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Interpretation of triacylglycerol profiles of palm oil, palm kernel oil and their binary blends

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Abstract

The effects of lipase-catalyzed interesterification (IE) on changes in the chemical composition of palm oil (PO), palm kernel oil (PKO) and their binary blends at 3:1, 1:1 and 1:3 (w/w) ratios, using both 1,3 specific *Rhizomucor miehei*, (Lipozyme™) and non-specific *Pseudomonas* sp. lipases were evaluated. IE of the native PO and PKO showed very distinct chemical composition changes. Catalysis of PO, using both lipases, caused synthesis of more medium and long chain triacylglycerols (TAG), with MMM/OLL, MMP, OOO and PPP (M, myristic acid; O, oleic acid; L, linoleic acid; P, palmitic acid) increasing in concentration. In contrast, IE of PKO resulted in the formation of more short and medium chain TAG, with LaLaO and LaMO (La, lauric acid; C, capric acid) experiencing noteworthy increments. Both *Rhizomucor miehei* and *Pseudomonas* sp. lipases showed high affinity in hydrolyzing PO fatty acids, resulting in high TAG losses and formation of high percentages of partial glycerides while these lipases were found to enhance the synthesis process in IE of PKO. Catalysis of the three binary blends caused similar TAG compositional changes where the synthesis process focussed on the medium chain TAG, while hydrolysis was observed in the short and long chain TAG that showed corresponding decreases. Catalysis of the three blends was influenced by the major fraction of these blends. Among these blends, PO: PKO at a 1:1 ratio exhibited the highest degree of IE. The diversity and quantity of available TAG are postulated to be the main causes of the different catalytic activities in these binary blends with *Pseudomonas* sp. lipase showing a higher degree and rate of IE than *R. miehei*.

Keywords: Binary blends; Fatty acid preference; Lipase-catalyzed interesterification; Palm oil; Palm kernel oil; Positional specificity; *Pseudomonas* sp.; *Rhizomucor miehei*; Triacylglycerol composition changes

1. Introduction

Most native vegetable oils, such as PO and PKO, have limited applications in their original forms due to their specific chemical composition. To widen their commercial use vegetable oils are modified, either physically by fractionation or blending, or chemically, by hydrogenation or IE (Anderson, 1996; Hauman, 1994). Blending among vegetable oils gives the manufacturer greater flexibility to tailor the products to accomplish specific functional properties or satisfy nutritional requirements. However, blending does not result in the chemical modification of the TAG composition. Further modifications of the TAG are necessary when the blended oils have very different physical properties that could result in fractionation.

Fats can also be suitably modified by hydrogenation. However, hydrogenation produces *trans*-fatty acids. Excessive intake of *trans*-fatty acids had been recognized to be associated with the increased risks of coronary heart disease, cardiovascular and other diseases (Enig, 1996; Lichtenstein, 1993; Weststrate, 1995). Consequently, the food industry is replacing hydrogenation with other processes.

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Other alternatives that can give fats and oils the desired functionality included chemical- and lipase-catalyzed IE.

The use of lipases in fats and oils modification has many benefits compared to chemical processes. Lipase is well known for its efficacy under mild conditions (pH and temperature), thus reducing cost and energy consumption. The use of lipases in a natural reaction system could reduce environmental pollution. The availability of lipases from a wide range of sources offers a tremendous potential in postproduction modification for industrial application (Yamane, 1987). However, the most important property of lipases that has led to their overwhelming interest is their specificity. Lipases impart specificity to a reaction that offers greater control over the positional distribution of fatty acid in fats and oils (Marangoni & Rousseau, 1995; Willis & Marangoni, 1998), which has been shown to be a versatile tool for the preparation of a wide variety of novel TAG (Ghazali, Hamidah, & Che Man, 1995).

A range of microbial lipases can catalyze IE reactions to various degrees, with *Pseudomonas* sp. and *R. miehei* lipases among the most reactive (Ghazali et al., 1995; Lai, Ghazali, & Chong, 1998; Liew, Ghazali, Long, Lai, & Yazid, 2001). However, Lipozyme IM (Novozyme, Denmark) has been the only commercial immobilized lipase used for the production of specific structured TAG (Xu, 2000). Lipase-catalyzed IE of PO and PKO with other oils had been investigated, including palm stearin–palm kernel olein (Lai, Ghazali, & Chong, 1995), palm kernel olein–anhydrous milk fat (Liew et al., 2001), palm stearin–sunflower oil (Lai et al., 1998, Lai, Ghazali, & Chong, 1999), and palm stearin–anhydrous milk fat (Lai, Ghazali, Cho, & Chong, 2000a, 2000b). There is hardly any literature available on the binary blends of PO and PKO and the present work aims to fill that void.

In this study, the chemical modification of transesterified PO, PKO and their binary blends (3:1, 1:1 and 1:3), using the 1,3-specific *R. miehei* and non-specific *Pseudomonas* sp. lipases is reported. The efficacy and catalytic nature of the lipases with different regiospecificities and the changes in chemical composition when using different substrates were of particular interest.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorized PO (RBD PO) and RBD PKO were supplied by the Malaysia Palm Oil Board (MPOB). The oils were first melted and homogenized completely at 70 °C. This was followed by hot filtration under suction through a Whatman No. 1 filter paper, to remove any fibrous solid particles prior to use. Binary blends of hot liquefied PO and PKO at 60 °C were made in the following mass (w/w) ratios of PO to PKO (PO:PKO): (i) 3:1; (ii) 1:1; (iii) 1:3.

Pseudomonas sp. lipase, in powder form (Lipase PS), was a generous gift from Amano Pharmaceutical Co. Ltd. (Nagoya, Japan), while *R. miehei* lipase (Lipozyme IM60) was obtained in immobilized form (immobilized on a macroporous anionic exchange resin with a particle diameter of 0.2–0.6 μm; moisture content, 2–3%) from Novozyme (Copenhagen, Denmark). Celite, used as the carrier for the immobilization of the *Pseudomonas* sp., was purchased from BDH Ltd. (Poole, England). All other chemicals were either of analytical or high-performance liquid chromatography (HPLC) grades.

2.2. Methods

2.2.1. Lipase immobilization

The immobilization of *Pseudomonas* sp. lipase on Celite was done according to the method described by Ghazali et al. (1995). 0.1 g of enzyme powder was first dissolved in 100 μl of cold deionized water, followed by thorough mixing with 0.25 g Celite. The preparation was lyophilized for 4 h at −43 °C using an Alpha 1–4 Christ LDC-1 freeze-dryer (B. Braun, Melsungen, Germany) prior to the IE process. *R. miehei* lipase was used as is in its immobilized form.

2.2.2. Interesterification reaction

The reaction mixtures composed of 5 g of PO, PKO or their binary blends were placed in a 50 ml conical flask. An equivalent 0.05 g of lyophilized immobilized lipase preparation (Celite-bound *Pseudomonas* sp. or Lipozyme IM60) was added to the oil samples. The reaction mixture was then agitated in an oven orbital shaker at 200 rpm and 50 °C, where each reaction was done in triplicate. Individual sample flasks were removed and analyzed at various time periods of 0, 3, 6, 9, 12, 24 and 48 h. The immobilized enzyme was removed from the mixtures by simple filtration using Whatman No.1 filter paper. The TAG profiles of the reacted sample at various times were analyzed by HPLC without the removal of free fatty acids (FFA). A control containing no added enzymes was prepared.

2.2.3. Hydrolytic activity

To determine the degree of hydrolysis throughout the IE reaction at various incubation periods, 2 g of filtered oil sample from the reaction mixtures were dissolved in 20 ml of acetone/ethanol (1:1). The amount of FFA present in the reaction mixture was then determined by titration with 0.1 N NaOH to the phenolphthalein end-point. Results reported are the averages, of triplicates. Degree of hydrolysis was thus expressed as the percentage of FFA liberated per gramme of oil sample (Cocks & van Rede, 1966)

$$\text{Percentage of FFA} = \frac{(V_s - V_c)NM}{10W},$$

where V_s is the volume of NaOH required for sample (ml), V_c is the volume of NaOH required for control (ml), N is the normality of NaOH (0.1 N), M is the Molecular weight of the most abundant FFA in the oil (256) and W is the Weight of oil used for titration (g).

2.2.4. TAG analysis

The TAG profiles of PO, PKO and their binary blends (control and lipase-catalyzed samples) were analyzed by non-aqueous reversed-phase HPLC in a Shimadzu liquid chromatograph LC-10AD and SLC-10A chloroform (w/w) were injected into the HPLC (Ghazali et al., 1995). The total run time was 45 min. Figs. 1(a,b) and Fig. 7 illustrated the typical TAG profiles of PO, PKO and the 1:1 binary blends where the peaks were previously identified with various TAG standards in the works of Swe, Che Man, and Ghazali (1995), Ghazali et al. (1995), Haryati, Che Man, Ghazali, Asbi, and Buana (1998) and Tan and Che Man (2000).

