**Chemical Transesterification of Flaxseed Oil and Medium Chain Triacylglycerols: MLCT yield, DAG content, Physicochemical properties, Minor compounds and Oxidation stability**

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Chemical Transesterification of Flaxseed Oil and Medium Chain Triacylglycerols:
MLCT yield, DAG content, Physicochemical properties, Minor compounds and
Oxidation stability

Running title: CTE of Flaxseed Oil and MCTs

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2. Jiangsu Xingfumen Grain and Oil Co. LTD

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Abbreviations used
ALA, α-linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid;
FFA, free fatty acid; PCCs, Pearson correlation coefficients; FA, fatty acid; SL, structured lipid;
MLCT, medium- and-long chain triacylglycerol; MCT, medium-chain fatty acid
ABSTRACT

A type of n-3 medium-and-long chain triacylglycerols (MLCT) rich in α-linolenic acid (ALA) were produced by chemical transesterification of medium chain triacylglycerols (MCT) and flaxseed oils. Under the optimal conditions of substrate of 60%, catalyst loading of 0.3% at 60 °C for 15 min, the product contained 75.07% of MLCT, 37.26% of ALA and 10.33% of DAG. Thereafter, fatty acid composition, physiochemical properties and minor compounds of the substrate and MLCT products were comparatively investigated. The correlations of these factors on oxidative stability were analyzed. The factors can be divided into positive groups and negative groups. The results of this work suggested that DAG, a major byproduct, can slow down oil oxidant at high content, which meant it is better to retain a higher level of DAG rather than remove it via purification.

Keywords: chemical transesterification, medium-and-long chain triacylglycerols, diacylglycerol, oxidation stability, minor compounds
1. Introduction

Structured lipids (SL) are functional lipids which have unique characters and great applications in foods, nutrition and therapeutics (Akoh & Min, 2008). There are many different types of SLs including medium-chain fatty acid triacylglycerols (MCTs), medium- and-long chain triacylglycerols (MLCTs), phospholipids and so on (Kim & Akoh, 2015). Many studies have focused on altering fatty acids (FA) composition and positional distribution of natural oils to satisfying consumers particular demands (Kim & Akoh, 2015). The n-3 polyunsaturated fatty acids (PUFA) are essential fatty acids (EFAs) and play an important role in the prevention and treatment of cardiovascular and inflammatory diseases (Baker, Miles, Burdge, Yaqoob, & Calder, 2016). Since then, more attention has been paid to preparing SLs rich in n-3 PUFAs such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Korma et al., 2018; Zou et al., 2020). For example, MLCTs rich in n-3 PUFA desirable as health-care products for supplementation in infant formula or as food supplement for adults to enhance overall health (Henry, 2009).

Generally, there are many different approaches (Y.-Y. Lee, Tang, & Lai, 2012) to prepare SLs while the most widely used in the food industry is transesterification especially chemical transesterification (CTE) since there are still several challenges and uncertainties such as hydrodynamics, mass transfer, and the packing efficiency of enzyme in the larger reactor during the enzymatic producing (Akoh & Min, 2008; Zhang et al., 2020). What’s more, sodium methoxide catalyzed CTE produce a satisfied yield in 15 min to 1h under low temperature while enzymatic one always takes more than 3h (Khodadadi & Kermasha, 2014a, 2014b;
Korma et al., 2018). The n-3 PUFA are all easy oxidized and deteriorated when heated for a long time. A study showed that the peroxide value (POV) of flaxseed oil, rich in n-3PUFA, increased a lot after being heated at 50°C for less than 18 hours (T. Lu et al., 2020). By taking all of these indices into consideration, it seems that sodium methoxide catalyzed CTE is more appropriate to preparing commercial MLCTs rich in n-3 PUFA.

