

## Research Article

# Functional monoesters of jojoba oil can be produced by enzymatic interesterification: Reaction analysis and structural characterization

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Long-chain monounsaturated fatty acid ethyl esters (LMFAEE) and fatty alcohol acetates (LMFAA) are important functional compounds in industry, and new sources are needed. In this study, jojoba oil was interesterified with ethyl acetate (EA) for the combined production of LMFAEE and LMFAA. Lipozyme RM IM and Novozym 435 were employed as the biocatalyst and their efficiencies were compared. Aliquot removal and subsequent  $^1\text{H}$  NMR analysis were used to monitor reaction progress and determine the interesterification yield (IY, %). Optimization of this reaction was conducted for both lipases with respect to four experimental parameters: temperature, EA content, immobilized lipase load, and reaction time. The highest IY in the presence of Novozym 435 was 97%, which was achieved after 9 hours of reaction at 37°C with 75 wt% EA and 5.00 wt% lipase load at 300 rpm, whereas the IY was 76% in the case of Lipozyme RM IM. The interesterified product was purified by preparative TLC and characterized by  $^1\text{H}$  NMR, FT-IR and GC-MS, indicating the main components are ethyl *cis*-11-eicosenoate (44%), *cis*-11-eicosenyl acetate (25%) and *cis*-13-docosenyl acetate (22%). Novozym 435 exhibited excellent operational stability over 17 interesterification cycles with ca. 12% IY decrease.

**Practical applications:** Conversion of the rare LMF-acid/alcohol source from jojoba oil to LMFAEE and LMFAA was realized in a one step interesterification reaction. The obtained jojobyl LMFAEE and LMFAA, in mixture or purified form, can serve as functional ingredients in various upmarket products, such as functional lipids, fragrances, cosmetics, pharmaceuticals and lubricants. *Cis*-11-eicosenyl acetate and *cis*-13-docosenyl acetate are also important insect pheromones in biological study. Additionally, the lipase reuse can help reduce cost significantly, making jojoba oil more economically attractive for industrial application.

**Keywords:** Interesterification / Jojoba oil / Lipase / Long-chain monounsaturated fatty acid ethyl esters / Long-chain monounsaturated fatty alcohol acetate

Received: September 15, 2014 / Revised: October 29, 2014 / Accepted: October 31, 2014

DOI: 10.1002/ejlt.201400458



Additional Supporting information online <http://dx.doi.org/10.1002/ejlt.201400458>.

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**Abbreviations:** LMF-acid, long-chain monounsaturated fatty acid; LMFAEE, long-chain monounsaturated fatty acid ethyl ester; LMF-Alcohol, long-chain monounsaturated fatty alcohol; LMFAA, long-chain monounsaturated fatty alcohol acetate; EA, ethyl acetate; IY, interesterification yield

## 1 Introduction

Long-chain monounsaturated fatty acid ethyl ester (LMFAEE) and fatty alcohol acetate (LMFAA) are extensively utilized in many high-end products, including senior lubricants, paints, cosmetics, fragrances and pharmaceutical formulations, in addition to their traditional use as functional lipids and food additives [1–4]. Ethyl oleate is found to be efficacious in weight management by inducing a sensation of satiety and controlling appetite [5]. It is also a regular delivery vehicle and viscosity-reducing solvent for intramuscular drug

preparations [6–8]. Apart from fruit coating and flavoring, oleyl acetate is incorporated into various skin care and personal care products for its anti-lipase ability [9–12]. Acetylated monounsaturated fatty alcohols with 18 to 22 carbons are insect pheromones of exceptional value for insecta mate study and social behavior investigation [13, 14]. Given the continuous expansion of their application scope and quantity demanded in both industry and academia, interest was provoked to search for new and convenient sources of long-chain monounsaturated fatty acid/alcohol (LMF-acid/alcohol) and their LMFAEE and LMFAA derivatives.