The total concentration of TAG present in reaction mixtures was calculated by subtracting the concentration of FFA, monoacylglycerols (MAG) and diacylglycerols (DAG) from the total concentration of all acylglycerols recorded on the HPLC chromatogram. TAG concentration of PO was calculated, based on the acylglycerols eluted after 12 min (Ghazali et al., 1995; Swe et al., 1995). TAG were calculated, based on the acylglycerols eluted after 5 min for PKO (Liew et al., 2001). Percentage TAG (% TAG) remaining was then calculated, based on the total concentration of TAG after reaction compared with that of the unreacted mixtures (Ghazali et al., 1995; Liew et al., 2001). The concentration of TAG that had increased in value [$TAGI_t$] at reaction time, t, was calculated, using the following formula:

$$[TAGI_t] = \frac{\text{Total peak area of TAG that increased in concentration}}{\text{Total peak area of TAG in the reaction mixture}} \times 100.$$

The degree of IE (% IE) is defined as the change of TAG peak areas that showed continuous and constant increases in value during the course of reaction [$TAGI_t$], with respect to the value at the start of the reaction [$TAGI_0$] (Ghazali et al., 1995; Liew et al., 2001)

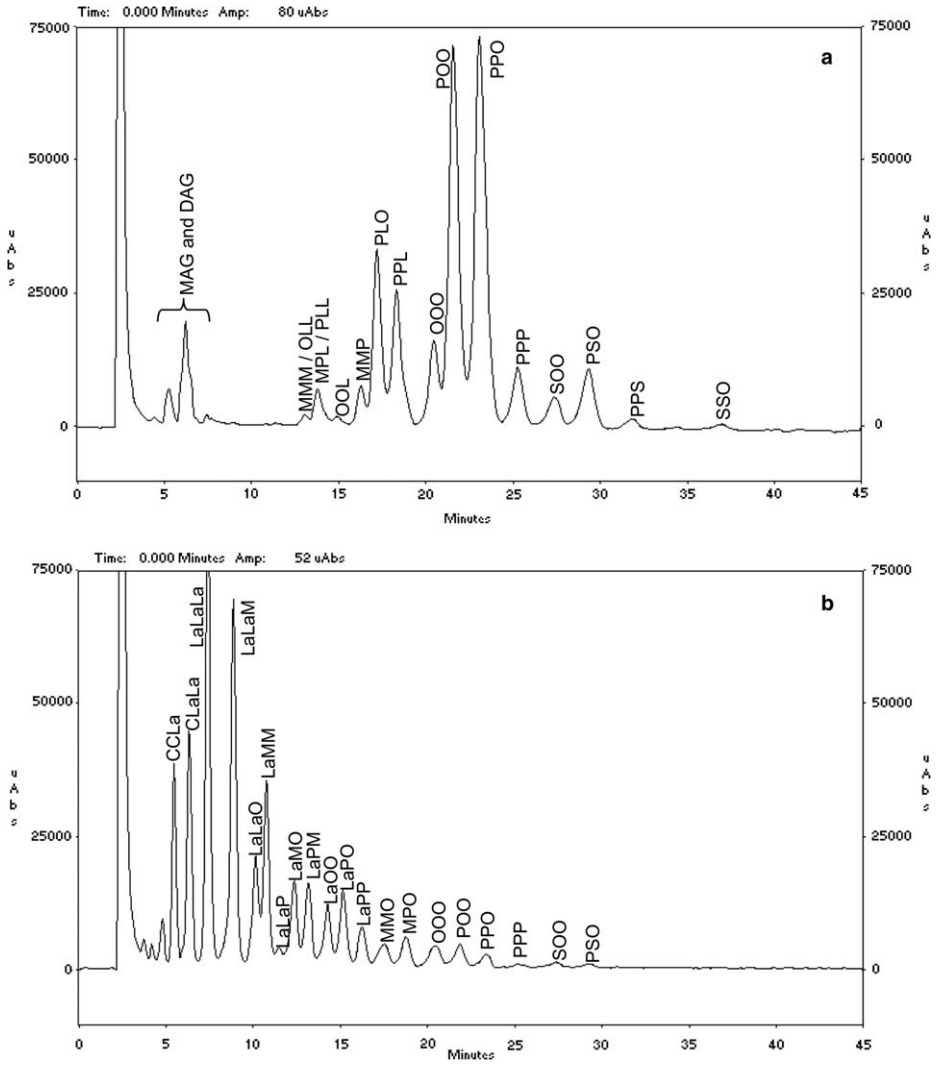

Fig. 1. TAG profiles of (a) PO and (b) PKO. (*Abbreviations*. MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerol; PO, palm oil; PKO, palm kernel oil; C, capric; La, lauric; M, myristic; P, palmitic; S, stearic; O, oleic; L, linoleic).

$$\% \text{ IE} = \frac{\sum[\text{TAGI}_t]}{\sum[\text{TAGI}_0]}.$$

3. Results and discussion

3.1. Interesterification of palm oil

Fig. 2(a)–(c) shows the changes in the TAG profiles of PO before (Fig. 2(a), control) and after 12 h (Fig. 2(b) and (c)) of reaction time, using lipases of *Pseudomonas* sp. and *R. miehei*, respectively. Both lipases caused similar changes in the PO TAG profile, with several TAG increasing in relative concentration (marked as arrow up) while others decreasing (marked as arrow down). Among the TAG that experienced continuous increases in concentration ($[\text{TAGI}_t]$) with reaction time were MMM/OLL, MMP, OOO and PPP while those showing major decreases were PPL, POO and PPO. Visual observation showed that PO became more solid-like (more easily solidified at room temperature) as the reaction time increased, which is most probably due to the increased formation of PPP in PO, resulting in a substantially harder product. Formation of intermediates, such as MAG and DAG (peaks eluted before 12 min), was observed to increase with reaction time.

Although both *Pseudomonas* sp. and *R. miehei* lipases caused a similar trend in TAG changes, the degrees of these changes were different. Visual appearance alone suggested that *Pseudomonas* sp. lipase caused a higher degree of TAG changes, where the oil was more solid compared to *R. miehei* lipase. Fig. 3(a) shows the manner in which the two lipases effected the cumulative summation of TAG that had increased in concentration ($\sum[\text{TAGI}_t]$) namely MMM/OLL, MMP, OOO and PPP, with respect to the total TAG present in PO. Lipase of *Pseudomonas* sp. had caused higher degree and rate of increase in the $\sum[\text{TAGI}_t]$ where it reached equilibrium after 24 h of reaction, but the reaction using *R. miehei* only accelerated after 12 h (Fig. 3(a)). After 48 h of IE, *Pseudomonas* sp. lipase had caused the sum of these TAG (namely MMM/OLL, MMP, OOO and PPP) to increase by 1.73-fold (relative to their initial concentrations) while *R. miehei* could only achieve a maximum increment of 1.47-fold.

Although IE could be judged by observing increases in the concentration of a pre-existing TAG or formation of new TAG (Ghazali et al., 1995), it is also important to consider the % TAG remaining and the % FFA generated, as IE occurs in parallel with hydrolysis (Ghazali et al., 1995; Lai et al., 1998). Both lipases used in the present study caused a high degree of TAG losses (more than 15%; Fig. 3(b)), due to the increased rate of hydrolysis (more than 5.20% FFA liberated; Fig. 3(c)) after 12 h of reaction with PO. Similarly, in works of Lai et al. (1995, 1998) as well as Lai et al. (2000a, 2000b), *Pseudomonas* sp. lipase was found to cause a greater decline in TAG concentration and generated more FFA in comparison. A drastic loss in TAG and an increase in FFA were observed in PO catalyzed by *Pseudomonas* sp. lipase after 24 h of reaction (Fig. 3(c)), indicating that the IE reaction had reached equilibrium (Fig. 3(a)) while hydrolysis started to predominate after that (Fig. 3(b) and (c)).

3.2. Palm kernel oil interesterification

Fig. 4(a)–(c) shows the changes in TAG profiles of PKO after IE with *R. miehei* and *Pseudomonas* sp. lipase, respectively, at 0 h (Fig. 4(a), control sample) and 12 h (Fig. 4(b) and (c)) of reaction times. Similar to PO, *Pseudomonas* sp. lipase caused more drastic TAG changes in PKO than did *R. miehei* lipase. IE caused an increment in the concentration of the preexisting TAG. Medium-chain TAG eluted between 7 and 14 min in the PKO profiles (Fig. 4), including LaLaM, LaLaO, LaMM, LaLaP, LaMO and LaPM were observed to increase after catalysis, although only two of these TAG, namely LaLaO and LaMO, experienced noteworthy increments. The rest of the TAG decreased correspondingly, with concentrations of CLaLa, LaLaLa and POO experiencing higher degrees of reduction.