Oxidative stability is essential to lipids products as lipids oxidation would cause deterioration of quality, rancidity of FA and loss of nutrition (Koh et al., 2009; Martin, Reglero, & Señoráns, 2010). Lipids oxidation is a complex reaction (Akoh & Min, 2008) which under the influence of FA composition and minor compounds which including DAG, MAG, FFA, peroxides, tocopherols, phytosterols, oryzanol and so on (Adhikari & Hu, 2012). The rate of lipids oxidant also depends on the interactions of these compounds. DAG is a major byproduct of CTE which caused the decrease in oxidative stability (Shinohara, Ogawa, Kasai, & Aoyama, 2005), and some studies indicated that DAG had bad effect on MLCT oxidation (Q. Wang, Xie, Li, Miao, & Wu, 2019). Thus, the content of DAG is expected to be as low as possible by optimization of reaction or purification (J. Lu, Jin, Wang, & Wang, 2017; Xu, Skands, Adler-Nissen, & Hoy, 1998). Hence, correlation of the yield of MLCT, the content of DAG, oxidative stability, FA composition, minor compounds and their relationships after CTE are crucial to PUFA rich MLCT production and deserve effective and systematical evaluation.

Flaxseed oil, a vegetable oil which contains 45%-65% of ALA (Symoniuk, Ratusz, & Krygier, 2017), is an ideal substrate for the catalyst of MLCTs rich in n-3 PUFA via transesterification with MCTs. Flaxseed oils contain some minor compounds, such as
tocopherols, phytosterols and oryzanol (Tanska, Roszkowska, Skrajda, & Dabrowski, 2016). These compounds show great potential in anti-oxidation. However, the FA profile of flaxseed oil, high rate of PUFA, has an undesirable effect on oxidative stability which was evaluated as oxidation induction time (the time it takes for oil to deteriorate). So, flaxseed oil is a good model to study the effect of CTE on oxidative stability of MLCT product rich in n-3 PUFA.

The purpose of this study was to found the best conditions to prepare high quality MLCT rich in ALA. Meanwhile, it aimed at explore the changes in DAG content during the reaction and the changes of FA composition, minor compounds after CTE and correlate these changes with oxidation induction time. A greater understanding of the changes in oxidative stability will benefit the MLCT preparing and purifying.

2. Materials and methods

2.1 Materials

Refined flaxseed oil without antioxidants was provided by Junxingfang Food Science and Technology CO., LTD (Wuzhong, China). MCTs were obtained from Jiande Qiandao Fine Chemical Industry Co., Ltd (Jiande, China). Sodium methoxide solution was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Fatty acid methyl ester standards were obtained from Sigma-Aldrich Chemical Co. Ltd. (Shanghai, China). Acetonitrile, isopropanol and n-hexane were supplied by J&K Chemical Ltd (Shanghai, China). All solvents used were HPLC grade.

2.2 Chemical transesterification flaxseed oil of with MCTs

A 100 g binary blend of flaxseed oil and MCTs was added into a three-necked flask. The
blend was dried at 80°C for 30 min to remove residual trace water, and then was held at reaction temperature for 15 min before adding the catalyst. The reaction was stopped by adding boiling distilled water. The sample mixed with water was washed by hot distilled water in a separating funnel for three times until it became neutral (pH=7.0). The washed sample was dried at 95°C for 30 min and centrifuged to remove soap. For purpose of preventing oils from oxidization, heating processes were all controlled under a high vacuum.

The effect of four different parameters including substrate weight ratio, reaction temperature, catalyst loading and reaction time were examined. Substrate weight ratio of flaxseed oil and MCTs was taken as the weight percent of flaxseed oil, ranged from 40% to 80%. Meanwhile, the reaction temperature was set from 40°C to 80°C. The catalyst loading was defined as the weight percent of sodium methylate and changed from 0.1% to 0.5%.

What's more, the reaction time contained six points consisted of 5, 10, 15, 20, 30, 40 min. Other parameters were kept the same when one parameter was optimized and the selected value was used for the next examination. Each factor optimization was replicated for three times.

2.3 Triacylglycerols composition analysis

Method to analysis triacylglycerols (TAGs) composition was the improvement of the method reported by Lu (J. Lu et al., 2017). The dried sample was analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) (Agilent 1260 Infinity, Agilent Technologies, Inc., USA) equipped with evaporative light scattering detector (ELSD) (Alltech 3300, Grace Davison Discovery Sciences, USA) and a Lichrospher C18 column (5 μm, 4.6 mm × 250 mm; Hanbon Science & Technology Co., Ltd., Jiangsu, China). The test
sample was diluted by mixing 20 μL MLCT product with 1 mL acetonitrile and eluted with a binary gradient of solvent A (100% acetonitrile) and solvent B (100% isopropanol) at a solvent flow rate of 1 mL/min, with the following gradient: 0 min, 90% A; 20 min, 70% A; 35 min, 60% A; 40 min, 55% A; 55 min, 70% A; 65 min, 90% A and staying 90% A for 10 min. The volume of the injected sample was 20 μL and the column temperature was set at 30°C. Parameters of ELSD were set at 55°C, air gas flow rate of 1.8 mL/min and gain of 1.

