High Oleic Enhancement of Palm Olein via Enzymatic Interesterification

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Abstract: Acidolysis to incorporate oleic acid into refined, bleached and deodorized (RBD) palm olein (IV 56) using various lipases (enzymes) as catalysts to increase the oleic content of the oil was investigated. Immobilised lipases (lipase PLG, Lipozyme TL IM, Lipozyme RM IM and Novozym 435) and non-immobilised lipase (lipase PL) were used in this study to compare the effectiveness of the selected lipases in catalyzing the reaction to produce a high oleic oil. The results showed that the TAG of OLO/OOL content was increased at least 4 fold and OOO content was increased at least 3 fold when a 5% enzyme load was used. Lipase PL showed the greatest increase in tri-unsaturated triacylglycerols (TAGs) content. A pilot scale experiment conducted using TL IM enzyme, followed by recovery of the oil and fractionation allows the production of oils with varying oleic contents. A high oleic content of 56% was achievable.

Key words: high oleic oil, acidolysis, lipases, RBD palm olein, triacylglycerol composition

1 INTRODUCTION

Oleic acid (also called cis–9–octadecenoic acid) is a mono–unsaturated fatty acid found in animal and vegetable oils. It occurs naturally in greater quantities than any other fatty acid. It is an omega–nine fatty acid, and considered one of the healthier fatty acids in the diet. High oleic (HO) oils such as HO sunflower oil fetch higher premium over the normal oil.

Numerous studies have been carried out to investigate the benefits of HO oils, particularly against coronary heart disease. High concentrations of oleic acid can lower blood levels of cholesterol, and raise levels of high–density lipoproteins (HDLs) while lowering low–density lipoproteins (LDLs), known as the “bad” cholesterol1,2). It has been reported that oleic acid protects against LDL oxidation, which has been suggested to play an important role in atherogenesis3–5). Therefore, high oleic oils have a real opportunity to substitute existing oils of higher saturated fatty acids, while providing certain functionality and nutritive values.

Oleic acid can be incorporated into oils to increase the oleic content of the mother oil to offer desirable properties related to both health benefits and stability characteristics. Several researchers have also studied the incorporation of oleic acid into oils especially butterfat6–8), tristearin9,10), soybean oil11) and tallow12) to produce more value–added lipids. Palm oil and palm olein have moderate contents of oleic acid. Increasing the oleic contents by selective breeding and/or genetic engineering takes many years of research and is nowhere near commercial ventures as yet. A much faster route will be via enzymatic acidolysis, which forms the research shown in this paper.

Lipases are enzymes that hydrolyze ester bonds of long chain fatty acids and alcohols and also catalyze esterification and transesterification (acidolysis, alcoholysis and interesterification). They are powerful tools for syntheses of structured lipids (SLs) which are triacylglycerols (TAGs) modified to change the fatty acid (FA) composition and/or the positional distribution in the glycerol backbone. Although chemical interesterification catalyzed by metal alkoxides is simple and inexpensive, it is not capable of modifying specific positions due to the random nature of the reactions. The 2–position of the TAGs in palm oil is preferentially occupied by an unsaturated fatty acid, mainly oleic acid. Therefore, 1,3–specific enzymes are more favourable for positionally specific modification of palm oil products, retaining the oleic acid at the 2–position of the TAGs. The advantages of enzymatic reactions are selectivity, mild reaction conditions, little or no unwanted side reactions or by–product isomerisation, ease of product

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Accepted July 21, 2009 (received for review April 28, 2009)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/
recovery, easy control over the process and less waste disposal\textsuperscript{13–15}.

Most of the work on acidolysis of oils have been on other oils. Very little work has been on palm oil products, in particular with respect to using oleic acid. In this work, several enzymes were tried, with the final selection of one of the enzymes for a pilot scale study.