Jojoba plant (*Simmondsia chinensis* Link Schneider) is commonly grown and harvested in desert regions of the US, Mexico, Israel, Argentina, Australia and Middle East [15, 16]. The jojoba seeds contain 40% to 65% of their weight in oil [15], of which the features differ fundamentally from other plant oils and animal fats, as it is a mixture of long straight chain monoesters of LMF-Acid and LMF-Alcohol (Figure 1) instead of TAG [16, 17]. In particular, about 80% of jojoba oil is composed by C<sub>40</sub> and C<sub>42</sub> monoesters of *cis*-11-eicosenoic acid and *cis*-11-eicosenol and *cis*-13-docosenol [17]. It is such structure imparts jojoba oil unique nature of a liquid wax with low and constant viscosity over different temperatures, excellent lubricity and other physicochemical properties (e.g., low chemical reactivity, high boiling, smoke, flash and fire point, etc.) [18, 19]. To date, jojoba oil has replaced sperm whale oil as major natural source of liquid wax esters in lubricants and other industrial applications [16].

In the last two decades, jojoba oil has been modified in various methods for new applications [16]. It could be easily hydrogenated into lustrous pearly-white solid wax, which can be used in polish waxes, penicillin drugs, fruit waxing and candle coating [16]. Hydrolyzed jojoba esters, produced from the reaction of jojoba oil with potassium hydroxide, is commercialized as emollient that can help skin remain consistent moisture, enhance formulation barrier function and sensory silky-feel when incorporated into cosmetic and personal care products [20]. However, to the best of our

knowledge, little attention has been paid to jojoba oil as a rarely enriched source of *cis*-11-eicosenoic acid (71.3% in total fatty acid content), *cis*-11-eicosenol and *cis*-13-docosenol (43.8% and 44.9%, respectively, in total fatty alcohol content) for functional LMFAEE and LMFAA production [21].

In this study, the LMF-acid/alcohol of jojoba oil is fully utilized via one step enzymatic interesterification with ethyl acetate (EA), for the combined production of jojobyl LMFAEE and LMFAA. The reaction was catalyzed by two commercialized lipases, Lipozyme RM IM and Novozym 435, respectively (Figure 1). <sup>1</sup>H NMR spectroscopy, a convenient and fast technique recently used in many investigations to quantify lipid production [22–25], was utilized in the current study to monitor the progress of reaction and determine the interesterification yield (IY, %). The product was purified by preparative TLC and characterized by <sup>1</sup>H NMR, FT-IR and GC-MS. To optimize the reaction conditions, time course and the effects of reaction temperature, EA content and immobilized lipase load on the IY were examined for both lipases. Operational stability of Novozym 435 was also evaluated. The produced jojobyl LMFAEE and LMFAA are of great potential and versatility in a wide range of industrial applications. Meanwhile, *cis*-11-eicosenyl acetate and *cis*-13-docosenyl acetate, the main components of jojobyl LMFAA, are valuable compounds for insecta biological study.

## 2 Materials and methods

### 2.1 Oils and reagents

Jojoba oil was purchased from NOW<sup>®</sup> Solution (IL, USA) and characterized by <sup>1</sup>H NMR (Figure S1, Table S1). EA was purchased from RCI Labscan Limited (Bangkok, Thailand). Lipozyme RM IM (*Rhizomucor miehei* lipase, immobilized on an anion exchange resin) and Novozym 435 (Lipase acrylic resin from *Candida antarctica* B) were generous gifts from Novo Nordisk A/S, Bagsvaerd, Denmark. Deuterated chloroform (CDCl<sub>3</sub>) with tetramethylsilane (TMS, v/v 0.03%) was purchased from Cambridge Isotope Laboratories Inc. (MA, USA).

### 2.2 Enzymatic interesterification

Three grams of jojoba oil and a certain amount of EA were added into a sealed round bottom flask. After stirring the substrates at given temperature for 5 minutes, a certain amount of Lipozyme RM IM or Novozym 435 was added to start the reaction. Reaction conditions were optimized via different sets of experiments when reaction temperature, EA amount, immobilized lipase load and reaction time were varied, respectively. Aliquots (700 μL) were collected periodically from the reaction system, evaporated under

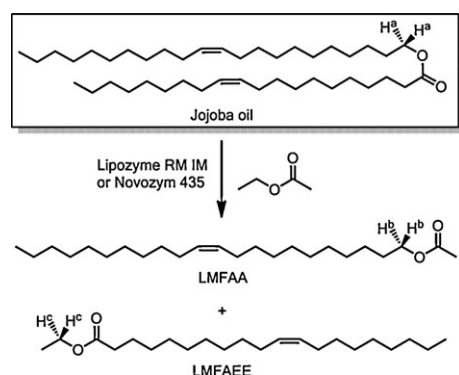


**Figure 1.** Typical structure of jojoba oil ester and its interesterification with EA catalyzed by Lipozyme RM IM and Novozym 435, respectively.