The TAGs profile after transesterification changed and equivalent carbon number (ECN) was used to predict the elution order. ECN was calculated as ECN = CN (total number of carbon atoms in a TAG molecule except the three glycerol carbons) - 2DB (total number of double bonds in the fatty acids). The yield of MLCTs was calculated as followed:

\[ \text{Yield of MLCTs(\%) = } \frac{\text{Weight of MLCTs}}{\text{Weight of total reactants}} \times 100 \]

2.4 Fatty acid composition and sn-2 position analysis

The fatty acid composition of MCTs, flaxseed oil and MLCT product was determined by gas chromatography (GC) after conversion of fatty acid methyl esters. The method of converting fatty acid to fatty acid methyl ester referred to the method reported by Korma (Korma et al., 2018) and the composition of sn-2 fatty acid was according to the AOCS Official Method Ch 3–91 (2011). The fatty acid methyl esters were separated by an ionic liquid capillary column (TRACE TR-FAME, 60 m × 0.25 mm × 0.25 μm, Thermo Fisher, USA), carrier gas was nitrogen, flow rate 1.2 mL/min, injector and detector temperatures were 250°C, split ratio of 1:20. The initial oven temperature was 60°C, held for 3 min, then programmed to 175°C at 5°C/min and maintained for 15 min, then increased to 220°C at
2°C/min and stayed at 220°C for 10 min. The type and content of FAs was determined by the retention times of fatty acid methyl ester standards and peak area percentage respectively.

2.5 Determination of DAG content

The content of DAGs was analyzed by Normal Phase HPLC (NP-HPLC) which had been reported by Zeng (Zeng, Qi, Xin, Yang, & Wang, 2015).

2.6 Determination of physicochemical properties

Physicochemical properties determined in this work contain Acid value (AV) and peroxide value. AV and POV was assessed via AOSC office methods by the determination of their acid value (Method Ca 5a-40), peroxide value (Method Cd 8b-90).

2.7 Determination of minor compounds and oxidative stability index

The minor compounds determined in this study contain tocopherols, phytosterols, oryzanol. The determinations of tocopherols, phytosterols and oxidation induction time were according to AOSC office methods, tocopherols (Method Ce 8-89), phytosterols (Method Ce 12-16), oxidative stability index ((Method Cd 12b-92). Detailed analysis method of oryzanol was described in our previous study (Chen, Jin, Zheng, Hu, & Jin, 2014).

2.8 Statistical analysis

All the experiments were performed in triplicate and presented as mean ± standard deviation (SD). Significant difference among groups was performed statistically by one-way analysis of variance (ANOVA) combined with Duncan’s multiple-range test using the SPSS 22.0. Statistical significance was expressed at $p<0.05$.

3. Result and Discussion
3.1 Effect of parameters on the yield of MLCT

The substrate weight ratio has a critical influence on the final equilibrium which is performed as yield of MLCT (Y.-Y. Lee et al., 2012). As shown in Fig. 1a., the yield of each substrate ratio reached the highest point at 15 min and then remained constant until the reaction ended. When the ratio changed from 40% to 60% the yield increased, while the ratio kept increasing from 60% to 80%, the yield decreased. The highest yield was 72.29% and was obtained by substrate ratio 60% at 20 min. Higher ratio of flaxseed oil leads to higher ALA content in final product and a decrease in MLCT yield and oxidation stability of the finally product. Substrate ratio had a significant effect ($p<0.05$) the yield of MLCT while showed no influence on reaction rate and catalyst activity. Therefore, the ratio of 60% was selected for subsequent experiments.

Reaction temperature was another considerable factor (Konishi, Neff, & Mounts, 1993). A large number of studied had been published and showed that temperature has impact on MLCT yield, catalyst activity and reaction rate (Korma et al., 2018; Yang et al., 2014). It was illustrated in Fig. 1b. that increasing reaction temperature would improve catalyst activity which also led to reaction rate increasing. The reaction rate increased markedly when temperature increased from 40°C to 60°C, ($p<0.05$); but keeping increasing temperature showed no positive improve on the rate. The interaction of substrates was poor at low temperature as a result of the viscosity decreasing the mass transfer rate, which led to a low reaction rate. When temperature reached at a high level, 60°C in this study, the viscosity and the catalyst activity stayed constant. Hence, keeping increasing temperature showed no
benefit on improving yield, but impaired the oxidative stability of PUFAs.

