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Diacylglycerol synthesis by lipase-catalyzed partial hydrolysis of palm oil under microwave irradiation and continuous flow conditions

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ABSTRACT

Diacylglycerols (DAG) are commonly used in different purity levels for food, medicine and cosmetic industries. Several approaches are found over the literature on DAG production under lipase-catalyzed reactions among which are highlighted: glycerol sterification, vegetable oils' glycerolysis and selective hydrolysis. Results obtained palm oil partial hydrolysis catalyzed by PS Amano IM under microwave irradiation show that DAG can be produced through short-term reactions and moderated yields (5 min, 30%). The DAG production using packed bed reactors under conventional heating and continuous flow conditions is more efficient allowing us to produce 128 g in 24 h with flow rate as a key feature.

1. Introduction

Diacylglycerols (DAG) are commonly used in different purity levels as additives for enhancing fats' plasticity or as bases for food, medicine and cosmetic industries. Mixtures of monoacylglycerols (MAG) and DAG are often used for such applications as they are cheap and provide proper performance [1]. DAG are also used as stranger oils to separate materials from moulds and as fat crystals adjuster [2], being precursors for organic synthesis of products such as phospholipids, glycolipids, lipoproteins [3,4], pro-drugs such as DAG-conjugated chlorambucil for lymphoma treatment [5,6], (*S*)-(3,4-dihydroxyphenyl) alanine (LDOPA) for Parkinson's disease treatment [7] and many others. More recently, DAG-rich oil has been used as a functional cooking oil with 80% of 1,3-DAG content [8].

Several approaches can be found on the literature for lipase-catalyzed DAG production. Sterification of free fatty acids (FFA) with glycerol [9–12], glycerolysis of plant oils [13] and selective hydrolysis [14] are the most used approaches. The lipase-catalyzed partial hydrolysis of plant oils under microwave irradiation has not been well investigated in the literature while the continuous flow procedure has just a few examples.Despite the great advantage of using microwave irradiation technology in small-scale organic reactions, these benefits are lost during the scale-up of a selected process due to the inability to reproduce the microwave heating rates and power density obtained on a small scale [15]. As proposed by Kappe et al., these scale-up problems can be overcome for some reactions by applying conventional heating on continuous flow process, since the high surface to volume ratio allows a rapid heat exchange that mimics the small scale microwave irradiation [16].

In our continuous work on lipase-catalyzed reactions [17,18] and DAG production under enzymatic catalysis [19], we performed the lipase-catalyzed partial hydrolysis of palm oil assisted by microwave irradiation. The scale-up for this process was done under conventional heating continuous flow conditions using a packed bed reactor with lipases.

2. Results and discussion

In order to evaluate the DAG production reaction profile under microwave irradiation we investigated the time reaction and catalyst loading influence (PS Amano SD) on the palm oil partial hydrolysis at 80 °C; results are summarized in Fig. 1. It was used 5% (w/w) water content for the reaction and a blank experiment was also performed not leading to any improvement on the palm oil's DAG content.

As shown in Graph 1, the PS Amano SD enzyme was able to perform the palm oil partial hydrolysis at moderate yields and 80 °C. The best yield was obtained at 60 min (26%) using 1% enzyme while with 10% enzyme the best yield was 27% after 30 min. These results are in agreement with the acidity measured in each sam-

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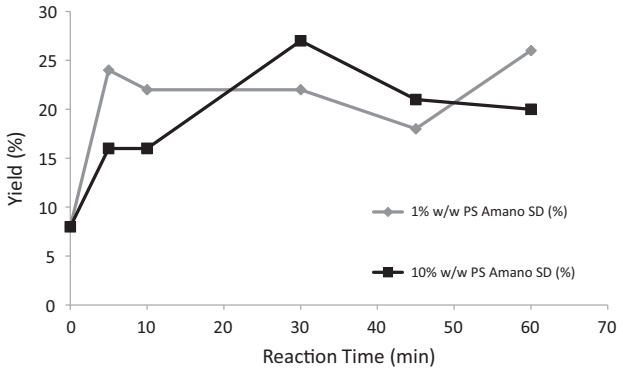


Graph 1. Time reaction and catalyst loading evaluation on the palm oil partial hydrolysis at 80 °C with PS Amano SD (free enzyme).