vacuum to remove surplus EA, and then prepared for  $^1\text{H}$  NMR analysis. The reaction ended when lipase were removed by filtration. The resulted jojobyl LMFAEE and LMFAA were purified from the reaction mixture via preparative TLC (Eluent: *n*-hexane/EA = 25:1 v/v) and characterized by  $^1\text{H}$  NMR (Figure S2, S3) and FT-IR.

Jojobyl LMFAEE:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 5.34 (m, 2H,  $-\text{CH}=\text{CH}-$ ), 4.11 (q,  $J_1 = 7.2$  Hz,  $J_2 = 14.4$  Hz, 2H,  $-\text{CH}_2\text{OCOR}$ ), 2.28 (t,  $J = 7.6$  Hz, 2H,  $-\text{CH}_2\text{COOR}$ ), 2.00 (m, 4H,  $=\text{CHCH}_2-$ ), 1.61 (m, 2.6H,  $-\text{CH}_2\text{CH}_2\text{COOR}$  and  $\text{H}_2\text{O}$ ), 1.27 (m, 27.5H,  $-(\text{CH}_2)_n-$  and  $\text{CH}_3\text{CH}_2\text{OCOR}$ ), 0.88 (t,  $J = 6.8$  Hz, 3H,  $\text{CH}_3(\text{CH}_2)_n-$ ). FT-IR ( $\text{cm}^{-1}$ , air): 3154, 2957, 2928, 2857, 2253, 1730, 1466, 1375, 1250, 1217, 1096, 1045, 912, 905, 745, 727, 650.

Jojobyl LMFAA:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 5.34 (m, 2H,  $-\text{CH}=\text{CH}-$ ), 4.05 (t,  $J = 6.8$  Hz, 2H,  $-\text{CH}_2\text{OCOR}$ ), 2.00 (m, 7H,  $=\text{CHCH}_2-$  and  $\text{CH}_3\text{COOR}$ ), 1.61 (m, 2.9H,  $-\text{CH}_2\text{CH}_2\text{OCOR}$  and  $\text{H}_2\text{O}$ ), 1.27 (m, 30.9H,  $-(\text{CH}_2)_n-$ ), 0.88 (t,  $J = 6.8$  Hz, 3H,  $\text{CH}_3\text{CH}_2-$ ). FT-IR ( $\text{cm}^{-1}$ , air): 3154, 2926, 2855, 2253, 1726, 1466, 1379, 1252, 1215, 1096, 1036, 908, 735, 650.

### 2.3 IY quantification by $^1\text{H}$ NMR

The samples were dissolved in  $\text{CDCl}_3$  at a ratio of 1:5 by volume and analyzed in 5 mm NMR tubes. Chemical shifts were referred indirectly to TMS signal ( $\delta = 0.0$  ppm) by calibrating the residual signal from monoprotonated  $\text{CHD}_2\text{Cl}_3$  to 7.26 ppm.  $^1\text{H}$  NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz with QNP probe (5 mm). Figures were processed using Bruker's TopSpin<sup>TM</sup> software.

The region between 4.00 and 4.15 ppm of the  $^1\text{H}$  NMR spectra of jojoba oil and the treated reaction aliquots exhibited the signals generated by  $\text{H}^a$  and combination of  $\text{H}^{a-c}$ , respectively (Figure 1). Specifically, in the spectra of the treated interesterification reaction mixture, signals of  $\text{H}^a$  and  $\text{H}^b$  overlapped perfectly around 4.05 ppm as one triplet, and signal of  $\text{H}^c$  was a quartet around 4.11 ppm. No overlap has been observed and both of the triplet and the quartet are perfect as its own (Figure 2). Hence, the IY, or the total yield of jojobyl LMFAEE and LMFAA, can be quantified in terms of the percentage of reacted jojoba oil, namely the jojobyl LMFAEE content divided by the total content of unreacted jojoba oil and jojobyl LMFAA. It is obtained directly from the area (A) ratio of the selected signals,  $\text{IY} (\%) = A_q/A_t \cdot 100$ , where  $A_q$  and  $A_t$  are the areas of signals of the quartet and the triplet, respectively.