There was also formation of a new TAG (marked as "N" in Fig. 4(b) and (c)) attached as a shoulder to the LaLaM peak. This new peak was not well resolved from the latter, but the increase in concentration of this new peak was obvious, especially after reaction using *Pseudomonas* sp. lipase. The new peak could also be a minor pre-existing TAG which was not well resolved that increased in concentration after the IE reaction. Identification of the new peak was unclear, but it could be either a medium-chain TAG with calculated carbon number close to LaLaM, such as CMM or CLaP, or it could also be TAG with higher calculated carbon number with a certain degree of unsaturation, e.g., CLaO (40:1). The new peak could also be MAG or DAG which resulted from the hydrolysis. However, since no MAG or DAG with a calculated partition number and unsaturation could fit into that particular retention time, the possibility seems low.

The cumulative summation of PKO TAG that increased in concentration, i.e., $\sum[\text{TAGI}_t]$ of LaLaO and LaMO (Fig. 5(a)) showed that catalysis with *Pseudomonas* sp. lipase reached its equilibrium at around 9 h (1.85-fold in increment relative to the initial concentration) and maximum increment in concentration of 1.92-fold at 48 h while *R. miehei* lipase could only reach similar concentration changes at about 48 h (Fig. 5(a)). As both *Pseudomonas* sp. and *R. miehei* lipases caused similar % TAG remaining and rate of hydrolysis (Fig. 5(b) and (c)) for PKO, the differences in the increment of $\sum[\text{TAGI}_t]$ thus become the sole factor that signified *Pseudomonas* sp. lipase had a higher IE degree and rate than *R. miehei* lipase. This phenomenon also occurs in PO.

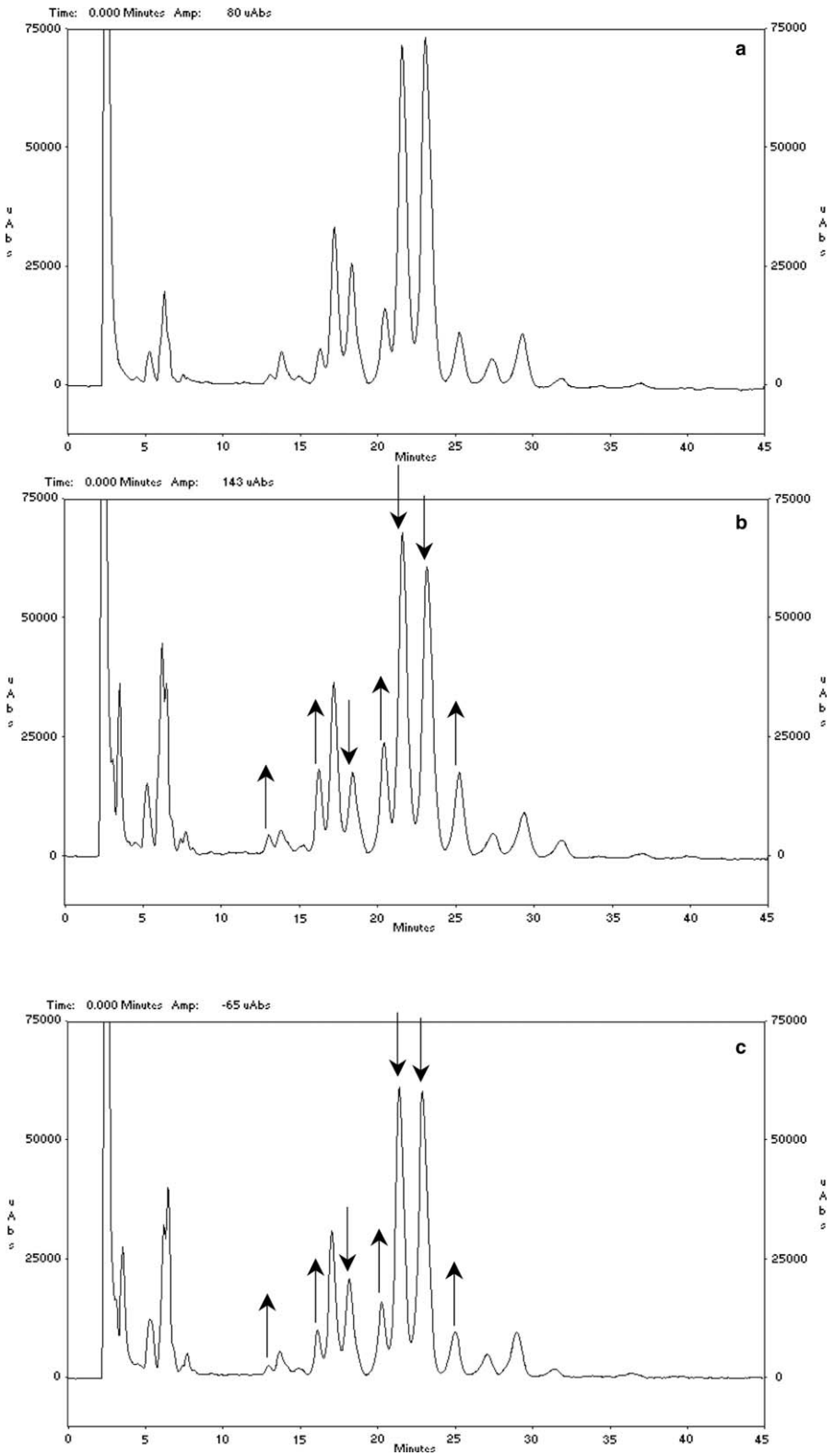

Fig. 2. TAG profiles of PO at (a) 0 h (control) and 12 h of IE using immobilized (b) *Pseudomonas sp*. and (c) *Rhizomucor miehei* liases, respectively. (*Abbreviations*. Arrow up indicates increases in concentration relative to control while arrow down indicates the opposite trend).

3.3. Catalytic nature of the lipases

Changes in chemical composition of PO and PKO showed that both oils exhibited similar increases in hydrolysis rate (% FFA liberated; Figs. 3 and 5(c)). At all reaction times, % TAG remaining of PO (Fig. 3(b)) was lower than PKO (Fig. 5(b)), while the percentage changes in $\sum[TAGI_t]$ were higher for the latter using