2 MATERIALS AND METHODS

2.1 Materials

The substrates used were refined, bleached and deodorized palm olein (iodine value 56) and oleic acid (88% purity). Refined, bleached and deodorized palm olein (IV 56) and standard refined, bleached and deodorized palm oil were purchased from Sime Darby Plantations Sdn Bhd., Malaysia. Oleic acid was purchased from Merck Sdn. Bhd., Malaysia. The catalysts used were lipases such as lipase PL, lipase PLG, Lipozyme TL IM, Lipozyme RM IM and Novozym 435. Lipase PL (Alcaligenes sp.) and lipase PLG (Alcaligenes sp. immobilized on granulated diatomaceous earth) were a generous gift from Meito Sangyo Co., Ltd., Japan. Lipozyme TL IM (Thermomyces lanuginosus lipase immobilized on a granulated silica), Lipozyme RM IM (Rhi-

2.2 Methods

2.2.1 Reaction protocol

Acidolysis of refined, bleached and deodorized palm olein (IV 56) was carried out to incorporate oleic acid into the glycerol backbone, catalysed by the chosen lipases. Reaction was monitored by reduction in POP content of the oil (Fig. 1).

The optimum temperature for enzymes may vary, however, in order to avoid crystalisation or solidification of the oils, a higher temperature of 60°C was selected. Palm oil products have higher melting points, and during the reaction, higher melting TAGs may be formed, resulting in some solidification if temperature of reaction is too low. The enzymes used in this study were Meito PLG (immobilized), Meito PL (non–immobilized) and Lipozyme TL IM (immobilized). The amount of substrate, palm olein:oleic acid used was 3:1 mol/mol. This ratio was selected based on earlier trials indicating reasonable amounts of oleic acid could be incorporated, and also based on cost effectiveness.

A dosage of 5% was chosen for comparison, being the common dosage used for immobilised type enzymes. For the non–immobilised enzymes, this dosage may be considered too high. However, for the purpose of a quick comparison, the dosage of enzymes is kept at 5%. When the need to study or effectively select the most appropriate enzyme for commercial application, further consideration should be taken into account the most appropriate dosage or process. The resultant fatty acids remaining in the oil had to be removed by short path distillations. These experiments were carried out using the rotary evaporator for 14 hours for time course profile. No vacuum was applied. Samples were collected every two h. by removing a small aliquot and filtering over cotton wool. The oil is retained for HPLC analysis of the TAGs. TAG data obtained from 8 h reaction were compared and shown in Figs. 2a–d.

The pilot scale experiment (1.8 kg) was carried out at 60°C using TL IM enzyme in a 5kg reactor fitted with a stirrer. Reaction was carried out for 8 h. The enzyme load was 5% w/w. After reaction, the oil was filtered through a vacuum filter flask. Free fatty acids (FFA) were removed by short path distillations using a VTA 70 from VTA GmbH Germany. The major part of the equipment was constructed from glass. The heating of the evaporator was provided by a jacket circulated with thermal oil from an oil bath. The main components are short path evaporator integral condenser, cooling trap for filling of liquid nitrogen and the vacuum system which is included oil diffusion pump and rotary vane pump. The evaporator surface is 0.04 m², with vacuum achieved up to 1 x 10\textsuperscript{–3} mbar. 1.5 kg of oil was preheated to 80°C and taken into the distillation column by a pump. The residue (Oil) and distillate (FFA) were collected in round bottomed flasks at temperature of 160°C.

2.2.2 Fractionation

Fractionation was carried out on 100g sample, using a small container, fitted with a stirrer. The container was placed in a waterbath, where the temperature control is as
Figs. 2a-d  Tri-unsaturated, Mono-saturated, Mono-unsaturated and Tri-saturated TAGs Content in RBD Palm Olein (IV 56) at 60°C.
follows: After fractionation, the oil is filtered through a fil-
tered paper, under vacuum. Filtration is carried out under
cool conditions to avoid remelting of crystals.