The effect of catalyst loading, an important factor, was also investigated. As Fig. 1c. showed, when catalyst loading was in the range of 0.1%-0.3%, increasing catalyst loading would significantly improve MLCT yield ($p<0.05$) and reaction rate. Conversely, catalyst loading showed no effect on MLCT yield and reaction rate in the range of 0.3%-0.5%. The highest yield (74.07%) was reached at 0.3% catalyst ration at 15 min. For two low catalyst loading, 0.1% and 0.2%, prolonging reaction distinctly improved yield from 7.04% to 32.11% and 28.92% to 64.92%, respectively. This agreed well with previous studies (Korma et al., 2018; J. Lu et al., 2017). However, the yield decreased from 70.03% to 66.09% at 40 min when catalyst loading was 0.5%. This may relate to the forming of byproducts which discussed later.

3.2 Effect of parameters on DAG content

DAG was a main byproduct of transesterification which produced together with free fatty acid (FFA) and had negative effect on product (Farmani, Safari, & Hamedi, 2009). It was necessary to monitor the content of DAG rather than FFA because of FFA could be partly removed through washing period. The DAG content of these five substrate ratios during forty minutes were showed in Fig. 1d. The contents of different substrate ratio showed the same trend that DAG content decreased to their lowest levels in 15 min, and then kept increasing. The DAG content of different substrate ratios in this reaction range from 11.24% to 14.36%. Substrate ratio showed no conspicuous effect on the content of DAG and side reaction rate.
DAG content increasing obviously with the increasing of reaction time rather than the improving of temperature as side products need time to accumulate. As Fig. 1e showed, DAG content increased faster as reaction temperature increased, while there was no significant difference in increasing rate observed in the first 10 min. The lowest content of DAG was 10.23% at 5 min when temperature was 60°C. The trend in DAG content was similar to what has been reported by Kowalshi, Tarnowska and Gruczynska (Kowalski, Tarnowska, & Gruczynska, 2005), but the content of DAG was higher than their result as the oil blend was different. Take the MLCT yield into consideration, it is important to limit reaction time together with temperature such as control temperature at 60°C and react for 15 min.

Fig. 1f. displays that the content of DAG, produced by side reaction, kept increasing when increasing the catalyst ratio, which was different from the changing of MLCT content. When reacting for 40 min, the DAG content, except for 0.3% catalyst loading, fluctuated slightly. The lowest content of DAG was 4.01% (0.1% catalyst loading and 5 min) while the highest one was 19.71% (0.5% catalyst ratio and 40 min). The catalyst loading had a significant effect on the DAG content. This may be attributed to the mechanisms of Chemical transesterification catalyzed by sodium methoxide. In the first period of CTE catalyzed by sodium methoxide, it took seconds or minutes for sodium methoxide to work with glycerides to produce the “real catalyst” which was called intermediate (Akoh & Min, 2008). DAG was formed when β-keto ester, converted from intermediate, underwent acid cleavage (Liu, 2004). Thus, it was clear that the more sodium methoxide was added the more DAG was created. According to this, 0.3% catalyst loading was chosen for the further transesterification.
Considering the yield of MLCT, the content of DAG and oxidative stability for industrial application, the substrate ratio of 60% flaxseed oil, catalyst loading of 0.3%, temperature of 60°C and reaction time of 15 min was selected as optimal.

3.3 Change in fatty acid composition and sn-2 fatty acid distribution

The difference in the fatty acid composition, sn-2 fatty acid distribution and the percentage of selected fatty acids in sn-2-position in relation to the whole acid content of product prepared under optimal parameters was showed in Tab.1. The fatty acid composition of the substrate before and after CTE was similar since the CTE showed no selectivity in fatty acid. What’s more, the percentage of ALA was 37.26% which exceed many kinds of edible vegetable oils like soybean oil, rapeseed oil, corn oil, sunflower oil. However, the sn-2 fatty acid distribution changed a lot as the percentage of saturated fatty acid (SFA) increased to 37.71% while PUFA decreased from 60.13% to 49.38%. As a past study showed (Ledochowska & Eilczynska, 1998), CTE would lead to a random distribution in FA distribution which agreed with the result that the percentage of each FA in sn-2 position was about 33% in this study. Hence, CTE is an efficient reaction which can completely randomize FA position in just 15 minutes.