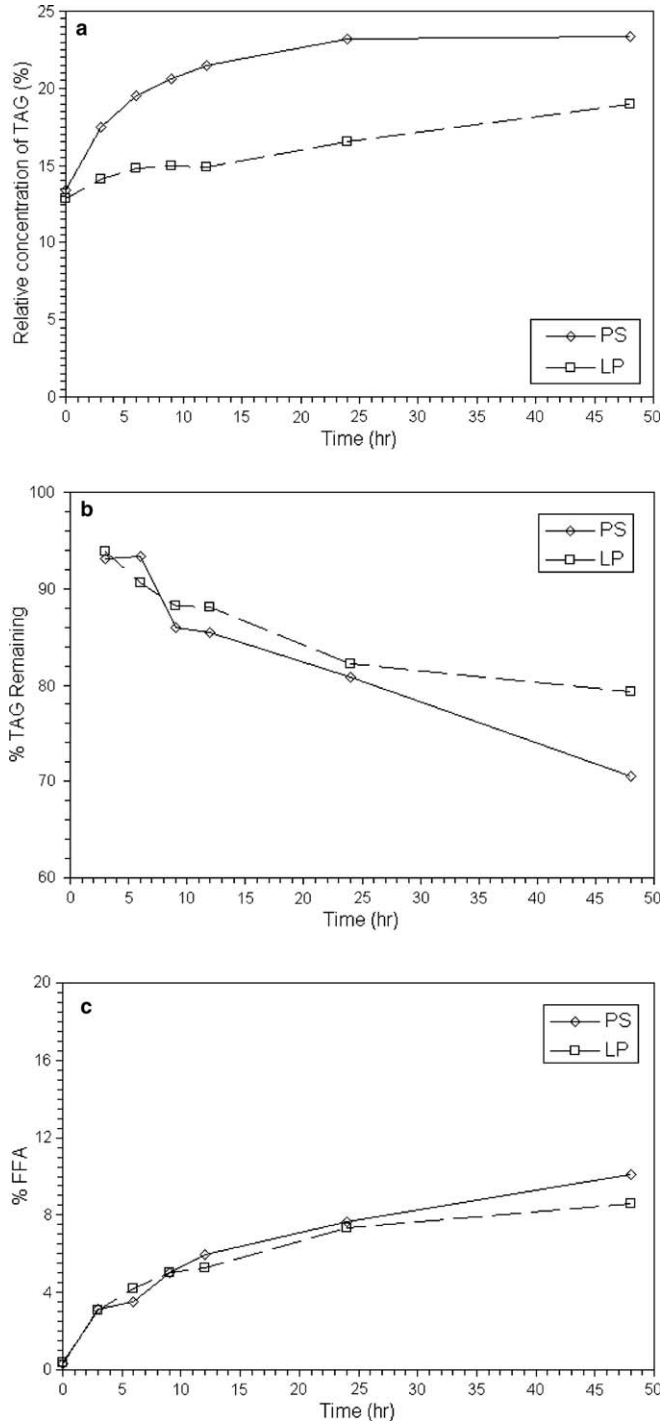

Fig. 3. Effect of different lipases on IE of PO: (a) cumulative summation of TAG that increased in concentration, $\sum[TAGI_t]$; (b) % TAG remaining; and (c) hydrolysis rate (% FFA liberated). (*Abbreviations*. LP, *R. miehei* (Lipozyme IM60) lipase; PS, *Pseudomonas sp*. lipase).

both lipases (Fig. 6), indicating that a relatively larger amount of TAG in PO had experienced hydrolysis, forming partial glycerides and FFA, than had PKO. The higher % TAG remaining in PKO could be due to the FFA generated that had been re-esterified to form TAG.

Another possibility is that the DAG liberated were continuously being hydrolyzed, forming MAG and eventually FFA and glycerol backbone, before the lipases hydrolyzed the other TAG. The chemical compositional differences of the two oils after IE suggested that both lipases had higher preference for short- and medium-chain TAG of PKO in term of IE while TAG of PO tended to promote hydrolysis by forming more partial glycerides. These observations also suggested that lipase preference for acylglycerols of PO, in terms of hydrolysis, was in decreasing order: TAG > DAG > MAG, while the opposite order could be true for PKO.

In this study, the effects of lipase regiospecificity (i.e., 1,3- and non-specific) were not obvious in the modification of either PO or PKO chemical composition. Indeed, both the oils exhibited very similar TAG profile changes (i.e., for PO, synthesis focussed on TAGs of MMM/OLL, MMP, OOO and PPP while hydrolysis was on PPL, POO and PPO, see Fig. 2; for PKO, synthesis focussed on LaLaM, LaLaO, LaMM, LaLaP, LaMO, LaPM and a new peak that could be either CMM, CLaP or CLaO while hydrolysis was CLaLa, LaLaLa and POO, see Fig. 4) although at different IE rates when their $\sum[TAGI_t]$, % TAG remaining and % FFA were compared. For all conditions, *Pseudomonas* sp. lipase always exhibited a higher degree and rate of catalysis than did *R. miehei*. Based on the difference in IE activity and similar TAG trend changes (namely $\sum[TAGI_t]$, % TAG remaining and the % FFA), it is postulated that IEs, using *R. miehei* and *Pseudomonas* sp. lipases for the same reaction time period, are similar to catalysis of the two oils using one of these lipases at different reaction times.

The differences in the lipases' catalytic rates could be considered from two aspects: the regiospecificity and fatty acid preferences of the two lipases. Since both lipases, with differing regiospecificities, caused similar trends in TAG changes (Figs. 2(b,c) and 4(b,c)) albeit different catalytic rates, it is postulated that regiospecificity could be the main cause of the different catalytic rates. For the two oils catalyzed, the regiospecificity of *R. miehei* lipase on 1,3 positions narrowed its catalytic option while non-specific *Pseudomonas* sp. lipase, that showed no discrimination toward catalysis position, had a wider selection of fatty acids, thus leading to the higher catalytic rate.

The fatty acid preferences of the two lipases could be another factor that contributed to the difference in catalytic rate. Xu (2000) indicated that *R. miehei* had higher preferences for short-chain fatty acids than medium and long-chain fatty acids while *Pseudomonas* sp. showed no discrimination. Since PO consisted mainly of medium- and long-chain TAG and these were not the *R. miehei* lipase's preference, the lower catalytic activity of *R. miehei* lipase compared to *Pseudomonas* sp. might be expected. Thus, the IE of PKO, that consisted of TAG with short and medium chain length fatty acids, using *R. miehei* lipase, would increase the changes in $\sum[TAGI_t]$ near to

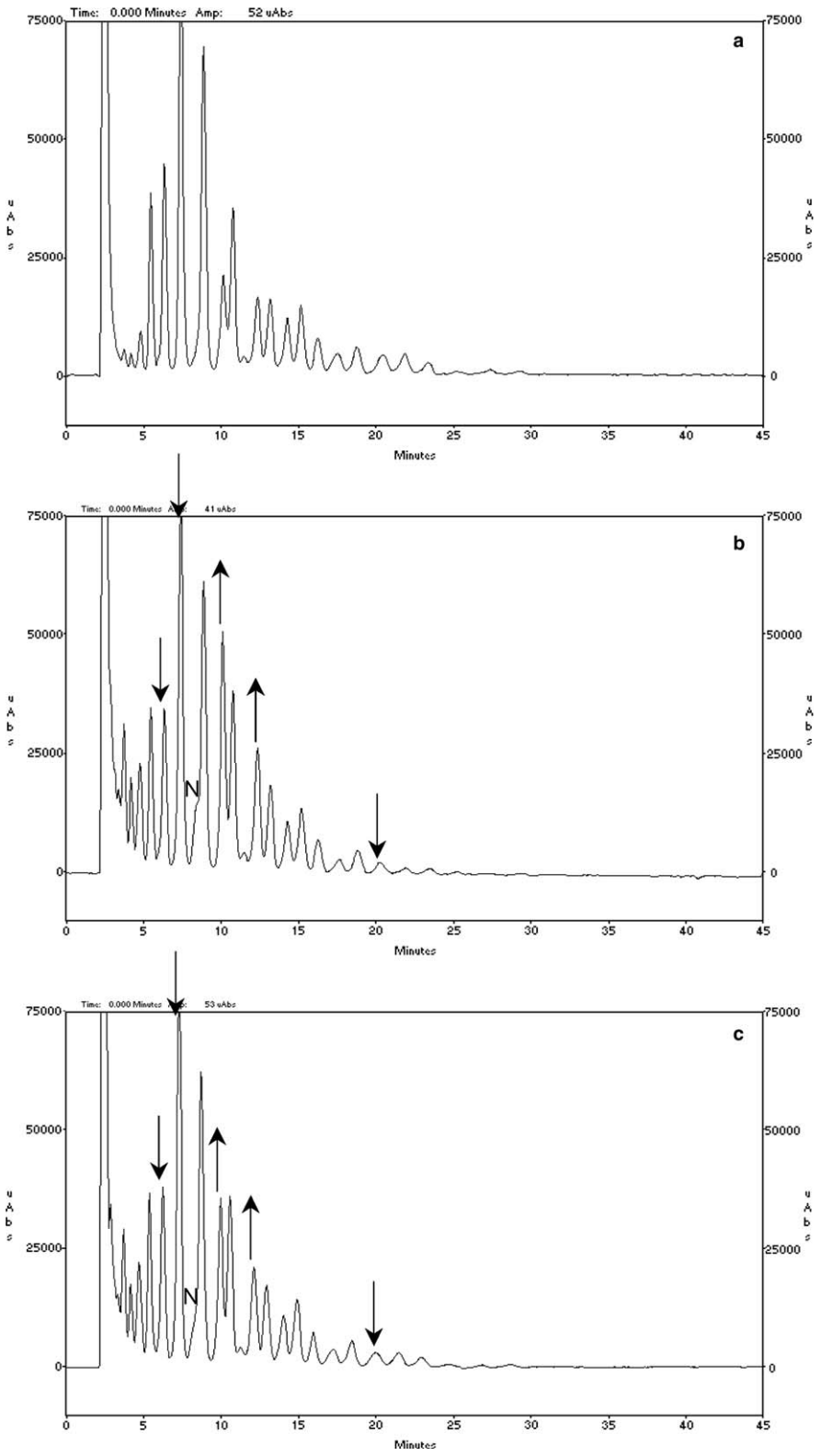

Fig. 4. TAG profiles of PKO at (a) 0 h (control) and 12 h of IE using immobilized (b) *Pseudomonas sp*. and (c) *Rhizomucor miehei* lipases, respectively. (*Abbreviation*. N indicates the formation of new peak; for other abbreviations see Fig. 2).

that of *Pseudomonas* sp. lipase (1.85- and 1.92-fold, respectively in Fig. 6). The increment of PKO catalyzed by *R. miehei* lipase, that surpassed the catalytic performance of *Pseudomonas* sp. lipase in catalysis of PO after 20 h of IE reaction (Fig. 6), could also be contributed by this fatty acid preference of the two lipases.

