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Fatty acids residue from palm oil refining process as feedstock for lipase catalyzed monoacylglicerol production under batch and continuous flow conditions

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ABSTRACT

Free fatty acids are used in many cases for the production of soaps, candles and assist processing of rubber products, but we believe that new process technology should be developed to produce products with higher added value. Monoacylglycerols (MAGs) are nonionic surfactant, highly hydrophobic and has been used as controlled release systems for drugs. The results presented here for the lipase-catalyzed MAG production show that both batch and continuous flow conditions can lead to the desired product in short reaction time and high yield (70–95%) but the use of packed bed reactors (PBR) shows higher efficiency when compared to batch reactors.

1. Introduction

The Twelve Principles of Green Chemistry reflect the need for definitive action with regard to the development of processes, whether in universities and industry [1]. In this context, the availability of cheap raw material and renewable energy from nature is the basis for industrial sustainability, as well as a better use of industrial waste. In this sense, strategies to develop clean technologies for chemical processes aim to balance economic and environmental aspects [2].

In recent years, the group of four main countries that grow soybeans comprising Brazil, Argentina, Bolivia and Paraguay recorded 92% increase in production and 66% increase in planted area with soybeans and palm oil, which represents several tons fiber and by-products of the refining procedure.

Palm oil has a wide range of applications, about 80% is used for food applications (cooking oil), while the rest is raw material for a series of non-food applications [3]. Among food uses, olein refined, bleached and deodorized is mainly used in cooking and frying oils, fats and margarines. Many mixtures have been developed to produce solid fats with zero content of trans-fatty acids [4].

The refining process removes free fatty acids, phosphatides, odoriferous matter, water and impurities from the crude palm oil to produce high quality edible oil that meets industry standards. To achieve this chemical or physical objective, refining can be used as shown in Scheme 1 and in both cases free fatty acids are obtained as a byproduct of the refining processes.

Free fatty acids are used in many cases for the production of soaps, candles and assist processing of rubber products, but we believe that new process technology should be developed to produce products with higher added value [5]. Monoacylglycerols (MAGs) are important molecules that find applications in different fields. In food industry are widely used in the preparation of bakers, pastry and margarine [6]. They are also used in pharmaceutical [7] and cosmetic industry as drug carriers and to increase the consistency of creams and lotions [8].

Monoacylglycerols (MAGs) are nonionic surfactant, highly hydrophobic and has been used as controlled release systems for

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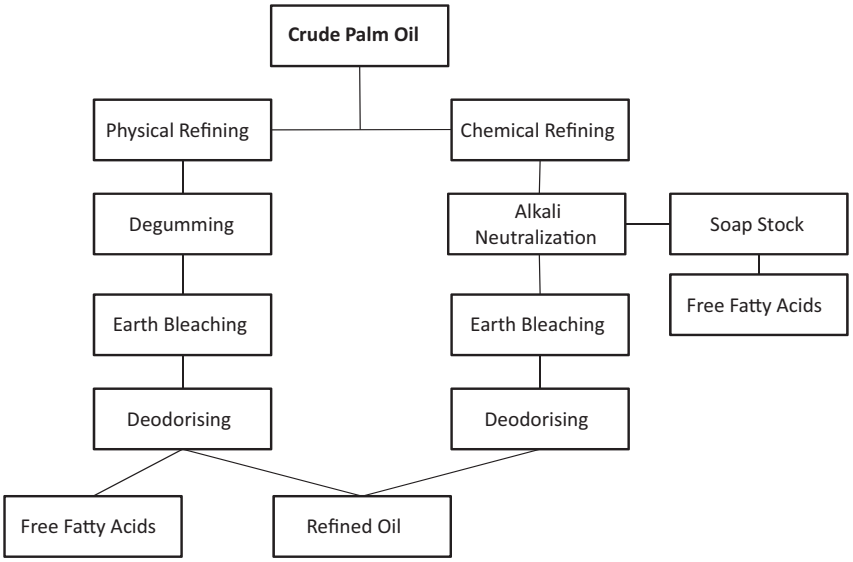