3.4 Change in minor compounds, physicochemical properties and oxidative stability

Tocopherols are important minor compounds who play an important role as natural antioxidants in vegetable oils. Tocopherols can scavenge radicals in membrane and lipoprotein particles and prevent lipid peroxidation (Mohanan, Nickerson, & Ghosh, 2018). Compositions and contents of tocopherols were tabulated in Tab. 2, and it is clearly that there
were four isomeric forms of tocopherols existed. The content of \(\gamma\)-tocopherols was higher than \(\alpha\), \(\beta\), and \(\delta\)-tocopherols, decreasing from 257.1 to 142.80 mg/kg. It could be seen that the content of total tocopherols decreased significantly \((p<0.05)\) as they are more sensitive to heat and base. The initial content of total tocopherols was 285.15 mg/kg, whereas the content decreased to 167.36 mg/kg after CTE. This result here was mostly in accordance with those from previous studies by Ledóchowska and Wilczynska (Ledochowska & Eilczynska, 1998) and Zhao, Hu, Zhu and Li (Zhao et al., 2014).

Oryzanol can scavenge free radicals and inhibit lipid peroxidation and was consider as an important minor compounds in rice brain oils (Juliano, Cossu, Alamanni, & Piu, 2005). As demonstrated in Tab.2, the content of CTE product was significant lower than the substrate before CTE, 143.51 and 269.29 mg/kg, respectively. Such reduction might be due to loss of oryzanol during CTE for it is sensitive to high temperature and base (Akoh & Min, 2008).

The content of phytosterols were measured for reasons that some studies reported that there were interactions of phytosterols, tocopherols and oryzanol on oxidative stability (Akoh & Min, 2008). The total content of phytosterols were 3390.74-3478.38 mg/kg and cycloartenol was the primary form in both samples. There was no significant change showed in compositions and contents of phytosterols since they are stable to both heat and base environment (Akoh & Min, 2008).

Tab.2 also showed the oxidation induction time, AV and POV of substrate and CTE production. POV is an important index to evaluate peroxides while AV can represent the content of free fatty acids which are easily oxidized to hydroperoxides. The POV of all
samples were lower than 5 meq/kg oil. The AV increased from 0.19 to 0.87 after CTE.

The rancimat tests performed for all the samples showed that the induction time was remarkably increased from 9.91 to 11.39 h. The increase in induction time was different from past studies (Kowalski et al., 2005; T. Wang, Jiang, & Hammond, 2005) that the induction time of CTE products always decreased due to the higher content of FFA, DAG and MAG and the reduction in minor compounds, mainly tocopherols. This suggested that different experimental conditions, including the yield of MLCT, the content of DAG, minor compounds and FA distribution, may play an important role in the effect of randomization on TAG oxidative stability. Hence, the correlation between oxidation induction time and these factors may be evaluated more effectively and systematically.

3.5 Correlations between oxidation induction time, DAG content, FA distribution, AV, POV

A Pearson correlation coefficients (PCC) analysis was conducted in order to investigate the degree of linear correlation between every two parameters of the substrate and CTE product. As Tab.3 showed, the induction time was positively correlated with MLCT yield (r=0.985) and DAG content (r=0.987) while was negatively correlated with the percentage of PUFA in sn-2 position (r=-0.817), total tocopherols (r=-0.980), oryzanol (r=-0.964) and AV (r=-0.983).

In most studies, DAG was a major byproduct which had pro-oxidant activity and was effective in reducing oxidative stability. However, the result of this studies indicated that there was a highly significant correlation between DAG content and oxidation induction time.
It seems that the high level of DAG has benefit in inhibiting oil from oxidation. Mistry and Min (Mistry & Min, 1988) pointed that whether DAGs acted as pro-oxidant or anti-oxidant depended on its concentrations. The low doses of DAGs can reduce the surface tension and increase the diffusion of oxygen in oils while high doses of DAGs accumulate at the interface of oils to form a barrier and prevent further dissolution of oxygen (T. Wang et al., 2005).