### 2.4 GC-MS analysis of jojobyl LMFAEE and LMFAA compositions

The samples were analyzed on a Thermo TR-5MS column (30 m  $\times$  0.25 mm I.D.  $\times$  0.25  $\mu\text{m}$  film), in a ThermoFinnigan

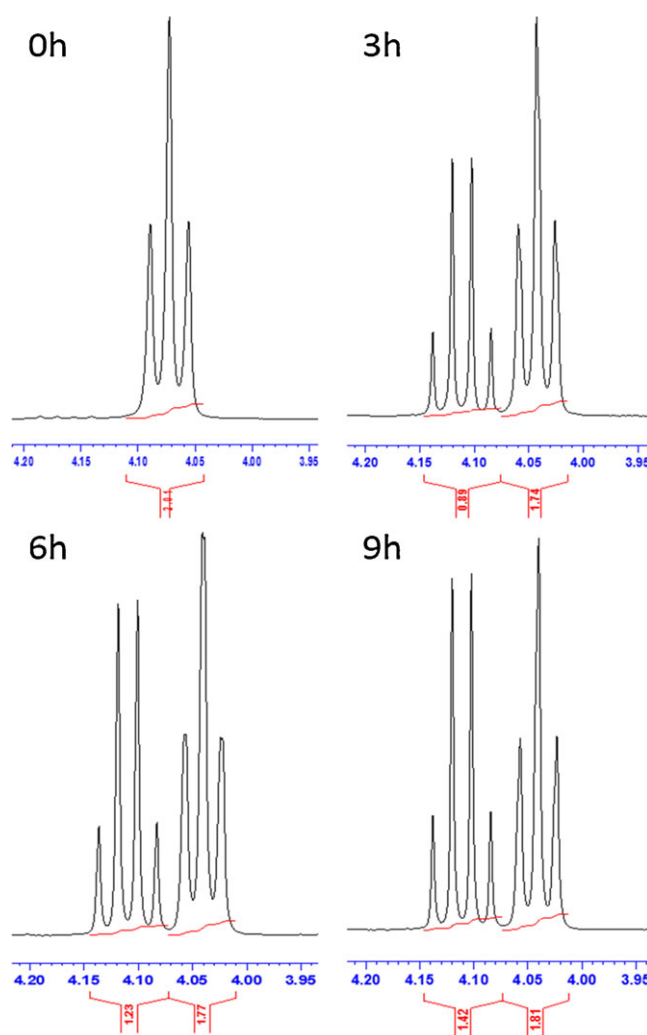


**Figure 2.** Expansion of the  $\delta$  4.00–4.15 regions of the  $^1\text{H}$  NMR spectra (400 MHz, 8 scans,  $\text{CDCl}_3$ ) of the treated reaction mixture as a function of time on the interesterification of jojoba oil with EA.

PolarisQ MS system which is equipped with Thermo Trace GC. The injector and detector temperature were set at 300°C, and the column temperature was programmed from 80°C to 180°C at 25°C/min, from 180°C to 280°C at 5°C/min, from 280°C to 300°C at 15°C/min, and finally kept at 300°C for 8 min. The injection volume is 0.5  $\mu\text{L}$  and sample concentration is 1 ppm.

## 3 Results and discussion

### 3.1 IY quantification by $^1\text{H}$ NMR

Since the methylene hydrogen atoms within EA can also generate a quartet in the same region as the  $\text{H}^c$  within jojobyl LMFAEE, the remaining EA in reaction mixture would affect

the quantification of IY. Thus, it must be completely removed before  $^1\text{H}$  NMR analysis.

Direct quantification for the yield of jojobyl LMFAEE and LMFAA in the EA-removed interesterification reaction mixture, was realized by the quantitative  $^1\text{H}$  NMR method described in section 2.3. The agreement between the values calculated from  $^1\text{H}$  NMR spectra data and those from the weights was good (within 2% of error). To confirm the results, each set of reaction was triplicated and the data shown are expressed as the mean values (within 5% experimental uncertainties).