Scheme 1. Palm oil refining process.

drugs [7]. Its application is mainly due to hydrophobic–hydrophilic balance in its structures. In addition, plasticizers and lubricants properties of monoacylglycerols allow its use in textile processes and formulation of oils for different types of machines. These compounds are also used as stabilizers and antifoam. It is estimated that mixtures of these with MAG and Diacylglycerol (DAG) representing 75% of world production of emulsifiers [9]. MAG is also of great interest in synthetic organic chemistry, which is used as synthetic intermediate and chiral building blocks. In addition, monoacylglycerols are considered safe by the Food and Drug Administration (FDA), increasing the interest in its use and development of new synthetic methods.

In order to add value to this residue of fatty acids from palm oil industry, it was decided to make a comparative study on the esterification reaction catalyzed by lipase between 1,2-O-isopropylidene glycerol and free fatty acids derived from palm oil refining (acid residue) under batch and continuous flow conditions using the surface response methodology (RSM) [10] for optimization of reaction conditions. For industrial purposes, the continuous flow system is preferred to batch reactors due to its greater process control, high productivity and improvement of quality/purity and yield [11,12]. Several types of reactor can be used in continuous operation, among those reactors, packed bed reactors (PBR) are the most popular due to its high efficiency, low cost and ease of construction, operation and maintenance [13–15].

2. Results and discussion

In order to evaluate the best biocatalyst for the esterification of 1,2-O-isopropylidene glycerol with acid residue from palm oil refining process (Fig. 1), immobilized lipase from *Rhizomucor miehei* (RMIM) and *Thermomyces lanuginosus* (TL IM) were chosen, which are recognized in the literature by having relative specificity against a large number of substrates and remarkable esterification activity [16].

First, to identify variables with important effect in our reaction system under batch conditions we use design of experiments with a central composite design (CCD), composed of four variables, varying on two levels, which was used to maximize the acid residual esterification catalyzed by lipases from *R. miehei* (RMIM) and *T. lanuginosus* (TL IM). Thus, the need for a greater amount of experiments is reduced without compromising the results. Temperature, amount of enzyme, substrate concentration and stirring were considered critical variables (independent) of process and therefore assessed in planning. The reaction time was not considered a significant variable in experimental designs, since past experience shows that after 4 h of reaction no progress in reaction yield was achieved.

For each enzyme a 2^4 CCD was applied with triplicate central points to calculate the experimental error. The four variables and their coded and real levels for the two enzymes evaluated in batch reactions are presented in parentheses on Table 1.

In the batch reaction experiments, the reactions were carried out using a stock solution containing 1,2-O-isopropylidene glycerol and acid residue at 1:1 (equivalents) ratio, dissolved in n-heptane. In 2 mL-ependorfs was added 1 mL reaction medium followed by the addition of appropriate enzyme (1%, w/w) (Scheme 2). Conversion was monitored using a modified method of Lowrey and Tinsley [17] and confirmed by GC–MS analysis as described in detail in Section 4.

Results of the experimental design with coded and real values for both lipases evaluated are presented in Table 1. The first 16 entries in Table 1 are sufficient for determining the mathematical model and the experiments of entries from 17 to 19 contain the central point triplicate.

Both enzymes were able to catalyze the 1,2-O-isopropylidene glycerol esterification with the acid residue at high conversions, as shown in entries 8 and 16 (Table 1) for *T. lanuginosus* (TL IM) and *R. miehei* (RMIM), respectively.

At this stage, there was a small influence of stirring for both lipases, since small variations in conversion percentages were found between reactions carried out under the same reaction temperature. This phenomenon can be more easily understood by examining the estimated effects (Table 2) that shows stirring as the smallest effect among variables studied (−2.17 and −3.08 for *R. miehei* (RMIM) and *T. lanuginosus* (TL IM), respectively). Another important effect is the amount of enzyme used in each reaction, since reactions with higher amounts of catalyst always lead to major improvements in reaction conversions, as shown in Table 1 (entries 10 and 12).