Thus, it is not always necessary to reduce DAG content of MLCT products by purification to improve its stability. Similarly, MLCT yield was highly correlated to oxidation induction time. The MLCT and DAG had same effect on induction time since they coming from one same CTE reaction which had high correlation with each other (r=1.00).

The PUFA is less stable in oxidation than SFA and the higher percentage of PUFA in sn-2 position leads to higher oxidation stability as a past study found that the oxidative stability of ALA at the sn-2 position was higher than that at the sn-1,3 positions (Dote, Yamamoto, & Hara, 2016). Therefore, the percentage of PUFA in sn-2 position showed a negative effect on induction time. It was evident in numerous studies, conducted with a focus on CTE reaction, that the reduction of tocopherols showed significantly negative effect on oxidative stability. However, the result of this work indicated that the high content of tocopherols hasten oxidation which was also reported by Lee and Akoh (J. H. Lee, Akoh, & Lee, 2007). Bou, Codony, Baucells and Guardiola (Ballus, Meinhart, de Souza Campos Jr, & Godoy, 2015) found that excessive tocopherols could be easily oxidized, resulting in oxidation. Oryzanol also showed significantly negative effect on oxidative. What’s more, that interactions of minor compounds also played a part. Studies found they hindered the
antioxidant capacity of each other at specific concentrations. The AV reflects the content of FFAs which can accelerate oxidant and was negative correlated to induction time.

The result of the correlations analysis explained the relationships of changes in minor compounds, FA composition, AV and POV on oxidative stability. The factors can be divided into positive groups, including MLCT yield and DAG content, and negative groups, such as the percentage of PUFA in sn-2 position, tocopherols, oryzanol and AV. However, the correlation mechanism and optimal concentrations of these two group still require further research.

4. Conclusions

Chemical transesterification of flaxseed oil and MCT is a good method to prepare MLCT products rich in n-3 PUFA. Under the optimal conditions of substrate of 60%, catalyst loading of 0.3% at 60 °C for 15 min, the product contained 75.07% of MLCT, 37.26% of ALA and 10.33% of DAG. Moreover, the changes in the fatty acid composition, physicochemical properties, minor compounds and oxidation induction time were also observed. The correlations of these factors were also analyzed. Results indicated that the high concentrations of DAG and PUFA in sn-2 position can prolong the induction time while tocopherols, oryzanol and AV have contrary effects. It seems that there are composition effects of these factors on product oxidative stability which deserve further studies.

Acknowledgments

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Ethical Guidelines

Ethics approval was not required for this research.

Data Availability

Research data are not shared.

Conflict of interest statement

No conflict of interest exists for the submission of this manuscript, and manuscript is approved by all authors for publication.

Reference


Triacylglycerol and Soybean Oil Using a Pilot-Scale Solvent-Free Packed Bed Reactor.


### Tab. 1 Fatty acid composition of substrates and transesterification products

<table>
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<th>Fatty acids (%)</th>
<th>Before chemical transesterification</th>
<th>After chemical transesterification</th>
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<td></td>
<td>Total</td>
<td>Sn-2</td>
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<tr>
<td>Caprylic acid</td>
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<tr>
<td>Capric acid</td>
<td>14.52±0.01</td>
<td>13.71±0.18</td>
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<tr>
<td>Palmitic acid</td>
<td>3.96±0.07</td>
<td>1.60±0.29</td>
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<tr>
<td>Stearic acid</td>
<td>3.04±0.05</td>
<td>1.34±0.32</td>
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<tr>
<td>Oleic acid</td>
<td>12.39±0.08</td>
<td>17.43±0.95</td>
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<tr>
<td>Linoleic acid</td>
<td>10.37±0.05</td>
<td>17.87±0.10</td>
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<td>Linolenic acid</td>
<td>35.68±0.55</td>
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<td>ΣMCFA</td>
<td>34.58±0.19</td>
<td>17.00±1.31</td>
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<td>ΣSFA</td>
<td>41.58±0.31</td>
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<tr>
<td>ΣMUFA</td>
<td>12.39±0.05</td>
<td>17.43±0.03</td>
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<td>ΣPUFA</td>
<td>46.05±0.26</td>
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Tab. 2. Minor compounds, induction time, acid value and peroxide value of substrates and transesterification products