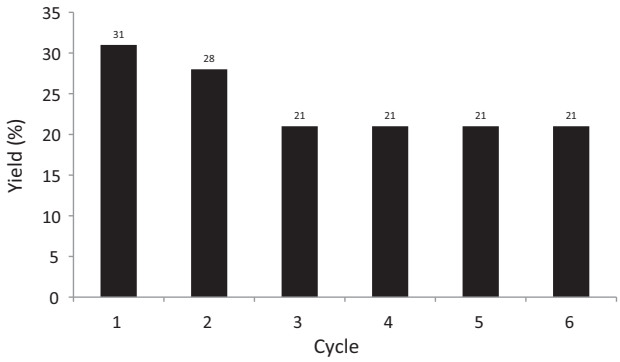


Chart 1. PS Amano IM recycle on the palm oil partial hydrolysis under microwave irradiation (60 °C).

ple, showing increased acidity on higher reaction times due to the acylglycerols hydrolysis.

If Graph 1 is looked carefully, it can be observed that 24% DAG yield is obtained with 1% PS Amano SD after 5 min; in contrast to just 16% obtained using 10% PS Amano SD. This result can be explained by enzymes difficult in building the interface at higher concentrations due to the powder agglomeration. Also this agglomerate can be a perfect site for "*hot spots*" during microwave irradiation leading to enzyme deactivation.

Results presented in Graph 1 were obtained under a "control mode temperature" where the magnetron's output power is controlled by the feedback temperature inside the reaction vessel measured through a fiber optic probe.

In order to evaluate the role of catalyst loading at different temperatures on the palm oil partial hydrolysis, it was chosen to use the immobilized form of PS Amano enzyme. Results are presented in Table 1.

Similar results were obtained at 80 °C compared to those using free PS Amano SD enzyme (Graph 1). Again, lower yields were obtained at higher concentrations probably due to the hot spots on the solid support surface (diatomaceous earth) as the aggregate formation in this type of enzyme preparation is minimized.

Since hot spots are temperature dependent, the same reaction conditions were performed at 60 °C. At this time it has just used the PS Amano IM since similar results were obtained to the free enzyme; the use of immobilized enzymes can be better in recyclability terms when using it in a packed bed reactor.

Results shows that better results are obtained with higher catalyst loadings at 60 °C promoting 31% DAG in 5 min under microwave irradiation at a control mode temperature.

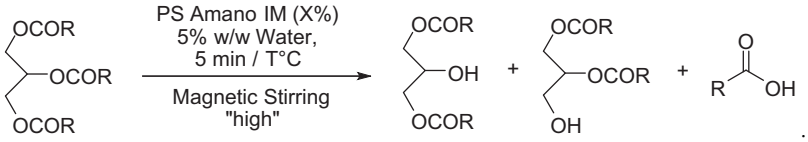
The PS Amano IM recyclability was also observed and results obtained are shown in Chart 1. 10% yield was lost after the third cycle compared to the first run but during the enzyme recycle was possible to identify that we were losing mass during the purification procedure. Thus, the enzyme amount was not the same after the first cycle.

Since results obtained under microwave irradiation lead only to moderated yields, it was investigated the use of a packed bed reactor with PS Amano IM under conventional heating continuous flow regime in order to obtain higher DAG amounts. The micro reactor system used for flow synthesis in this work was a high-temperature, high-pressure microtubular flow unit that can be used for processing homogeneous reaction mixtures (X-Cube, Thales Nanotechnology Inc.). This reactor uses stainless steel coils (i.d. 1000 μm) with variable lengths (4, 8, and 16 mL volume) that can be directly heated across their full length by electric resistance heating up to 350 °C. The reaction mixture is introduced to the reactor block containing the packed bed reactor and a heat exchanger via one or more standard HPLC pumps (flow rate: 0.1–3.0 mL/min). The system pressure valve sets and stabilizes the set pressure value between 5 and 150 bar pressures.