It was also found that the estimated effects for isolated variables for both enzymes were all statistically significant as shown by (p) values (Table 2). Variables showed similar effects for both enzymes in relation to its grandeur and influences. The substrate concentration showed the most positive effect among variables studied for *R. miehei* (RMIM) and *T. lanuginosus* (TL IM) (11.1868 and

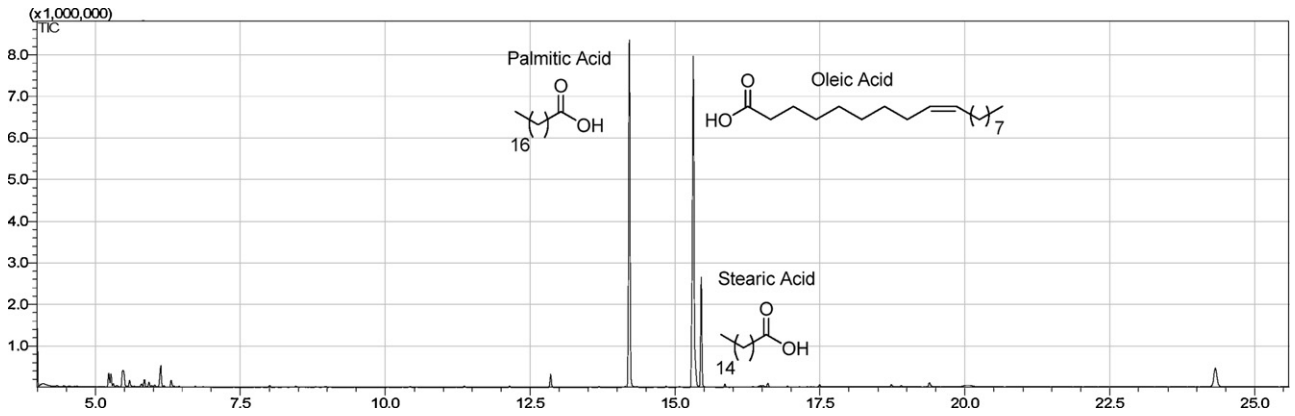


Fig. 1. Chromatogram of the fatty acid residue obtained from the Palm Oil refining process (fatty acid residue composition: 44% of palmitic acid, 42% of oleic acid and 14% of stearic acid).

Table 1
Experimental design and results of the factorial CCD for the esterification reaction between 1,2-O-isopropylidene glycerol and acid residue catalyzed by *Rhizomucor miehei* (RMIM) and *Thermomyces lanuginosus* (TL IM).

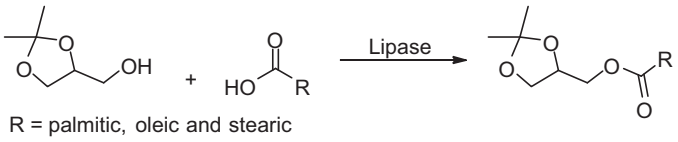
Entry	Temperature (°C)	[E] (%)[a]	[S] (mM)	Stirring (rpm)	Conversion (%)	
					RMIM	TLIM
1	−1 (40)	−1 (0.1)	−1 (50)	−1 (50)	58	60
2	1 (60)	−1 (0.1)	−1 (50)	−1 (50)	62	58
3	−1 (40)	1 (1)	−1 (50)	−1 (50)	75	69
4	+1 (60)	1(1)	−1 (50)	−1 (50)	78	70
5	−1 (40)	−1 (0.1)	+1(100)	−1 (50)	82	77
6	+1 (60)	−1 (0.1)	+1 (100)	−1 (50)	87	87
7	−1 (40)	+1 (1)	+1 (100)	−1 (50)	82	85
8	+1 (60)	+1 (1)	+1 (100)	−1 (50)	94	95
9	−1 (40)	−1 (0.1)	−1 (50)	+1 (250)	56	52
10	+1 (60)	−1 (0.1)	−1 (50)	+1 (250)	54	54
11	−1 (40)	+1(1)	−1 (50)	+1 (250)	62	63
12	+1 (60)	+1(1)	−1 (50)	+1 (250)	67	71
13	−1 (40)	−1(0.1)	+1 (100)	+1 (250)	80	75
14	+1 (60)	−1(0.1)	+1 (100)	+1 (250)	90	89
15	−1 (40)	+1(1)	+1 (100)	+1 (250)	78	81
16	+1 (60)	+1(1)	+1 (100)	+1 (250)	96	93
17	0 (50)	0(0.55)	0 (75)	0 (150)	72	75
18	0 (50)	0(0.55)	0 (75)	0 (150)	72	74
19	0 (50)	0(0.55)	0 (75)	0 (150)	72	75