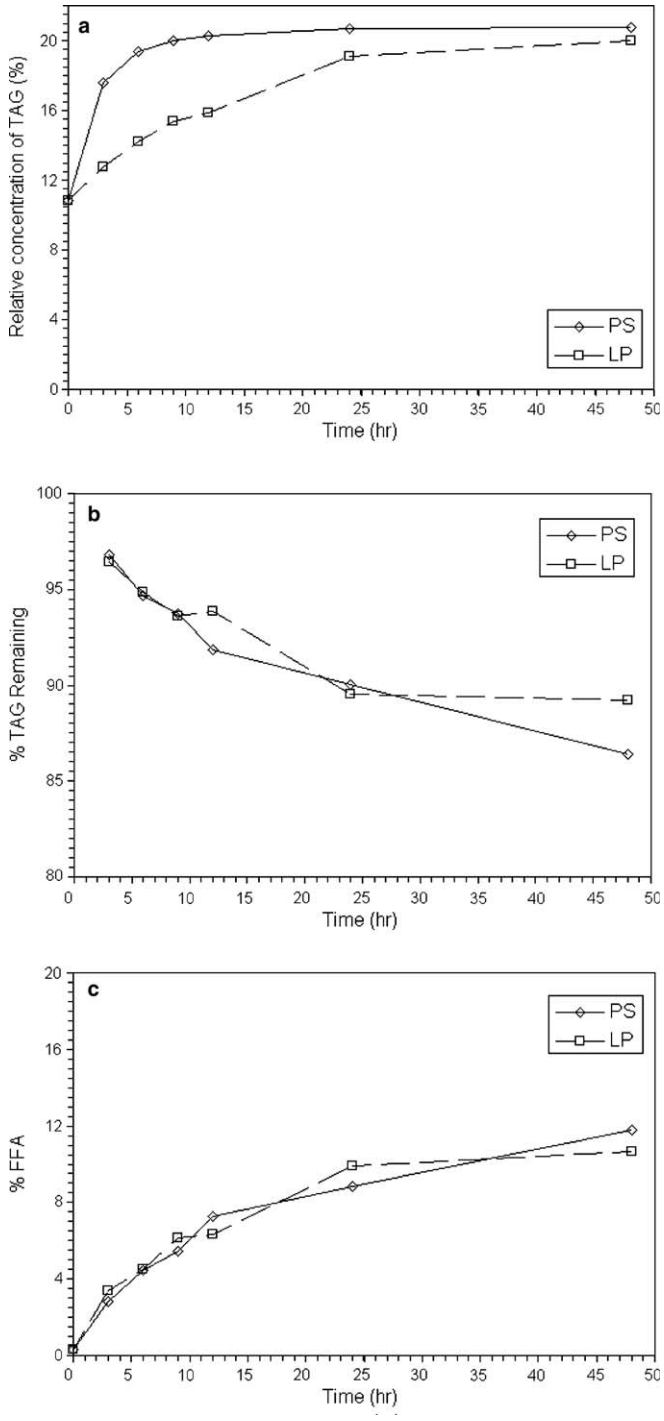

Fig. 5. Effect of IE using different lipases on PKO: (a) cumulative summation of TAG that increased in concentration, $\sum[TAGI_t]$; (b) % TAG remaining; (c) hydrolysis rate (% FFA liberated), (see Fig. 3 for abbreviations).

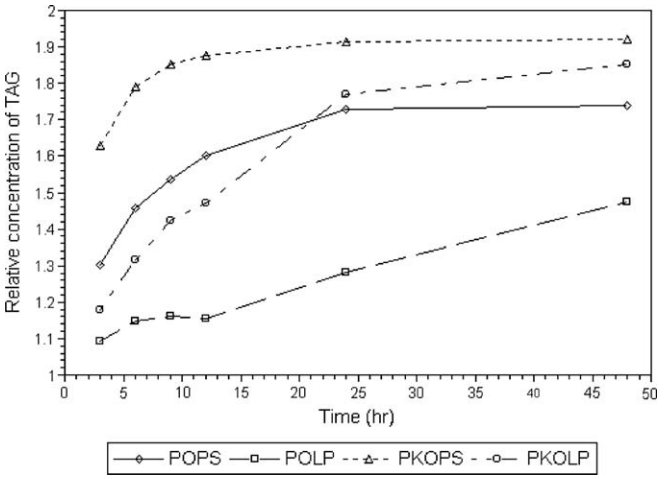

Fig. 6. Percentages of IE in terms of $\sum[TAGI_t]$ relative to their initial concentration, $\sum[TAGI_0]$ of PO and PKO catalyzed by *R. miehei* (Lipozyme IM60) (LP) and *Pseudomonas sp*. (PS) lipases.

3.4. TAG compositional analysis of the PO–PKO binary blends

The TAG profile of PO–PKO binary blend at 1:1 ratio is given in Fig. 7. Effect of blending on TAG composition changes is shown in Table 1. For the three blends, about 30 types of TAG (excluding isomers) were obtained. The TAG compositions of the binary fat blends represent a linear progression of the fat component in the blends, and, as the proportion of PKO increased, the proportion of the medium-chain TAG, such as CLaLa, LaLaLa and LaLaM, increased correspondingly. As the PO fraction increased, the proportion of POP, PLP, PLO and other TAG, that were representative of PO, increased instead.

Interpretation of TAG profiles for the PO–PKO binary blend mixtures was a difficult task and the difficulties encountered were related to the principle of TAG separation using the RP-18 column, i.e., based on their equivalent carbon number (ECN, i.e., molecular weight and degree of unsaturation). The greater the molecular weight of a TAG, the longer was the time needed before it could be eluted. However, the presence of unsaturation in a TAG increased its polarity, thus decreasing its retention time (Tan & Che Man, 2000). Within this principle, when the two oils were blended, some of the TAG, such as LaPM and MMM or LaOO and MPL, with the same calculated carbon number and degree of unsaturation, merged and appeared at the same retention time (Fig. 7), making it difficult and sometimes impossible to distinguish which TAG were experiencing concentration changes. The situation was further complicated as some of the unsaturated TAG, e.g., OLL may also elute at the same time as LaPM and MMM.

Another major difficulty was the calculation of the % TAG remaining for the three blends. The preliminary study of native oils, along with other literature works had shown that the TAG of PO eluted after 12 min while that of PKO eluted as early as 5 min (Ghazali et al., 1995; Liew et al., 2001; Swe et al., 1995). Blending of PO and PKO will cause most of the MAG and DAG of the PO (Fig. 1(a)) to co-elute with some of the early eluting TAG of PKO (i.e., CCLa and CLaLa, Fig. 1(a)), causing difficulties in calculating the % TAG remaining.

In order to reduce errors in calculation and interpretation, few assumptions were made. For TAG that eluted

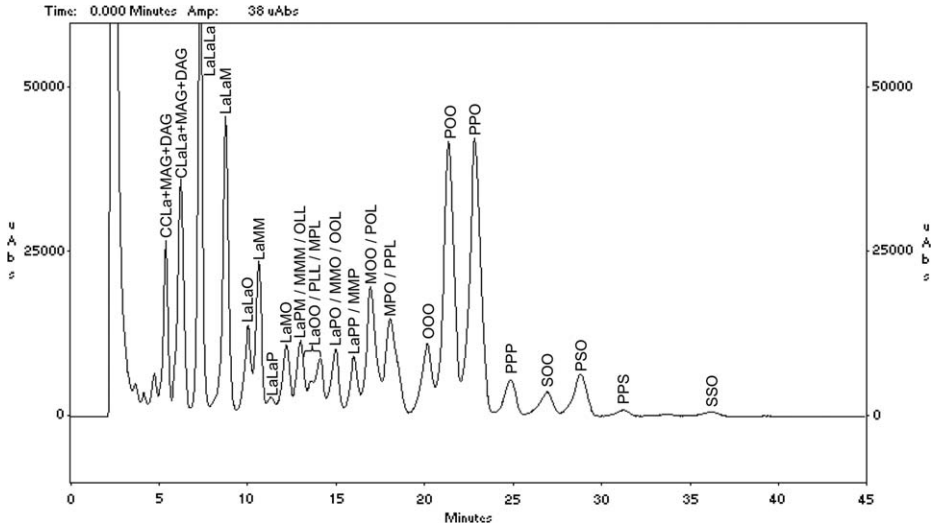

Fig. 7. TAG profiles of PO–PKO binary blend at 1:1 ratio (see Fig. 1 for abbreviations).