<table>
<thead>
<tr>
<th>Fractionation at 8°C</th>
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<th>Duration, min</th>
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<tr>
<td>2</td>
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<td>4</td>
<td>18</td>
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<td>5</td>
<td>15</td>
<td>140</td>
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2.2.3 HPLC analyses

The TAG composition of samples were determined by
reversed–phase HPLC from Gilson, France using refractive
index detector from Waters 2410, USA and two commer-
cially packed LiChrospher100 RP–18 column encapped (25
cm each) with 5 μm particle size from Merck Sdn. Bhd.,
Malaysia. Oil samples of about 0.040 g were weighed and
diluted in acetone for injection into the HPLC column. The
TAG composition were eluted with a mobile phase of ace-
tone:acetonitrile (7:3) at a flow rate of 1 mL/min. The
method of analysis was modified from AOCS Official
Method Ce 5c–93 (AOCS Official Method 1995). The TAG
peaks were identified according to the reference standards
and comparison with information on palm oil available in
the literature16. Quantification was based on area %. In
the analysis, the TAG of POP referred to mixtures of POP
and PPO, and do not differentiate between the isomers.

3 RESULTS AND DISCUSSION

3.1 Laboratory trials

The incorporation of oleic acid in an oil depends on the
type of enzyme being used, the ratio of oil to oleic acid as
well as optimum conditions. In this study, oil to oleic acid
ratio of 3:1 was chosen, as being more cost effective, taking
into account cost of oleic acid. A higher oleic acid ratio
would no doubt provide more oleic acid into the triacyl-
glycerols, however, the mixture of fatty acids that need to
be removed would be higher. Thus, after some considera-
tion, it was estimated that the 3:1 would provide sufficient
oleic for the reaction.

The TAG profiles of the acidolysed samples were com-
pared. From the HPLC analysis, TAGs were grouped into
tri–unsaturated, tri–saturated, mono–unsaturated and
mono–saturated TAGs. Graphs of the tri–unsaturated,
tri–saturated, mono–unsaturated and mono–saturated
TAGs before and after reactions with different types of
enzymes were plotted.

The reaction was monitored by evaluating the reduction
in POP triacylglycerol, the main triacylglycerol in the oil.
This method was used, as other TAGs with oleic contents
may not be reflective of the overall incorporation of the
oleic acid. Although the best way is to monitor the oleic
content of the oil directly after reaction, it can only be car-
rried out after removal of the fatty acids present in the oil.
Thus, a more direct method was utilised by checking the
POP content by HPLC. In actual fact all the TAGs were
monitored, but for the purpose of this paper, the POP
reduction is illustrated.

Figure 1 shows the reduction of the main triacylglycerol
component (POP) over 14 h. The results indicate that after
8–10 h. equilibrium is reached with the 5% dosage at 60°C
for PL, PLG and TL IM, but not for RM IM and N435. Table
1 shows that except for RM IM and N435, most other
enzymes attained equilibrium with almost similar oleic
contents at 60°C. PL can achieve a higher oleic content
under similar conditions, indicating the higher efficiency
or activity of the enzyme. Both RM IM and N435 require
high reaction temperature, thus oleic content tends to be
lower. Also, if PL were to be used, the equilibrium time is
shorter with the 5% dosage. It is possible to use a lower
dosage, and should be considered in further studies. When
using TL IM, the temperature within 50–70°C gave almost
similar results.

Palm olein IV 56 consists mainly of disaturated (POP and
PPO) and monosaturated (POO and OPO) TAGs, which are
28.9% and 23.7% respectively. There is at least 4 fold
increase in oleic–linoleic–oleic or oleic–oleic–linoleic TAGs
contents (OLO) and 3 fold increase in triolein (OOO) con-
tent. Lipozyme® TL IM gives a comparable yield of 12.6% of
triolein and can work as well as lipase PLG.