[a] By weight of total system.

23.0262, respectively) having a great influence on the conversion rate.

As observed that *R. miehei* (RMIM), showed overall better results than *T. lanuginosus* (TL IM), we decided to use this enzyme under continuous flow conditions since the use of flow reactors can help in improving the process under development.

To determine the optimal conditions for acid residue esterification and identify variables with important effect on the reaction catalyzed by immobilized lipase from *R. miehei* (RMIM) under flow conditions we use a complete factorial design with two independent variables: flow rate and substrate concentration, varying in two levels and three repetitions of the central point. The temperature used for these reactions was the same used in the best reaction profile obtained in batch, 60 °C (entry 16, Table 1).



Scheme 2. Esterification reaction between 1,2-O-isopropylidene glycerol and free fatty acids residue from palm oil refining process catalyzed by lipases.

The factorial design is presented in parentheses in Table 3 and shows variables with their respective levels used. To adjust a second order model, extra points (axial points) with the same distance from the central point was added to the matrix for this project.

However, the optimization for the batch process, experimental designs and analysis of results were performed using the software

Table 2
Estimated parameter effect for enzymes studied in CCD (batch process).

Variable	Effect		*p*-Value	
	RMIM	TLIM	RMIM	TLIM
Mean	75.1584	74.2894	<0.0001[a]	<0.0001[a]
Temperature (*T*)	3.4956	7.0137	0.0008[a]	0.0037[a]
Amount of enzyme (*E*)	3.9081	9.2487	0.0006[a]	0.0021[a]
Substrate concentration (*S*)	11.1868	23.0262	<0.0001[a]	0.0003[a]
Stirring (*St*)	−2.1756	−3.0887	0.0022[a]	0.0187[a]
T × *E*	1.2943	0.6862	0.0062[a]	0.2508
T × *S*	2.1781	4.3237	0.0022[a]	0.0097[a]
T × *St*	0.5231	2.2487	0.0364[a]	0.0345[a]
E × *S*	−2.4843	−2.9362	0.0017[a]	0.0206[a]
E × *St*	−1.0693	−0.0412	0.0090[a]	0.9321
S × *St*	1.9493	1.3212	0.0027[a]	0.0912

[a] Statistically significant at 95% of confidence level.

Table 3
Experimental factorial design 2^2 and results for the esterification reaction between the solketal and acid residue catalyzed by *Rhizomucor miehei* (RMIM) under continuous flow conditions.

Entry	Substrate (mM)	Flow (mL/min)	Conversion (%)
1	−1 (71.9)	−1 (0.4)	56
2	−1 (71.9)	+1 (0.8)	26
3	+1 (95.3)	−1 (0.4)	53
4	+1 (95.3)	+1 (0.8)	42
5	−1.41 (67.5)	0 (0.6)	73
6	+1.41 (100)	0 (0.6)	20
7	0 (83.7)	−1.41 (0.3)	28
8	0 (83.7)	+1.41 (0.9)	41
9	0 (83.7)	0 (0.6)	37
10	0 (83.7)	0 (0.6)	37
11	0 (83.7)	0 (0.6)	36

Statistica 6.0 (Statsoft, Inc., USA). The level of significance was set as 95% for the mathematical model and surface response. The significance of regression coefficients and associated probabilities $p(t)$ were determined by the Student's t test, the model equation significance was determined by Fisher's F test. The variance explained by the model is given by the coefficients of multiple determination, R^2.