Table 1
TAG compositions (% area) of PO, PKO and their binary blends

TAG	CN:DU	PO	PKO	PO:PKO		
				3:1	1:1	1:3
CCLa	32:0	ND	6.13	2.34[a]	3.38[a]	4.79[a]
CLaLa	34:0	ND	8.96	5.44[a]	6.43[a]	7.76[a]
LaLaLa	36:0	ND	21.8	5.75	10.5	16.4
LaLaM	38:0	ND	17.0	4.42	8.04	12.6
LaLaO	42:1	ND	5.82	1.46	2.68	4.25
LaMM	40:0	ND	8.90	2.26	4.16	6.64
LaLaP	40:0	ND	0.84	0.25	0.46	0.57
LaMO	44:1	ND	4.98	1.23	2.23	3.62
LaPM	42:0	ND	4.59	1.51	2.52	3.55
MMM	42:0	0.54	ND			
OLL	54:5		ND			
LaOO	48:2	ND	3.80	2.4	2.76	3.22
PLL	52:4	2.21	ND			
MPL	48:2		ND			
OOL	54:4	0.59	ND	1.48	2.36	3.44
LaOP	46:1	ND	4.57			
MMO	46:1	ND	ND			
LaPP	44:0	ND	2.40	1.99	2.17	2.23
MMP	44:0	2.02	ND			
MOO	50:2	ND	1.95	7.58	5.89	3.97
POL	52:3	9.92	ND			
PPL	50:2	9.00	ND	6.78	5.52	3.73
MOP	48:1	ND	2.15			
OOO	54:3	5.53	1.99	4.33	3.73	2.79
POO	52:2	25.6	1.97	18.9	13.9	7.95
PPO	50:1	29.9	1.10	21.7	15.6	8.39
PPP	48:0	4.74	0.30	2.88	2.17	1.3
SOO	54:2	2.98	0.48	2.25	1.83	1.1
PSO	52:1	5.40	0.31	3.96	3.12	1.64
PPS	50:0	1.03	ND	0.64	0.39	0.27
SSO	54:1	0.36	ND	0.50	0.26	ND

Abbreviations. CN: DU, calculated carbon number: degree of unsaturation; C, capric; La, lauric; M, myristic; P, palmitic; S, stearic; O, oleic; L, linoleic; ND: not detectable, see Fig. 1 for other abbreviations.

[a] TAG may contain small quantities of MAG and DAG from PO.

at similar retention times, the change in TAG concentration caused by IE was assumed to have the same effect for all the TAG unless otherwise specified. To obtain more accurate calculations of % TAG remaining, peak areas of MAG and DAG of PO that co-eluted with CCLa and CLaLa of PKO had to be subtracted. It was assumed that the total MAG and DAG in the blended sample was directly proportional to the PO (control) ratio, where

$$\sum[\mathrm{MAG}+\mathrm{DAG}]_{\mathrm{blend}} = \sum[\mathrm{MAG}+\mathrm{DAG}]_{\mathrm{PO}} \times \%\ \text{of PO in blend},$$

while MAG and DAG from PKO were found to be trace amounts, based on the chromatograms and % FFA calculated.

Based on the TAG profiles, changes of PO and PKO during IE, it was observed that the concentrations of MAG and DAG of PO increased as reaction time increased. In contrast, the two TAG from PKO that co-eluted with MAG and DAG of PO in the binary blends, namely CCLa and CLaLa, were observed to decrease in concentration as reaction time increased. Therefore, it could be assumed that any increases in concentration of these two TAG in the blends were most likely caused by increases in MAG and DAG concentrations, where these values were subtracted for the % TAG remaining calculation.

3.5. Palm oil–palm kernel oil blends interesterification

Figs. 8–10 show the TAG profile changes of the three PO–PKO binary blends at 0 h (control sample) and after 12 h of IE for catalysis using the two lipases. IE of binary blends caused more TAGs to increase in concentration compared to their native oils. Generally, eight TAG peaks, that eluted between 10 and 16 min, increased in concentra-

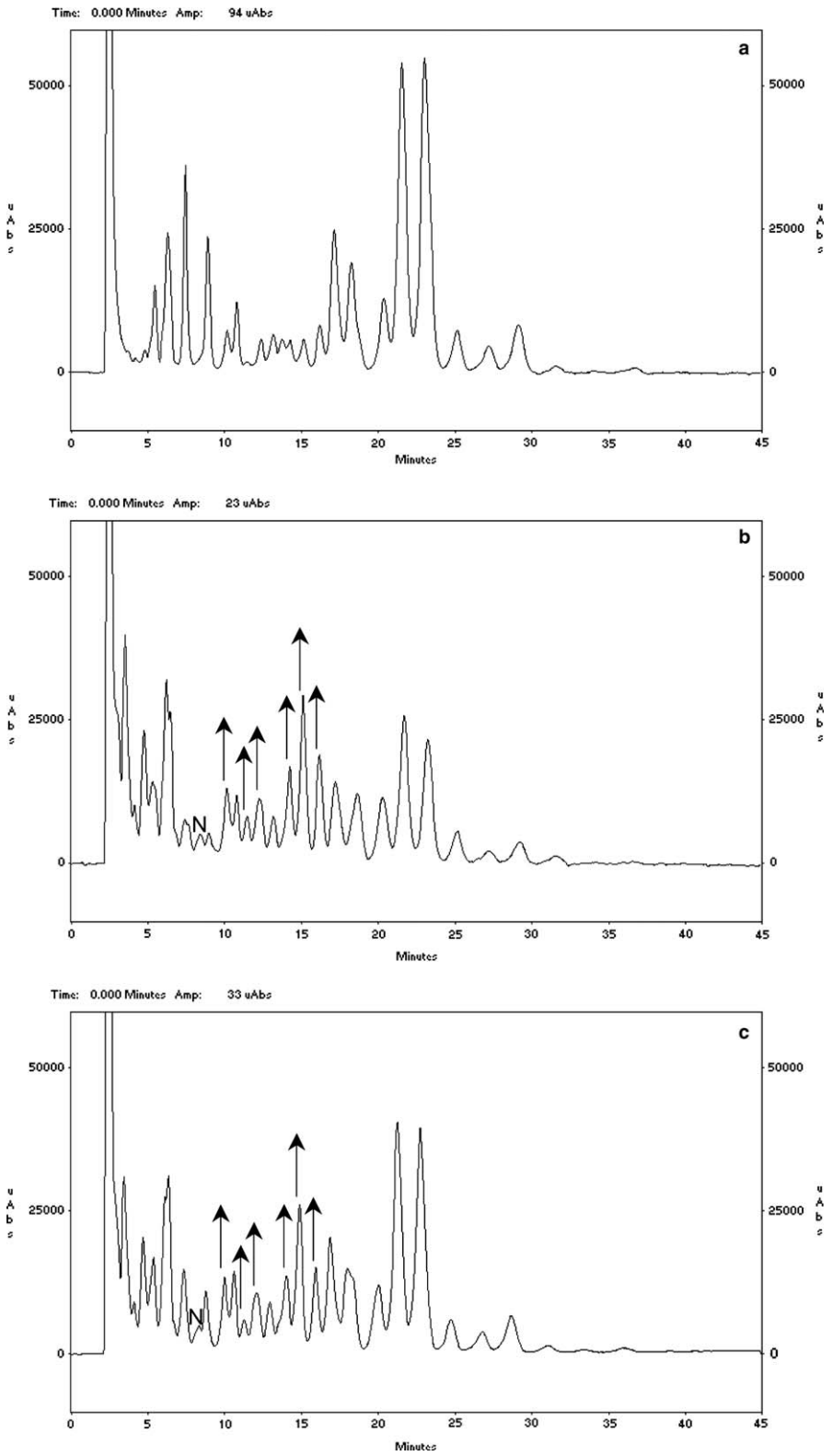

Fig. 8. TAG profiles of PO–PKO binary blends at 3:1 ratio at (a) 0 hour (control) and 12 h of IE using immobilized (b) *Pseudomonas sp*. and (c) *Rhizomucor miehei* (Lipozyme IM60), respectively. (see Fig. 2 for abbreviations).

tion during IE (Figs. 8(b,c), 9(b,c) and 10(b,c)). However, only six of these peaks showed noteworthy increments in concentration, namely LaLaO, LaLaP, LaMO, LaOO/MPL/PLL, OOL/LaOP/MMO, and LaPP/MMP. Triolein (OOO) experienced both decreases and increases concentration, depending on the PO ratio of the blend. However, as the change in concentration of OOO was relatively small, the effect to the total concentration was negligible.