Figures 2a – d show the different classes of TAG content
in the refined, bleached and deodorized palm olein (IV 56)
after incorporating oleic acid. Lipase PL showed the highest increase in tri-unsaturated TAGs. The activity of lipase PL is higher as it is a non-immobilized enzyme when compared to the immobilized enzymes. Although by using Lipase PL will generate the highest yield in tri-unsaturated TAGs, but tri-saturated TAGs content is also the highest among the 3 types of enzymes used. Therefore, at room temperature, the esterified oil by lipase PL will tend to crystallize faster than the other two samples, resulting in the oil becoming half solidified in room temperature. Lipase PLG and Lipozyme TL IM showed comparable results in tri-unsaturated TAGs. However, Lipozyme RM IM and Novozym 435 showed a much lower tri-unsaturated TAGs content.

Mono-saturated TAGs are of POO, and PLO, with SOO and PLL being minor components. The highest POO tends to come from N435 and RM IM, while for the mono-unsaturated TAGs, the lowest POP comes from PL and TL IM. In terms of saturated TAGs being formed, PL, PLG and TL IM provide the highest contents, resulting from the breakdown in POP, where the palmitic acid is re-esterified with the small amount of OPO normally present. As the cheapest enzyme for use is TL IM, further studies on pilot scale were carried out with this enzyme. As it is in the immobilised form, it is easier to carry out the reaction.

3.2 Pilot scale experiments

A pilot scale experiment was carried out using TL IM enzyme, followed by short path distillations to remove the free fatty acids. The final oil was analysed, and further fractionated at different temperatures of 8, 10 and 15 °C. The oils obtained illustrate compositions of high oleic palm oil products which are achievable with enzymatic processing and fractionation. Table 2 shows the fatty acid compositions of the original olein, and the final products obtained. The iodine values have been modified from IV 56 to 67.3, and with further fractionation, improved to 72.0 to 73.6. This amount to about 33% increase in the oleic content from 42.1% to 55.8%.

4 CONCLUSIONS

Lipase-catalyzed acidolysis (interesterification) reactions can be used to incorporate oleic acid into palm olein to increase its oleic content. From the study, the OLO content was increased at least 4 fold and the triolein (OOO) content was increased at least 3 fold. An increment of 33% oleic acid was achievable, resulting in high oleic content of 55–56% oleic in palm oil products. Currently, the available palm olein which has the highest oleic content is one with iodine value about 67, having oleic of only 49%. Further improvements could be obtained if fractionation is optimised to a lower temperature. Also, there is further need to optimise reaction conditions for any one of the enzymes, if to be utilised in commercial operation, as the paper only
Table 2  Fatty Acid Composition of Palm Olein IV 56, Enzymatically Modified Palm Olein and New Fractions at 8, 10 and 15˚C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C20:0</th>
<th>SFA</th>
<th>C16:1</th>
<th>C18:1</th>
<th>MUFA</th>
<th>C18:2</th>
<th>C18:3</th>
<th>PUFA</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olein IV 56</td>
<td>–</td>
<td>0.2</td>
<td>1.1</td>
<td>40.7</td>
<td>4.0</td>
<td>0.4</td>
<td>46.4</td>
<td>0.2</td>
<td>41.9</td>
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<td>11.2</td>
<td>0.3</td>
<td>11.5</td>
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<td>Modified olein</td>
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<td>0.9</td>
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<td>0.3</td>
<td>36.9</td>
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<td>12.8</td>
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<td>Olein 8</td>
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<td>0.8</td>
<td>25.1</td>
<td>2.7</td>
<td>0.3</td>
<td>29.5</td>
<td>0.2</td>
<td>55.8</td>
<td>56.0</td>
<td>14.5</td>
<td>0.2</td>
<td>14.7</td>
<td>73.6</td>
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<tr>
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<td>0.2</td>
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serve to provide a general estimate of the possible properties of a high oleic oil from palm products through enzymatic process.

ACKNOWLEDGEMENTS

The authors thank the Director–General of MPOB for permission to publish this work; Director of Product Development and Advisory Services Division for their support and other assistant research officers of the Analytical and Quality Development Unit for their technical assistance.

References