Experimental and matrix data for experimental designs for these reactions are presented in Table 3. Experiments presented in entries 1–4 show combinations of individual variables in the process; entries 5–8 refer to the axial points for constructing the quadratic model and inputs 9–11 are triplicates of the central point for obtaining the experimental error.

The highest conversion is shown in experiment 5 with 73% conversion. This value is considerably lower compared with results obtained in batch reactions, but the reaction time in the first experiments was much higher. In continuous flow equipment used, reaction proceeds in a microenvironment with 0.6 mL total volume. In entry 5 was applied 0.6 mL/min, which means that the contact time between substrate and enzyme was only 1 min, against 4 h by batch reactions. Thus, it was possible to obtain excellent conversion with reduced times under continuous flow conditions.

By using higher flow rates, the residence time for substrate is not enough to lead to good conversions and only moderate to poor results were obtained (entries 2 and 8, Table 3). It is important to note that substrate concentration is also a problem that must be taken into account. In this work high concentrations of substrates can lead to poor conversions, even at low flow rates (entries 6 and 11, Table 3) which may be related to enzyme inhibition by the substrate and also fatty acids deposition inside the channel as observed for some experiments.

Table 4
Estimated effect of the parameter 2^2 experimental design for the esterification reaction between solketal and acid residual catalyzed by *Rhizomucor miehei* (RMIM) under continuous flow.

Variables	Effect	p-Value
Mean	36.9000	<0.0001[a]
Flow	−28.8883	<0.0001[a]
Flow^2	11.6500	0.0006[a]
Substrate concentration	7.6462	0.0011[a]
Substrate concentration^2	−0.4500	0.2763
Flow × substrate concentration	9.4000	0.0015[a]

[a] Statistically significant at 95% of confidence level.

Table 4 shows the estimated effects for the 2^2 experimental design and p values. The variable flow, flow^2, substrate concentration and interaction between flow and substrate concentration showed $p < 0.05$ being significant in the process. The variable flow showed negative effect (−28.8883) as shown in Table 4, indicating that the better low flow is the substrate conversion to products.

Eqs. (1)–(3) represent the experimental models of conversion rate of acid residue to monoacylglicerols by enzymes *R. miehei* (RMIM) and *T. lanuginosus* (TL IM) in batch and continuous flow conditions, respectively, depending on variables studied. The model fit was performed by analysis of variance and parameter R^2.

$$Y = 75.1584 + 3.4956T + 3.9081E + 11.1868S - 2.1756St + 1.2943TxE + 2.1781TxS + 0.5231TxSt - 2.4843ExSt - 1.0693ExSt + 1.9493SxSt \quad (1)$$

$$Y = 74.2894 + 3.5068T + 4.6243E + 11.5131S - 1.5443St + 2.1618TxS + 1.1243TxSt - 1.4681ExS \quad (2)$$

$$Y = 36.6882 - 14.4442Q + 5.8912Q^2 + 3.8231S + 4.6500QxS \quad (3)$$

where Y is the conversion percentage and T, S, E, St and Q, not encoded values of temperature, substrate concentration, enzyme content, mechanical stirring and flow, respectively. Statistical tests of models were performed by Fisher's statistical test for ANOVA (Table S1, see supporting information).