### 3.2 Variation of lipases on IY

Two commercially available immobilized lipases, namely Lipozyme RM IM and Novozym 435, were studied on their catalytic efficiency on the current interesterification under different reaction conditions. An agitation speed of 300 rpm was maintained in the current experiments as it is high enough for both Lipozyme RM IM and Novozym 435 to minimize the limitations of mass transfer and devoid of significant mechanical destruction on the lipase carrier in interesterification reactions at the same time [26–28].

Time course of the reactions was recorded when the two lipases were employed under their own optimized reaction conditions. As presented in Figure 3, both of the IY curves rose very fast in the first 4 hours while it began to slow down in the period thereafter (5<sup>th</sup> – 8<sup>th</sup> hour) until stable conversion (9<sup>th</sup> hour onwards) was obtained. However, the IY values from Novozym 435 sets were much higher than those obtained from Lipozyme RM IM sets at each time point, and the highest IY in the presence of Novozym 435 was 97%, whereas it was only 76% for Lipozyme RM IM, suggesting a much stronger catalytic ability of Novozym 435 in this specific reaction system. Therefore, Novozym 435 was selected for further studies.

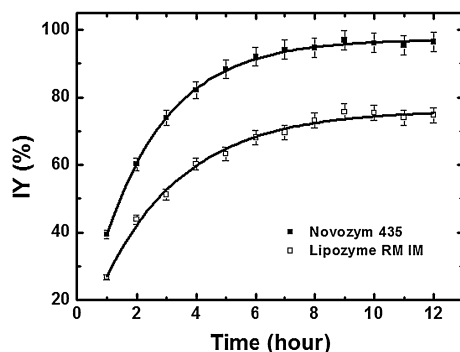


**Figure 3.** Time course of the optimized interesterification between jojoba oil and EA catalyzed by Lipozyme RM IM and Novozym 435, respectively. Reaction conditions: 9 hours, 75 wt% EA, 5.00 wt% lipase load, 300 rpm for both lipases, 30°C and 37°C for Lipozyme RM IM and Novozym 435, respectively. Optimization of reaction conditions for Lipozyme RM IM is demonstrated in Figure S4.

### 3.3 Effect of reaction temperature on IY

Temperature is an important factor in the enzymatic reaction, for it directly affects both stability and catalytic activity of lipase. As shown in Figure 4a, the IY value increased as the temperature was raised firstly, because high temperature accelerates the reaction by reducing the viscosity of lipid mixture and therefore enhancing the mass transfer on the surface and inside the lipase particles [26, 29]. However, higher temperature would also inactivate the lipase and reduce its operational stability and catalytic reactivity [27, 30]. Hence, it is reasonable that the IY value decreased after

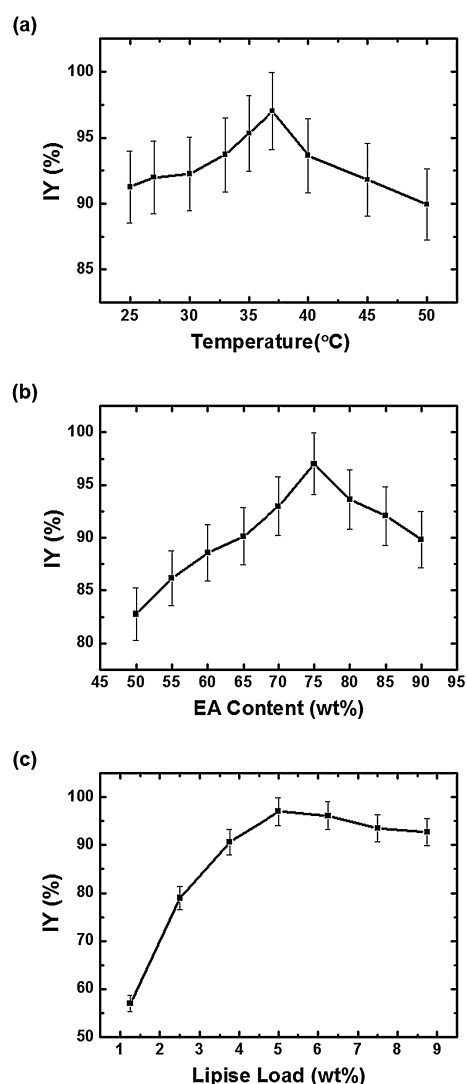


**Figure 4.** Effects of (a) reaction temperature, (b) EA content, and (c) immobilized lipase load on the interesterification between jojoba oil and EA catalyzed by Novozym 435. Reaction conditions: (a) 75 wt% EA, 5.00 wt% lipase load, 9 hours, 300 rpm; (b) 5.00 wt% lipase load, 9 hours, 37°C, 300 rpm; (c) 75 wt% EA, 9 hours, 37°C, 300 rpm.