Employing the X-Cube flow system we worked on two HPLC pumps, one for the palm oil and another to the water. Since palm oil is solid at room temperature it was placed a heater plate for pumping into the system. Unfortunately, palm oil viscosity was too high for pumping by the standard HPLC pumps/valves integrated into the X-Cube flow system. Another problem is that temperature at the stainless steel coils decreases after passing through the heater/packed bed reactor solidifying the oil and blocking the system. At this point we had no other choice than to adapt the microwave irradiation protocol to use it in our flow process.

Therefore, a palm oil solution in organic solvents (hexane and cyclohexane) was prepared for this purpose. First, 1 M solution with both hexane and cyclohexane was tested. Reactions were performed pumping the desired reaction mixture (organic solvent/palm oil) from pump A and water from pump B at 5% (w/w) water. These pumped solutions were mixed in 5 mL loop and then pumped through the packed bed reactor loaded with PS Amano IM

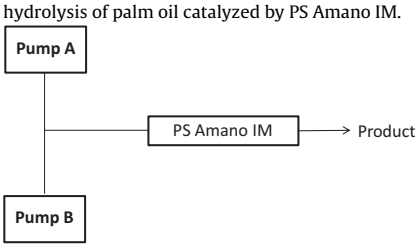
Table 1
Effect PS Amano IM loading on the lipase catalyzed partial hydrolysis of palm oil at different temperatures.



Entry	Loading	Temp. (°C)	Yield (%)[a]
1	1%	80	23
2	1%	60	10
3	10%	80	19
4	10%	60	31

[a] Based on GC (gas chromatography) analysis.

Table 2
Effect of solvents and flow rate on the continuous production of DAG by partial hydrolysis of palm oil catalyzed by PS Amano IM.



Entry	Solvent	Rate (mL/min)	Residence time	Yield (%)[a]
1	Hexane	0.5	1.2 min	48
2	Hexane	1.5	0.4 min	26
3	Hexane	3.0	0.2 min	16
4	Cyclohexane	0.5	1.2 min	45
5	Cyclohexane	1.5	0.4 min	23
6	Cyclohexane	3.0	0.2 min	16

[a] Based on GC (gas chromatography) analysis.

Table 3
Adjustment of flow rate to increase DAG production by partial hydrolysis of palm oil catalyzed by PS Amano IM.

Entry	Rate (mL/min)	Residence time	Yield (%)[a]
1	0.1	6 min	57
2	0.2	3 min	50
3	0.3	2 min	47
4	0.4	1.5 min	48

[a] Based on GC (gas chromatography) analysis.

heated at 60 °C as in the microwave irradiation setup. Results are summarized in Table 2.

As shown in Table 2, the use of flow system allow us to reach 48% DAG production under 0.5 mL/min rate flow and 60 °C. Higher rates do not lead to good results once residence time seems to be short enough for palm oil hydrolysis. Hexane and cyclohexane provided similar results and can be distilled from the final product and recycled in the process.

We have also tried to use higher palm oil solution concentrations but yields obtained for DAG production were lower than those obtained with 1 M solution.

After this initial result it was chosen to operate the flow system under a lower rate trying to find the perfect adjustment to the partial palm oil hydrolysis. A range of 0.1–0.4 mL/min was tested in the same reaction profile using hexane as solvent and results are summarized in Table 3.

As shown in Table 3, DAG production yield is dependent on the flow rate, where higher residence times can lead to better yields on the palm oil partial hydrolysis. DAG production achieved best results (128 g DAG enriched palm oil) with 0.1 mL/min after 24 h.

Since best results for DAG production were obtained under continuous flow condition, it was chosen to run the same reaction profile in packed bed reactors with different enzymes sources (Table 4).