F values calculated for all mathematical models were highly significant, being higher than those tabulated (Table S1, see supporting information). The suitability of a model can be verified by the coefficients of determination (R^2) and (R) correlation. The coefficients of determination $R^2 = 0.98$ for *R. miehei* (RMIM) in batch, $R^2 = 0.99$ for *T. lanuginosus* (TL IM) in batch and $R^2 = 0.91$ for *R. miehei*

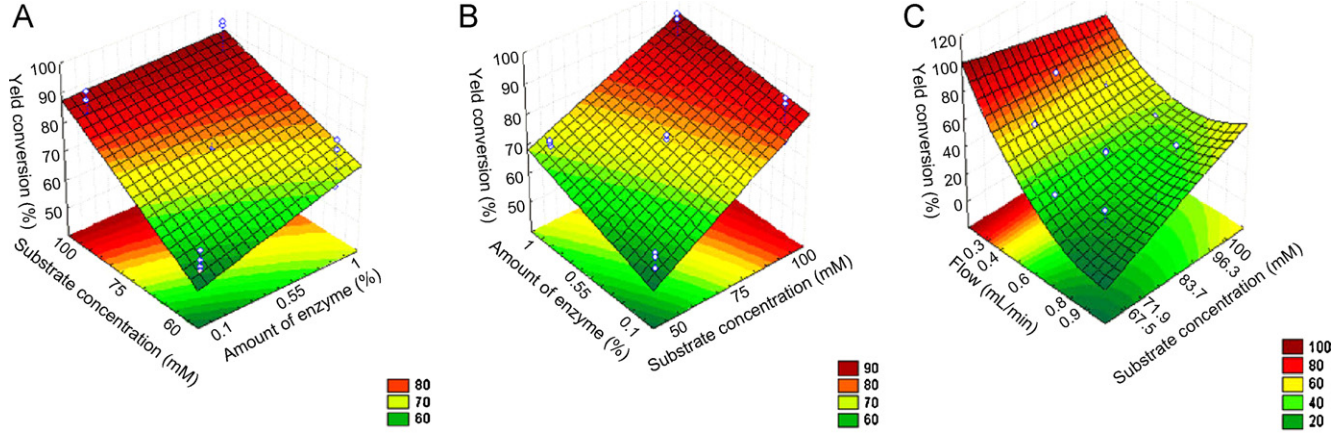


Fig. 2. Response surface figures for batch and continuous flow process [18].

(RMIM), reactions under continuous flow conditions imply that the sample variation sample of 98%, 99% and 91% for esters production is attributed to the independent variables and can be explained by the model accuracy.

R values as 0.99 for batch reaction to *R. miehei* (RMIM), 0.99 for batch reactions to *T. lanuginosus* (TL IM) and 0.95 for continuous flow conditions of *R. miehei* (RMIM) suggest a satisfactory representation of the process model and a good correlation between experimental results and theoretical values predicted by the model equation. The closer to 1 the *R* value (correlation coefficient) indicates the best correlation between experimental and predicted values.

Fig. 2A and B shows the surface response graphs for batch reactions of *R. miehei* (RMIM) and *T. lanuginosus* (TL IM), respectively. The surface is framed in the reaction under continuous flow conditions catalyzed by the enzyme *R. miehei* (RMIM), Fig. 2C shows the negative effect of variable flow that was discussed.

The final step for monostearin synthesis was accomplished by 1,2-O-isopropylidene cleavage using boric acid as standard procedure described over literature.

3. Conclusion

In conclusion, we developed an efficient biocatalytic method under continuous flow conditions that add value to an acid residue of oil palm refining process. Results presented show that both batch and continuous flow conditions can lead to the desired product, but the process flow productivity is better than the batch process, since good yields can be obtained (73%) with short residence times (1 min).

4. Experimental

4.1. Materials

Heptane was purchased from Tedia Co., (R,S)-1,2-isopropylidene glycerol from Sigma–Aldrich as well as all chromatographic standards. Stearic acid (>98%) was purchased from Vetec Ltda. Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Lipozyme IM-20, 25 BIU/g) from *R. miehei*, supported on a macroporous weak anionic exchange resin beads and Lipozyme TL IM (lipase from *T. lanuginosus*, 35 BIU/g) were purchased from Novozymes.