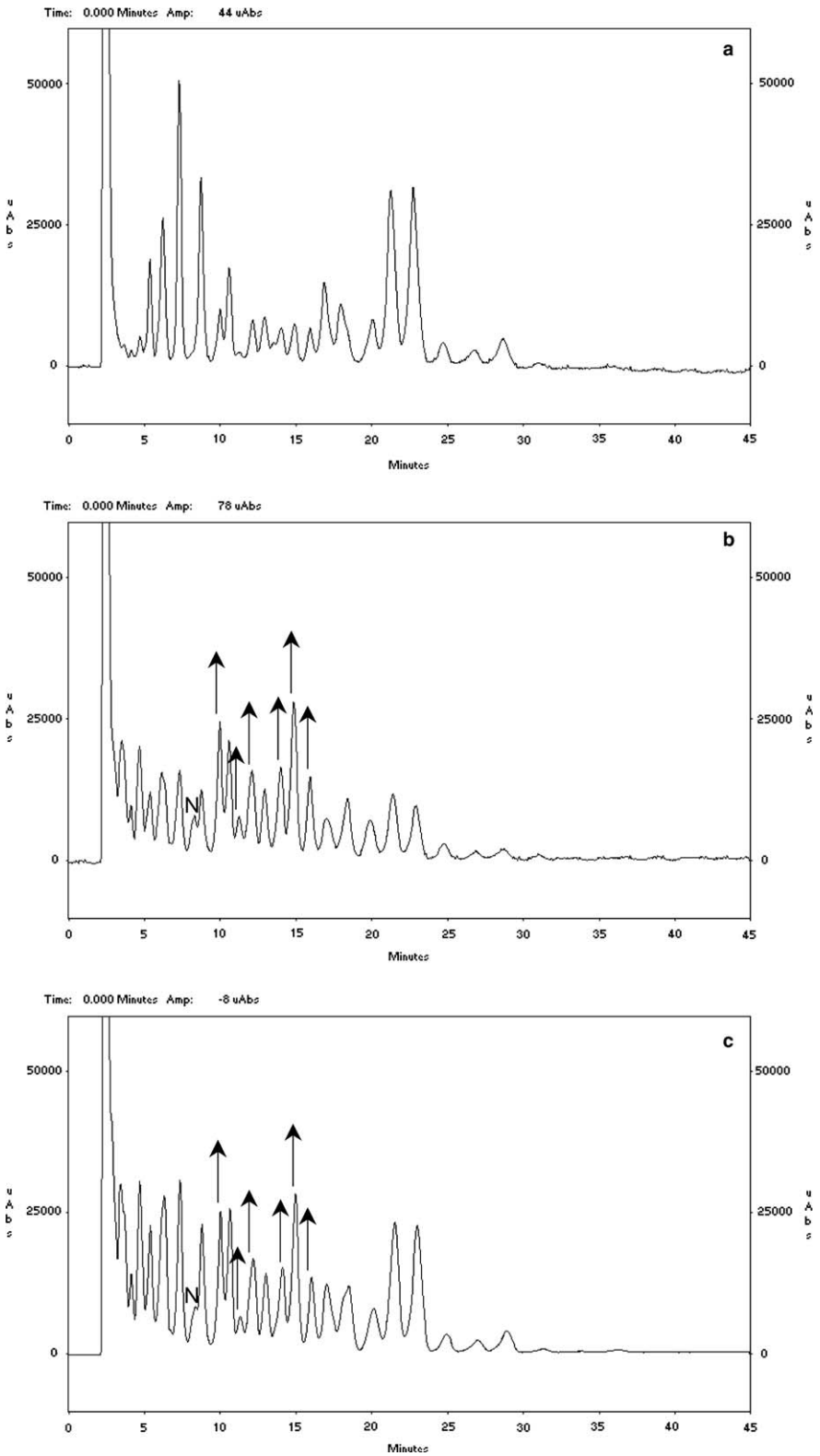

Fig. 9. TAG profiles of PO–PKO binary blends at 1:1 ratio at (a) 0 hour (control) and 12 h of IE using immobilized (b) *Pseudomonas sp*. and (c) *Rhizomucor miehei* (Lipozyme IM60), respectively. (see Figs. 2 and 4 for abbreviations).

The other TAG, except CCLa and CLaLa, showed corresponding decreases with reaction time where the increases of CCLa and CLaLa were recognized to be due to the formation of MAG and DAG as discussed earlier. There was also formation of a new peak (marked as "N" in Fig. 2) eluting as an attached shoulder peak before

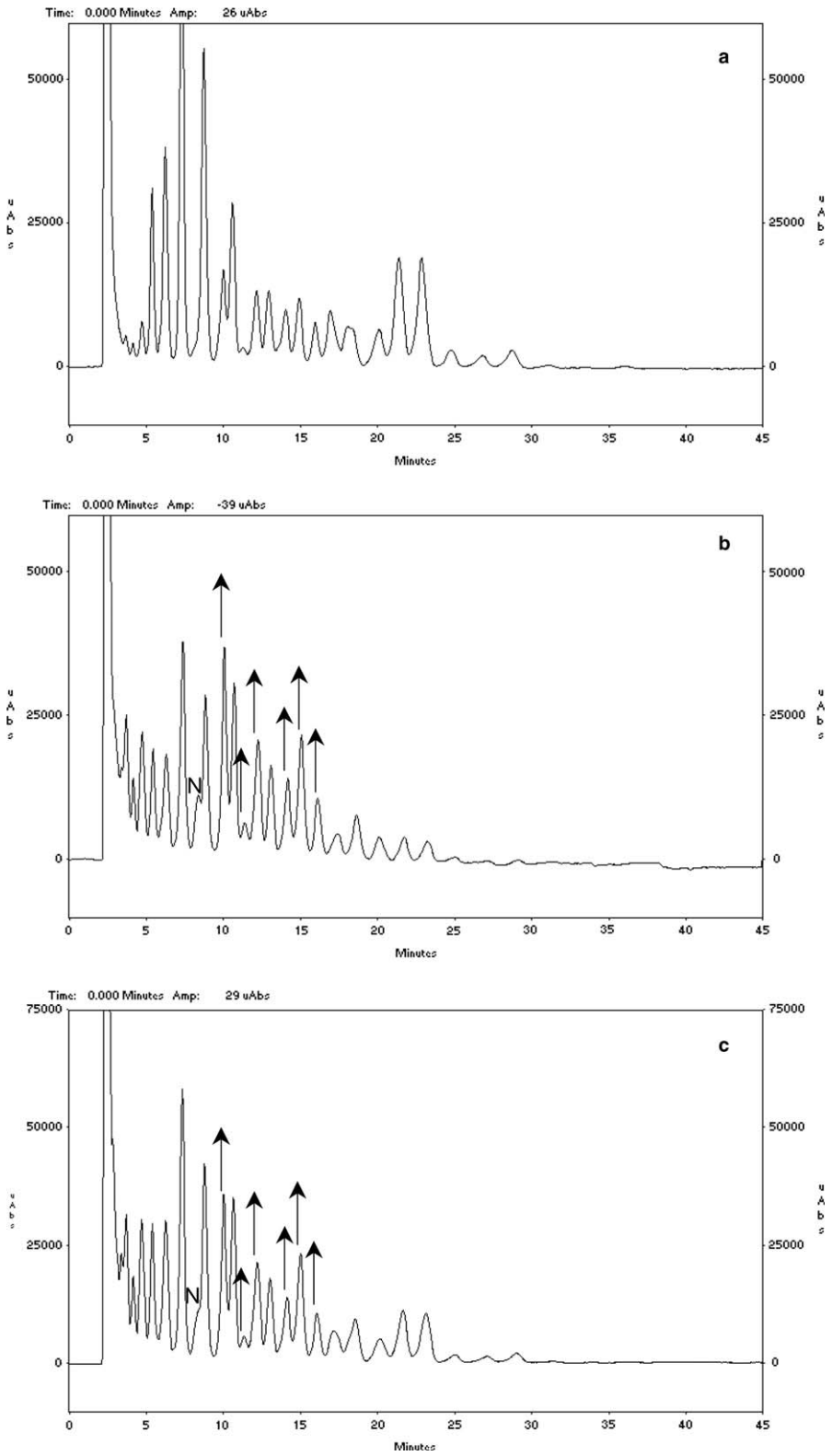

Fig. 10. TAG profiles of PO–PKO binary blends at 1:3 ratio catalyzed using immobilized (a) *Pseudomonas sp.* and (b) *Rhizomucor miehei* (Lipozyme IM60) lipases for 12 h of IE process (see Figs. 2 and 4 for abbreviations).