<table>
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<th>Compounds</th>
<th>Before chemical transesterification</th>
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<td><strong>Phytosterols (mg/kg)</strong></td>
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<td>Campsterol</td>
<td>488.75±59.28</td>
<td>489.93±59.38</td>
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<td>β-Sitosterol</td>
<td>1018.2±11.71</td>
<td>996.02±66.57</td>
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<td>Δ5-Avenaster</td>
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<td>254.88±13.58</td>
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<td>Cycloartenol</td>
<td>1103.67±107.78</td>
<td>1087.89±118.96</td>
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<td>2,4 Methylene cycloartenol</td>
<td>314.4±45.75</td>
<td>295.63±10.17</td>
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<td>Total phytosterols</td>
<td>3478.38±128.19</td>
<td>3390.74±49.93</td>
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<td><strong>Tocopherols (mg/kg)</strong></td>
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<td>α-Tocopherol</td>
<td>20.08±0.23</td>
<td>19.82±0.64</td>
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<tr>
<td>β-Tocopherol</td>
<td>2.95±0.11</td>
<td>3.37±0.13</td>
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<tr>
<td>γ-Tocopherol</td>
<td>257.10±8.36</td>
<td>142.80±3.34</td>
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<tr>
<td>δ-Tocopherol</td>
<td>5.03±0.88</td>
<td>1.37±0.03</td>
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<tr>
<td>Total tocopherols</td>
<td>285.15±7.14</td>
<td>167.36±2.86</td>
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<tr>
<td><strong>Oryzanol (mg/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>269.29±12.84</td>
<td>143.51±9.73</td>
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<tr>
<td><strong>Induction time (h)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.91±0.12</td>
<td>11.39±0.15</td>
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<tr>
<td><strong>Acid value (mgKOH/g oil)</strong></td>
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<tr>
<td></td>
<td>0.19±0.00</td>
<td>0.87±0.01</td>
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<tr>
<td><strong>Peroxide value (meq/kg oil)</strong></td>
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<tr>
<td></td>
<td>4.00±0.11</td>
<td>4.00±0.15</td>
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Tab. 3. Pearson correlation coefficients (PCCs) for oxidation induction time, DAG content, FA distribution, AV, POV and minor compounds.

<table>
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<tr>
<th></th>
<th>induction time</th>
<th>MLCT yield</th>
<th>DAG content</th>
<th>Total phytosterols</th>
<th>Total tocopherols</th>
<th>Oryzanol</th>
<th>AV</th>
<th>POV</th>
<th>PUFA in sn-2 position</th>
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<td>induction time</td>
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<tr>
<td>MLCT yield</td>
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<td>DAG content</td>
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<td>1.00**</td>
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<tr>
<td>Total phytosterols</td>
<td>0.435</td>
<td>0.340</td>
<td>0.352</td>
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<tr>
<td></td>
<td>Total tocopherols</td>
<td>Oryzanol</td>
<td>AV</td>
<td>POV</td>
<td>PUFA in sn-2 position</td>
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<td>-0.996**</td>
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<td>Oryzanol</td>
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<td>-0.988**</td>
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<td>AV</td>
<td>-0.983**</td>
<td>-0.999**</td>
<td>-0.999**</td>
<td>-0.341</td>
<td>0.998**</td>
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<td>POV</td>
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<td>-0.016</td>
<td>-0.828*</td>
<td>0.026</td>
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<td>-0.125</td>
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<td>PUFA in sn-2</td>
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<td>0.811</td>
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Note: *p < 0.05; **p < 0.01.

**Figure captions**

Fig.1. Effects of different factors on the changes of the yield of MLCT and DAG content. a: substrate ratio on MLCT yield; b: temperature on MLCT yield; c: catalyst loading on MLCT yield; d: substrate ratio on DAG content; e: temperature on DAG content; f: catalyst loading on MLCT yield.
Fig. 1. Effects of different factors on the changes of the yield of MLCT and DAG content. 

a: substrate ratio on MLCT yield; b: temperature on MLCT yield; c: catalyst loading on MLCT yield; d: substrate ratio on DAG content; e: temperature on DAG content; f: catalyst loading on MLCT yield.

279x151mm (150 x 150 DPI)
For Peer Review

Substrate

Flavored oil  MCT

Catalyst

Chemical Transesterification

Optimization of MLCTs preparing conditions

Analysis of Factors related to oxidative stability

379x302mm (150 x 150 DPI)