4.2. GC–MS analysis

GC–MS analysis was performed by using modified method from EN 14105. Free fatty acids and (R,S)-1,2-isopropylidene glycerol were transformed into more volatile silylated derivatives in presence of pyridine and N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA). All GC–MS measurements were carried out in duplicate using a DB 5–HT (Agilent, J & W. Scientific®, USA) capillary column (10 m × 0.32 mm × 0.1 μm). The quantifying was done based on calibration curves with internal standards. The GC–MS samples were prepared by dissolving 0.1 g of the final product on 1 mL of n-heptane. 100 μL of this solution and pyridine solutions of butanetriol (1 mg/mL) and tricaprine (8 mg/mL), used as internal standards, were added on a flask forward by an addition of 100 μL of MSTFA. After 15 min, these reactants were dissolved on 8 mL de n-heptane. 1 μL of this sample was then injected into a Shimadzu CG2010 equipment.

4.3. Lowry–Tinsley analysis

The esterification rate was also measured using a modification of the Lowry and Tinsley assay. The depletion of fatty acid was monitored as follows: 0.30 mL of the reaction solution (including the buffer solutions) was added to a tube containing 0.6 mL of n-heptane and 1 mL of cupric acetate-pyridine (5%, w/v, pH 6.0). The final solutions were vigorously mixed for 30 s in vortex, and the upper organic phase was measured by a UV/visible spectrophotometer at 715 nm. Each reaction was analyzed in triplicate, and content conversion, calculated according to the percentage difference for the absorbance shown by the stock solution.

4.4. Continuous flow reaction procedure

A 1 L HPLC bottle was equipped with desired reaction mixture in heptane and a stir bar. The starting mixture was stirred for 5 min, while the X-Cube (ThalesNano) instrument was equipped with the packed bed reactor containing immobilized lipase (0.6 mL volume, 70 mm × 4 mm). The reaction parameters/temperature (40–60 °C), 0.1–3.0 mL/min flow rate and pressure (10 bar) were selected on the flow reactor, and processing was started, whereby only pure solvent (heptane) was pumped through the system until the instrument had achieved the desired reaction parameters and stable processing was assured. At that point the inlet tube was switched from the solvent flask to the 1 L HPLC bottle containing the freshly prepared reaction mixture. After processing through the flow reactor, the inlet tube was dipped back into the flask containing pure heptane and processed for 10 min further, thus washing from the system any remaining reaction mixture. The excess of heptane was removed under vacuum, and the product was obtained and analyzed by GC.

4.5. Batch reactions procedure

The fatty acid residue was melted in a water bath at 60 °C until form a homogeneous oil mixture. Then, a stock solution was prepared in order to avoid pipeting errors. In a flask, the appropriate amount of fatty acid residue and R,S-1,2-O-isopropylidene glycerol in proportions of 1:1 (75 mM) were dissolved in n-heptane, enough to complete 100 mL. The esterification reactions were performed in 2 mL cryotubes, whose were poured 1 mL of stock solution, followed by the addition of appropriate enzyme (1%, w/w). Each reaction was done in triplicate. The cryotubes were finally incubated in shaker at 250 rpm and 60 °C during 4 h. The conversion were measured by a modification of the Lowry and Tinsley method and then analyzed by GC–MS to be confirmed.

4.6. Statistical analysis

The experimental designs and results analysis were carried out using the software Statistica 6.0 (Statsoft, Inc., USA), according with the significance level established to obtain the mathematical model. The significance of the regression coefficients and the associated probabilities, $p(t)$, were determined by Student's t test; the model equation significance was determined by Fisher's F test. The variance is given by the multiple determination coefficients, R^2.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2012.01.008.