LaLaM, similar to the case of lipase catalysis on native PKO. This new peak was, however, better resolved from the latter peak as compared to that of native PKO.

For TAG that increased in concentration during the IE reaction, it was observed that LaLaO and LaMO increased as the proportion of PKO increased, while

LaLaP experienced a maximum concentration increment in the blend containing equal ratio of the two oils (1:1). For peaks that consisted of mixtures of TAG, such as LaOO/MPL/PLL, OOL/LaOP/MMO and LaPP/MMP, the effect of IE on the changes of individual TAG concentrations was unclear. Collectively, these TAG experienced lower concentration increments for the 1:3 ratio blend while the other blends achieved higher but similar increments. Concentration of OOO increased as the PO fraction increased but decreased as the PKO fraction increased.

The effect of different blend ratios on the decreases in TAG concentrations was much more apparent. For TAG that eluted before 10 min (TAG from PKO, such as LaLaLa and LaLaM), the concentration decreased more drastically as the proportion of PKO increased (Fig. 10), while TAG eluted after 16 min (mostly TAG from PO) experienced greater decreases as the PO fraction increased (Fig. 8). The concentration of the new peak increased more when the PKO fraction was increased (Fig. 10). This is easily understood as the formation of the new peak was only observed in PKO alone but not in PO. This observation also suggested that the TAG concentration changes during IE of these binary blends were very much influenced by the blend fraction that is in excess.

The % FFA liberated increased as the PKO fraction in the blends increased (Fig. 11(c)), although the differences were not large. Initially, % FFA liberated for the three blends were relatively similar to each other but, after 24 h of IE, reaction using *Pseudomonas* sp. lipase resulted in relatively larger increases in % FFA (12.9–15.6% for *Pseudomonas* sp.; 10.9–12.4% for *R. miehei*), indicating the predomination of hydrolysis over IE. Surprisingly, when the PKO fraction increased, % TAG remaining (Fig. 11(b)) did not decrease as expected. This phenomenon is clearly due to the fatty acid preference of the lipases in terms hydrolysis and IE. Since both lipases showed a relatively higher tendency toward hydrolysis of PO TAG, as discussed earlier, it is postulated that TAG such as POO and PPO (mainly found in the PO fractions TAG_{PO}) were hydrolyzed to form MAG and DAG in the blend. When the amount of PO fraction was higher (i.e., 3:1 blend), there were excesses of TAG_{PO} available for hydrolysis, thus leading to lower % TAG remaining (Fig. 11(b)). However, in the blends where the PO Mg fraction was lower than PKO or the same (i.e., 1:1 and 1:3), TAG_{PO} available for hydrolysis by the lipases were limited. When the concentrations of these TAG declined to a certain level, the lipases started to hydrolyze the generated MAG and DAG to form more FFA instead of hydrolyzing the TAG from the PKO fraction (TAG_{PKO}), thus resulting in higher % TAG remaining in the 1:1 and 1:3 binary blends. This hypothesis could also explain why PKO had a higher % TAG remaining as compared to that of PO during IE using the two lipases.

As the same TAG from the three blends (i.e., TAG eluted between 10 and 16 mm) were found to increase and their initial concentrations were different in these blends, the factor of initial concentration differences had

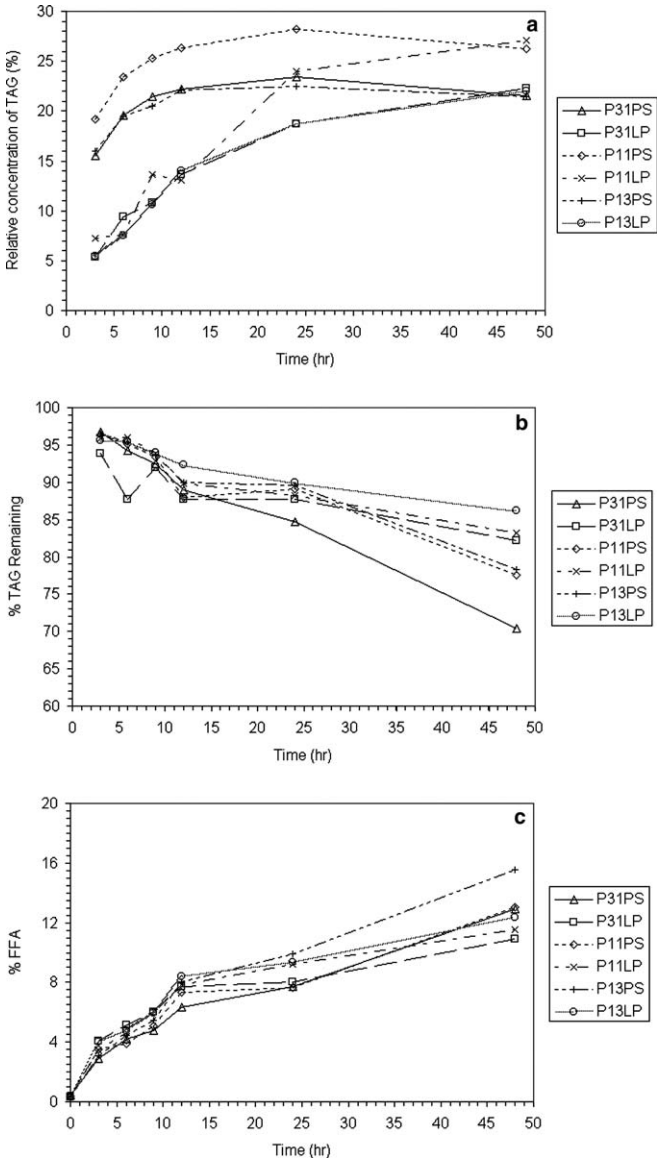

Fig. 11. Effect of IE using different lipases on PO:PKO blend mixtures of 3:1, P31; 1:1, P11 and 1:3, P13: (a) total concentration increases of $[TAGI_t]$; (b) % TAG remaining; and (c) hydrolysis rate (% FFA liberated) (see Fig. 3 for abbreviations).

to be considered for comparison purposes. Increases in $\sum[TAGI]$ (i.e., $\sum[TAGI_t] - \sum[TAGI_0]$) instead of percentage changes of the different blend ratios catalyzed by the two lipases are shown in Fig. 11(c). Among the three blends, PO:PKO at 1:1 ratio catalyzed by both lipases had the highest increase in $\sum[TAGI_t]$ while the other two blends had similar increases in $\sum[TAGI_t]$.

A possible explanation could be that, in the 3:1 and 1:3 (PO:PKO) blends, the IE process focussed on the major fraction. However, in the 1:1 blend, TAG from the two fractions available for IE (i.e., TAG available for hydrolysis were TAG that eluted before 10 min and after 16 min, mainly from PKO and PO fractions, respectively, where these hydrolyzed fatty acids were then re-esterified into TAG between 10 and 16 min), were in s equal proportions.

Under this condition, lipase (either *R. miehei* or *Pseudomonas* sp.) will catalyze both fractions with the same reaction rate, thus causing the highest increase in $\sum[TAGI_t]$. Within this postulation, when PO was in higher proportion in the blends, one would expect the concentration of TAG_{PO}, such as PLL, MPL (from peak consisted of LaOO, PLL and MPL; Fig. 7) and OOL (from peak consisting of LaPO, MMO and OOL; Fig. 7) to increase to a higher level after IE. In contrast, concentrations of TAG_{PKO}, such as LaOO and OOL, increased as the PKO fraction increased. In the PO–PKO blend (1:1), the TAG from both fractions increased at the same reaction rate, thus leading to a maximum increment in $\sum[TAGI_t]$.

For all the blends, *Pseudomonas* sp. lipase had a higher catalytic activity, both in terms of rapidly in changing the TAG concentration and the formation of a new peak. In most cases, IE using *Pseudomonas* sp. lipase had the $\sum[TAGI_t]$ and formation of a new peak reaching their maximum level after about 12 h while hydrolysis of the oil started after 24 h. IE using *R. miehei* lipase progressed to similar concentration changes at 48 h.

The present study showed that changes in chemical composition were very much influenced by the type of substrates as well as the major substrate in a blend. Regiospecificity of *Pseudomonas* sp. and *R. miehei* lipases did not cause different chemical composition changes to PO, PKO or their binary blends. Instead, the positional fatty acid specificity was found to affect the efficacy of IE of the various oils. IE of the native PO and PKO demonstrated that distinct chemical composition changes could be used to characterize changes in the TAG profiles of the binary blends, despite the difficulties encountered.

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