spectra (LRMS) spectrometry of DAG; C1: high-resolution mass spectra (HRMS) of MCD; C2, C3: HRMS of MLCD.



MCD ($C_{19}H_{36}O_5$) MLCD ($C_{27}H_{52}O_5$) MLCD ($C_{29}H_{56}O_5$)

Fig. 1

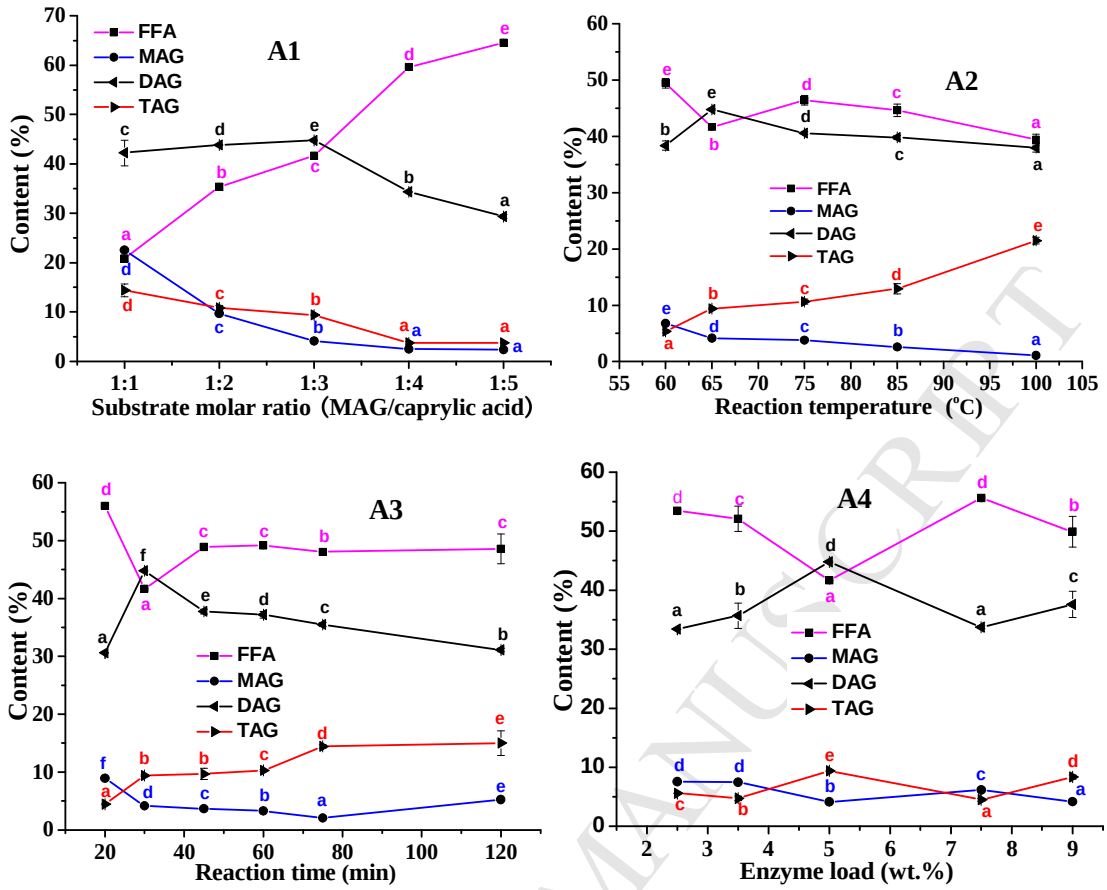


Fig. 2

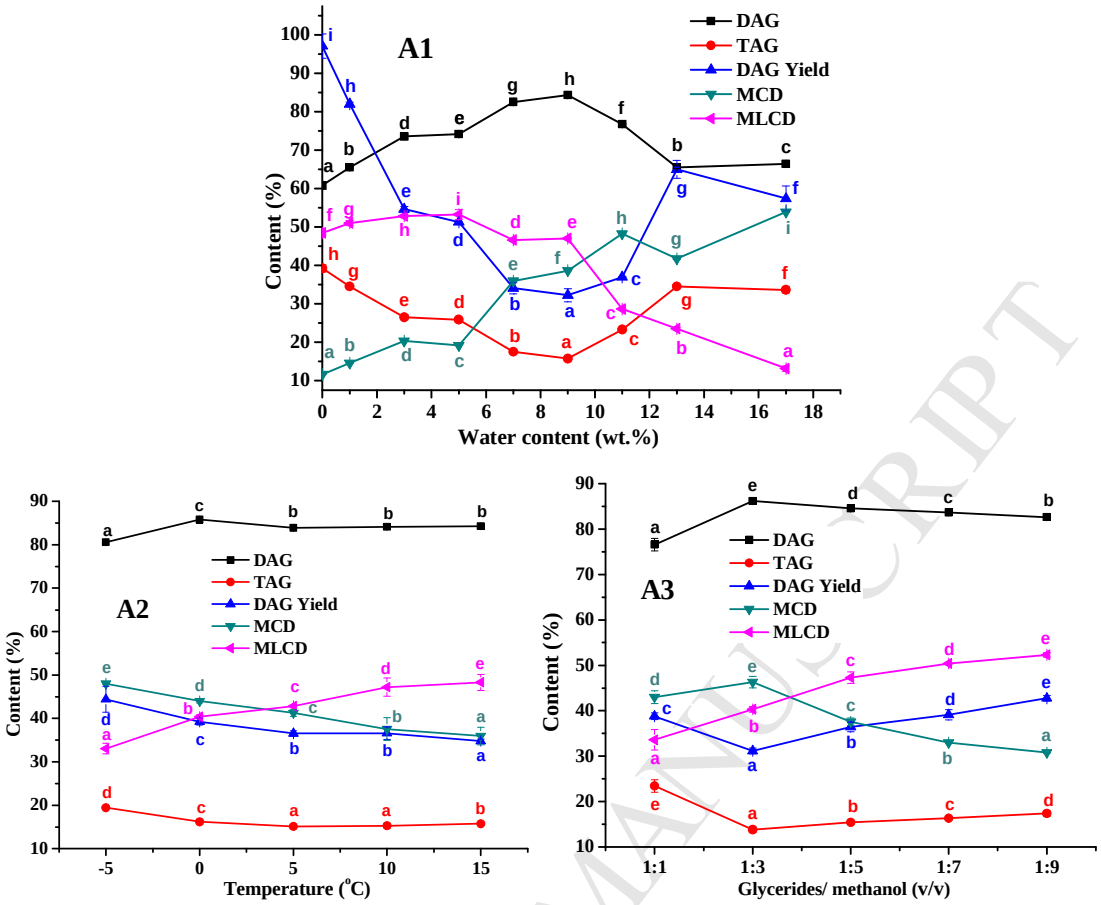