reaching the peak point at 37°C. Based on these results, 37°C is the optimum temperature for Novozym 435 to perform interesterification in this reaction system and it was fixed for subsequent reactions.

### 3.4 Effect of EA content on IY

Stoichiometrically, at least one mole of EA (12 wt%) is required to convert one mole of jojoba oil completely to the corresponding interesterified products. In the current experiment, the amount of EA was raised to 50–90 wt% in order to shift the interesterification equilibrium to give higher yields of jojobyl LMFAEE and LMFAA, and decrease the viscosity of the oil reaction system at the same time. As illustrated in Figure 4b, the maximum conversion was obtained when EA content was 75 wt%. EA amount exceeding this value led to lower IY as a result of over-dilution of the reaction mixture and inefficient collisions between substrates and lipase [31]. Therefore, the usage of EA was settled at 75 wt% in the following experiments.

### 3.5 Effect of immobilized lipase load on IY

In a further effort to study the interesterification conversion, the reaction was performed with different amount of Novozym 435. In Figure 4c, the IY value rose significantly when lipase load was increased from 1.25 to 5.00 wt%. Further addition of lipase would not elevate IY, because the reaction had come to a saturation point where the concentration of substrates cannot satisfy the lipase [29]. More importantly, in viscous oil reaction system, the lipase might block the carrier (macroporous acrylic resin) if high dosage is charged and the reaction mixture becomes more viscous, consequently resulting in a hindrance to the mass transfer between substrates and lipase [32, 33]. And this might explain the slight decrease in IY when lipase load was raised from 5.00 to 8.75 wt%. Thus, from the aspect of both efficiency and economy, the optimum immobilized lipase load was determined to be 5.00 wt%.

### 3.6 Stability of Novozym 435

Reuse of the lipase has significant implications for enzymatic process economics. In the current experiments, the operational stability of immobilized lipase Novozym 435 was examined under the optimized reaction conditions. Before reuse, the same lipase was regenerated by washing with hexane at the end of each interesterification cycle. From the data given in Figure 5, it is obvious that Novozym 435 exhibited excellent stability as an enzyme catalyst at least for 17 reaction cycles (153 hours) at 37°C, maintaining an average IY of 88%. As the cost of lipase can be reduced significantly by simple regeneration, this method is more economically attractive when industrialized for functional LMFAEE and LMFAA production.

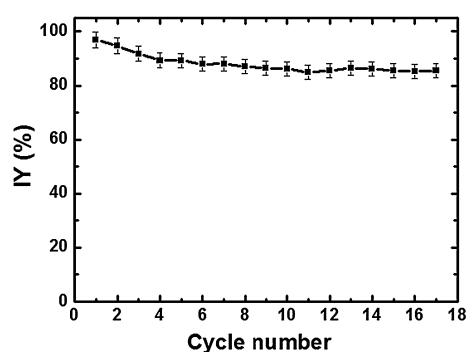


**Figure 5.** Operational stability of Novozym 435 in the interesterification between jojoba oil and EA. Reaction conditions: 75 wt% EA, 5.00 wt% lipase load, 9 hours, 37°C, 300 rpm.