References

[1] S.L.Y. Tang, R.L. Smith, M. Poliakoff, Green Chem. 7 (2005) 761–762.
[2] (a) M.J. Hernaiz, A.R. Alcantara, J.I. Garcia, J.V. Sinisterra, Chem. Eur. J. 16 (2010) 9422–9437;
(b) Y.L. Gu, F. Gerome, Green. Chem. 12 (2010) 1127–1138;
(c) P. Anastas, N. Eghbali, Chem. Soc. Rev. 39 (2010) 301–312;
(d) J.H. Clark, F.E.I. Deswarte, T.J. Farmer Biofuels, Bioprod. Biorefin. 3 (2009) 72–90.
[3] A. Salamiah, Non-Food Uses of Palm Oil and Palm Kernel Oil, in: MPOPC Palm Oil Information Series, Kuala Lumpur, 2000, 24 pp.
[4] K.G. Berger, Food Uses of Palm Oil, in: MPOPC Palm Oil Information Series, Kuala Lumpur, 1996, 25 pp.
[5] (a) I. Kralova, J. Sjoblom, J. Dispersion Sci. Technol. 30 (2009) 1363–1383;
(b) J.P. Jain, M. Sokolsky, N. Kumar, A.J. Domb, Polym. Rev. 48 (2008) 156–191;
(c) M.A.R. Meier, J.O. Metzger, U.S. Schubert, Chem. Soc. Rev. 36 (2007) 1788–1802;
(d) J.C. Mol, Green Chem. 4 (2002) 5–13;
(e) I. Johansson, M. Svevensson, Curr. Opin. Colloid Interface Sci. 6 (2001) 178–188.
[6] (a) M. Pernetti, K.F. Van Malssen, E. Floter, A. Bot, Curr. Opin. Colloid Interface Sci. 12 (2007) 221–231;
(b) E. Boyle, J.B. German, Crit. Rev. Food Sci. Nutr. 36 (1996) 785–805.
[7] T. Watanabe, N. Kosenki, H. Kyushiki, WO2004022091-A1, AU2003261950-A1.
[8] E. Martin, Trav. Chim. Aliment. Hyg. 79 (1988) 406–410.
[9] (a) S. Saadi, A.A. Ariffin, H.M. Ghazali, M.S. Miskandar, S.M. Abdulkarin, H.C. Boo, J. Food Sci. 76 (2011) C21–C31;
(b) P. Pacivova, I. Buresova, H. Bilkova, J. Sci. Food Agric. 90 (2010) 2282–2288;
(c) H. Szelag, W. Zwierzykowski, Colloids Surf. A 155 (1999) 349–357;
(d) P. Kohler, W. Grosch, J. Agric. Food Chem. 47 (1999) 1863–1869.
[10] E.R. Gunawan, M. Basri, M.B.A. Rahman, A.B. Salleh, R. Rahman, Enzyme Microb. Technol. 37 (2005) 739.
[11] C.G. Frost, L. Mutton, Green Chem. 12 (2010) 1687–1703.
[12] A. Pohar, I. Plazl, Chem. Biochem. Eng. Q. 23 (2009) 537–544.
[13] P. Watts, C. Wiles, Eur. J. Org. Chem. 10 (2008) 1655–1671.
[14] A. Kirschning, S. Ceylan, J. Wegner, Chem. Commun. 47 (2011) 4583–4592.
[15] (a) M.P. Dudukovic, F. Larachi, P.L. Mills, Chem. Eng. Sci. 54 (1999) 1975–1977;
(b) G.M. Whitesides, Nature 442 (2006) 368–370.
[16] (a) R. Fernandez-Lafuente, J. Mol. Catal. B 62 (2010) 197–212;
(b) R.C. Rodrigues, R. Fernandez-Lafuente, J. Mol. Catal. B 64 (2010) 1–22.
[17] R.R. Lowry, I.J. Tinsley, J. Am. Oil. Chem. Soc. 53 (1976) 470.
[18] R. Hess, U. Bornscheuer, A. Capewell, T. Scheper, Enzyme Microb. Technol. 17 (1995) 725.