Fig. 3

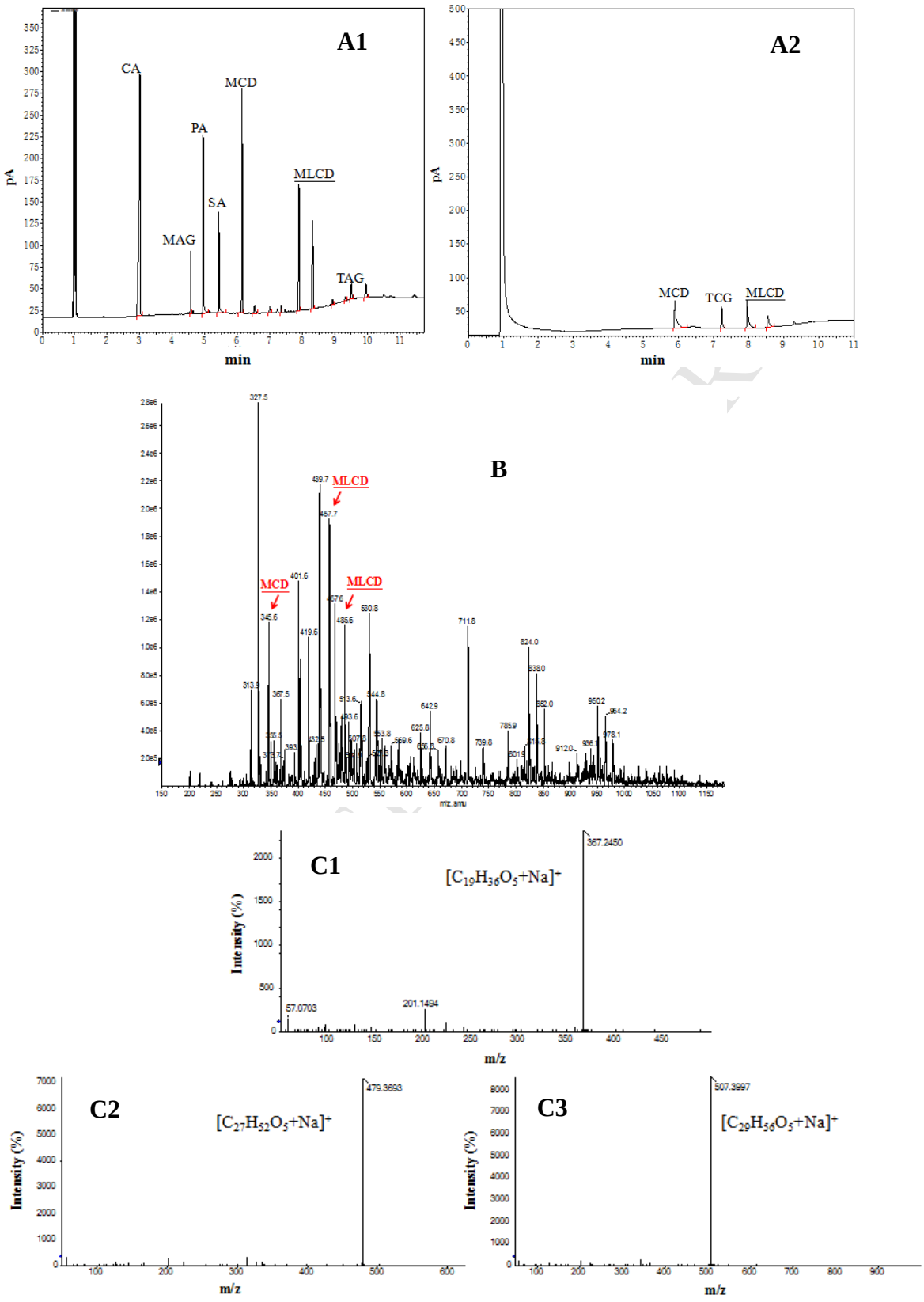

A1
CA
MAG
PA
SA
MCD
MLCD
TAG
pA
min
A2
MCD
TCG
MLCD
pA
min
B
MCD
MLCD
MCD
MLCD
m/z, amu
C1
[C19H36O5+Na]+
367.2450
57.0703
201.1494
Intensity (%)
m/z
C2
[C27H52O5+Na]+
479.3693
Intensity (%)
m/z
C3
[C29H56O5+Na]+
507.3997
Intensity (%)
m/z

Fig. 4

Highlights

- Enzymatic method was investigated to prepare the MCD- and MLCD-rich DAG.
- The content of MCD- and MLCD-rich DAG could reach 86.6±0.6% in our study.
- GC, LRMS and HRMS proved the efficiency of our structured DAG preparation process.