### 3.7 Composition analysis of jojobyl LMFAEE and LMFAA

The profiles of jojobyl LMFAEE and LMFAA produced in the current interesterification reaction are shown in Table 1 and 2, respectively. Absorption around 730 cm<sup>-1</sup> and the

**Table 1.** Composition of jojobyl LMFAEE analyzed by GC-MS

| Compound                            | Fatty acid component | R <sub>t</sub> (min) | Content (%) |
|-------------------------------------|----------------------|----------------------|-------------|
| Ethyl tetradecanoate                | C(14:0)              | 7.83                 | 0.01        |
| Ethyl <i>cis</i> -9-hexadecenoate   | C(16:1)              | 10.34                | 0.02        |
| Ethyl hexadecanoate                 | C(16:0)              | 10.62                | 0.49        |
| Ethyl <i>cis</i> -9-octadecenoate   | C(18:1)              | 13.19                | 3.25        |
| Ethyl <i>cis</i> -11-eicosenoate    | C(20:1)              | 16.28                | 88.37       |
| Ethyl eicosanoate                   | C(20:0)              | 16.75                | 0.11        |
| Ethyl <i>cis</i> -13-docosenoate    | C(22:1)              | 19.30                | 7.27        |
| Ethyl docosanoate                   | C(22:0)              | 19.67                | 0.18        |
| Ethyl <i>cis</i> -15-tetracosenoate | C(24:1)              | 22.21                | 0.27        |
| Ethyl tetracosanoate                | C(24:0)              | 22.56                | 0.02        |

**Table 2.** Composition of jojobyl LMFAA analyzed by GC-MS

| Compound                            | Fatty alcohol component | R <sub>t</sub> (min) | Content (%) |
|-------------------------------------|-------------------------|----------------------|-------------|
| <i>Cis</i> -9-tetradecenyl acetate  | C(14:1)                 | 7.91                 | 0.18        |
| 1-Hexadecanyl acetate               | C(16:0)                 | 10.60                | 0.03        |
| <i>Cis</i> -9-octadecenyl acetate   | C(18:1)                 | 13.56                | 0.59        |
| 1-Octadecanyl acetate               | C(18:0)                 | 13.84                | 0.12        |
| <i>Cis</i> -11-eicosenyl acetate    | C(20:1)                 | 16.51                | 49.66       |
| 1-Eicosanyl acetate                 | C(20:0)                 | 16.92                | 0.18        |
| <i>Cis</i> -13-docosenyl acetate    | C(22:1)                 | 19.54                | 44.26       |
| 1-Docosanyl acetate                 | C(22:0)                 | 19.87                | 0.59        |
| <i>Cis</i> -15-tetracosenyl acetate | C(24:1)                 | 22.41                | 4.30        |
| 1-Tetracosanyl acetate              | C(24:0)                 | 22.72                | 0.09        |

absence of absorption around  $970\text{ cm}^{-1}$  in FT-IR indicate that all ethylenic bonds are in *cis* configuration. Ethyl *cis*-11-eicosenoate predominates in the jojobyl LMFAEE with a content of ca. 88%. Meanwhile, the jojobyl LMFAA mainly consists of *cis*-11-eicosenyl acetate (ca. 50%) and *cis*-13-docosenyl acetate (ca. 44%). In other words, the main compositions of the total interesterified product are ethyl *cis*-11-eicosenoate (44%), *cis*-11-eicosenyl acetate (25%) and *cis*-13-docosenyl acetate (22%).

## 4 Conclusions

The jojobyl LMFAEE and LMFAA were produced through enzymatic interesterification of jojoba oil and EA. The highest IY of 97% in the presence of Novozym 435 was obtained under its optimized reaction conditions (9 hours,  $37^\circ\text{C}$ , 75 wt% EA, 5.00 wt% lipase load), which is much higher than that of Lipozyme RM IM (76%). The interesterified product mainly contains ethyl *cis*-11-eicosenoate (44%), *cis*-11-eicosenyl acetate (25%) and *cis*-13-docosenyl acetate (22%). Novozym 435 showed excellent stability over 17 interesterification cycles with a ca. 12% decrease in IY.

Our method produces functional LMFAEE and LMFAA in one simple reaction, avoids costly separation and purification, and maximizes the utilization of the LMF-acid/alcohol from jojoba oil. The resulting jojobyl LMFAEE and LMFAA, in mixture or purified form, have great potential and versatility for both industrial applications and academic studies.

*The authors wish to express their gratitude to Nanyang Technological University for financial supports through research grants, M4011125.110 (Tier 1), M4011062.110 (Tier 1) and M4081461.110 (iFood Research Grant), to Tianhu Li.*

*The authors have declared no conflict of interest.*

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