Results obtained show that other enzyme sources could be used for palm oil partial hydrolysis; however, PS Amano IM (entry 1, Table 3) provided best results for DAG production under optimized conditions compared to lipozyme RM IM, lipase Ca-A and lipase Ca-C (entries 1–3, Table 4).

Table 4
Effect of different enzymes on continuous flow production of DAG by partial hydrolysis of palm oil.

Entry	Enzyme source	Yield (%)[a]
1	Lipozyme RM IM	40
2	Lipase Ca-A	35
3	Lipase Ca-C	33

[a] Based on GC (gas chromatography) analysis.

3. Conclusion

In conclusion, we have performed the palm oil partial hydrolysis catalyzed by lipases under microwave irradiation with short reaction times and moderated yields. These results were used as starting points to development a conventional heating flow process for DAG production with packed bed reactors. Results obtained in the flow mode were better than those under microwave irradiation and produced 128 g in 24 h. Rate flow is an important parameter to get the best yield on the palm oil partial hydrolysis and the oil must be diluted in order to avoid blocking the system by crystallization.

4. Experimental

4.1. Microwave irradiation and continuous flow equipment

Reaction optimization on small scale was performed either in a Monowave 300 (Anton Paar GmbH, or a Discover LabMate (CEM Corp.) in Pyrex microwave process vials using standard procedures. All flow chemistry described herein was performed at X-Cube Flash stainless steel microreactor (ThalesNano Inc.) according to the general principles previously described.

4.2. General methods

All chemicals were purchased from commercial sources and used without further purification. Palm oil was donated by the Agropalma S/A Companhia Refinadora da Amazônia. The GC analysis was performed by using the modified method from EN 14105 (fat and oil derivatives-fatty acid methyl ésteres (FAME) – determination of free and total glycerol and mono, di, triglyceride contents – Reference method n°28, 2001). Mono-, di-, tri-glyceride, free and total glycerol were transformed into more volatile silylated derivatives in the presence of pyridine and N-methyl-N-trimethysilyltrifluoroacetamide (MSTFA). All GC measurements were carried out in duplicate (Dizge & Keskinler; 2008) using a DB 5–HT (Agilent, J & W. Scientific®, USA) capillary column (10 m × 0.32 mm × 0.1 μm). The quantification was performed based on calibration curves of monolein, diolein and triolein which were used as internal standards. GC samples were prepared by dissolving 0.1 g final product on 1 mL 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 100 μL MSTFA addition. After 15 min these reactants were dissolved into 8 mL n-heptane. 1 μL of this sample was then injected into a Shimadzu GC2010 equipped with on-column injector and flame ionization detector.

4.3. Microwave irradiation procedure

Palm oil hydrolysis catalyzed by lipase under microwave irradiation were carried out by adding palm oil (1 g), water (5% wt oil mass) and lipase (1%, w/w) to a 10 mL Pyrex microwave process vials. The microwave irradiation was performed under control mode temperature. All reactions were performed in closed vessels and the pressure monitored by the microwave equipment. The reaction was stopped by filtering the enzyme and the products were extracted using n-hexane and then being washed twice with water. The organic media was collected and the solvent was evaporated under reduced pressure. Final products were analyzed by gas-chromatography.

4.4. Continuous flow procedure

1-L HPLC bottle was equipped with palm oil and solvent (hexane/cyclohexane) and a stir bar. The starting mixture was stirred for 5 min, while the X-cube flash instrument was equipped with the packed bed reactor containing lipase (0.6 mL volume, 1.2 min residence time at 0.5 mL/min flow rate). The reaction parameters/temperature (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 (hexane/cyclohexane) 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 reaction mixture. After processing through the flow reactor, the inlet tube was dipped back into the flask containing pure hexane and processed for 10 min further, thus washing from the system any remaining reaction mixture. The Hexane excess was removed under vacuum, the DAG enriched palm oil was obtained and analyzed by GC.

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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.2011.04.021.

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